

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 BOD₅ 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 NH₃-N occurred in both 50% and 100% leachate. The highest percentage of NH₃-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 BOD₅, COD and NH₃-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% NH₃-N removal. A promising BOD₅, COD and NH₃-N leachate removal was obtained with the treatment using immobilized *G. australe* in flask followed by treatment with crude enzymes. The percentage removal of BOD₅ in leachate was 58.47% while for COD and NH₃-N were 57.02% and 62.17%, respectively. This findings suggested that in order to achieve optimum removal of leachate BOD₅, COD and NH₃-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, perkadarannya 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* and *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 $NH_3\text{-N}$ masing-masing adalah 57.02% dan 62.17%. Penemuan ini mencadangkan bagi mencapai penyingkiran BOD_5 , COD dan $NH_3\text{-N}$ bahan larut lesap yang optimum, rawatan gabungan antara *G. australis* pegun di dalam kelalang dan enzim bebas sel (=enzim mentah) mesti diaplikasikan.

ACKNOWLEDGEMENTS

Alhamdulillah.... praise to Allah SWT for his blessing and mercy by giving me an excellent health to enable me to complete my study.

During these years many people got involved in one way or another in my research project and for that contribution they will always have my sincere gratitude. However, I feel obliged to acknowledge special contributions of some of these persons who supported me continuously to complete this research.

First and foremost, I would like to thank both of my supervisors Associate Prof. Dr. Noor Zalina Mahmood and Prof. Dr Noorlidah Abdullah for providing excellent guidance and criticisms. Secondly, I would like to thank all my friends and staff in the Mycology Lab, University of Malaya and also in the Faculty of Applied Sciences, University Technology MARA for helping me through-out my study.

My special thanks also go to my family members who love and support me during my whole journey of education. I am thankful to my parents and parents' in-law for their blessing on me in achieving this important step in my professional career. Last but not least to my beloved husband - Shamzol Ropae, my childrens—Muhammad Amirul Shahmi, Nurhuda Aina, Nurhuda Afifah and Muhammad Amirul Shakir. Thank you for your support, understanding and courage to stand next to me all these years.

Finally, thank you very much for all the people that involved directly or indirectly in realizing this valuable thesis. May Allah reciprocate your deed.

CONTENTS

	PAGE
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	viii
LIST OF FIGURES	xv
LIST OF TABLES	xvii
LIST OF SYMBOLS AND ABBREVIATIONS	xix
CHAPTER ONE	
1.0 Background of study	1
1.1 Problem statements	6
1.2 Research objectives	8
1.3 Scope of study	9
CHAPTER TWO	
LITERATURE REVIEW	11
2.1 Solid Waste Management	11
2.2 Leachate	14
2.3 Characteristics of leachate	17
2.3.1 pH	18
2.3.2 BOD and COD	18
2.3.3 Nitrogen	20
2.3.4 Heavy metals	22
2.4 Treatments of leachate	22
2.4.1 Physical/Chemical treatments	24

2.4.1.1	Chemical treatment	24
2.4.1.2	Physical treatment	25
2.4.2	Membrane technology	26
2.4.3	Biological treatments	27
2.5	Bioremediation	31
2.5.1	Fungal in bioremediation	34
2.5.2	Bioremediation of leachate	36
2.5.3	Fungal remediation of leachate	37

CHAPTER THREE

CHARACTERIZATION OF LEACHATE COLLECTED FROM LANDFILL IN SELANGOR

3.0	INTRODUCTION	41
3.1	MATERIALS AND METHODS	44
3.1.1	Landfills description	44
3.1.2	Sampling procedure	46
3.1.3	Analysis of leachate	48
3.1.3.1	pH	48
3.1.3.2	Total suspended solid	48
3.1.3.3	Biological oxygen demand (BOD_5)	49
3.1.3.4	Chemical oxygen demand (COD)	49
3.1.3.5	Total nitrogen (TN)	50
3.1.3.6	Ammoniacal nitrogen (NH_3-N)	50
3.1.3.7	Total organic carbon (TOC)	50
3.1.3.8	Heavy metal	51

3.2	RESULTS AND DISCUSSION	
3.2.1	Preliminary study of leachate characteristic collected from sanitary and non-sanitary landfills	51
3.2.2	Comparison of leachate characteristic collected from active and closed landfills	55
3.3	CONCLUSION	61

CHAPTER FOUR

SCREENING OF FUNGI AS CANDIDATE FOR LEACHATE BIOREMEDIATION

4.0	INTRODUCTION	63
4.1	MATERIALS AND METHOD	66
4.1.1	Leachate sample	66
4.1.2	Fungal strains	66
4.1.3	Preparation of leachate medium	66
4.1.4	Effect of pH of leachate medium on growth of fungi	67
4.1.5	Effect of leachate concentration on growth of fungi	67
4.1.6	Growth of fungi on leachate medium	67
4.1.7	Statistical analysis	69
4.2	RESULTS AND DISCUSSION	69
4.3	CONCLUSION	75

CHAPTER FIVE

TREATMENT OF LEACHATE USING MYCELIA OF SELECTED WHITE-ROT FUNGI

5.0	INTRODUCTION	76
5.1	MATERIALS AND METHOD	78
5.1.1	Stock culture maintenance	78
5.1.2	Preparation of mycelial suspension	78
5.1.3	Leachate sample	78
5.1.4	Treatment of leachate with free fungal mycelium in batch culture at aerobic condition	79
5.1.5	Preparation of immobilized fungal mycelium	80
5.1.5.1	Sterilization of Ecomat	80
5.1.5.2	Immobilization of fungal mycelium on Ecomat	80
5.1.6	Treatment of leachate using immobilized mycelium in batch cultures	81
5.1.7	Treatment of leachate using immobilized <i>G.</i> <i>australe</i> mycelium on Ecomat in column	81
5.1.8	Leachate analysis	83
5.1.9	Statistical analysis	84
5.2	RESULTS AND DISCUSSION	84
5.2.1	Treatment of leachate with free cell mycelia of <i>Ganoderma australe</i> and <i>Trametes menziesii</i> in batch cultures at aerobic condition	84
5.2.2	Treatment of leachate using immobilized mycelia of <i>Ganoderma australe</i> and <i>Trametes menziesii</i> on	89

Ecomat in batch cultures	
5.2.3 Treatment of leachate by immobilized coculture	94
mycelia of <i>Ganoderma australe</i> and <i>Trametes menziesii</i> on Ecomat in batch cultures	
5.2.4 Treatment of leachate using Ecomat-immobilized mycelium of <i>G. australe</i> in column	98
5.3 CONCLUSION	104

CHAPTER SIX

TREATMENT OF LEACHATE USING ENZYMES PRODUCED BY *G. AUSTRALE* AND *T. MENZIESII*

6.0 INTRODUCTION	105
6.1 MATERIALS AND METHOD	108
6.1.1 Fungal cultures and maintenance	108
6.1.2 Preparation of mycelia suspension and inoculum	108
6.1.3 Profiling of extracellular enzymes and the effect of inducers on enzymes yield	109
6.1.4 Preparation of extracellular enzyme and crude enzyme by <i>G. australe</i>	109
6.1.5 Preparation of enzyme extract	110
6.1.6 Enzyme assays	110
6.1.6.1 Lignin peroxidase Activity	111
6.1.6.2 Manganese peroxidase Activity	111
6.1.6.3 Laccase Activity	111
6.1.6.4 Protease Activity	112
6.1.6.5 Lipase Activity	112

6.1.6.6 Amylase Activity	112
6.1.7 Remediation of leachate by crude enzyme	113
6.1.8 Remediation of leachate by immobilized <i>G. australe</i> followed by treatment with crude enzyme	114
6.2 RESULTS AND DISCUSSION	114
6.2.1 Profiling of extracellular enzymes and the effect of inducers on enzymes yield	115
6.2.2 Production of extracellular enzymes by <i>G. australe</i>	120
6.2.3 Remediation of leachate by crude enzyme at different parameters	122
6.2.4 Remediation of concentrated (100%) leachate by immobilized <i>G. australe</i> followed by treatment with crude enzyme	128
6.3 CONCLUSION	135
CHAPTER SEVEN	
CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK	
7.0 OVERALL CONCLUSION	136
7.1 RECOMMENDATIONS FOR FUTURE WORK	139
REFERENCES	141

APPENDIX

APPENDIX A: STATISTICAL ANALYSIS TABLE	158
APPENDIX B: LIST OF PUBLICATIONS/PAPER	185
PRESENTATIONS	

LIST OF FIGURES

Figures	Page
1.1 Waste Hierarchy (Current Status)	2
1.2 Waste Hierarchy (Targeted 2020)	2
1.3 Scope of Work	10
2.1 Compost Nitrogen Cycle	21
2.2 Current leachate treatments	24
2.3 Fungi used to treat leachate	38
3.1 Landfill sites included in the study	46
3.2 Example of sanitary landfills and non-sanitary in the study	47
4.1 Flow chart and method to determine growth of fungi on Petri dish	68
4.2 Growth rates of twelve different fungi on leachate medium containing 50% leachate with pH adjusted to 6.0 and unadjusted pH at 28° C	70
4.3 Growth of <i>G. australe</i> on MEA incorporated with 50% and 100% leachate after twelve days of incubation at 28° C	71
4.4 Growth rates of twelve different fungi on MEA, MEA with 50% leachate and MEA with 100% leachate at 28° C	72
5.1 Free fungal mycelium	80
5.2 Immobilization of fungal mycelium on Ecomat	80
5.3 Flow chart of experimental procedures for leachate treatment with fungal mycelia	82
5.4 Experimental set-up of column of immobilized <i>G. australe</i> on Ecomat for the leachate treatment	83
5.5 Percentage removal of 100% leachate BOD ₅ , COD, NH ₃ -N	91

	and pH changes by <i>T. menziesii</i> immobilized on Ecomat at weekly intervals for 28 days incubated at room temperature, shaking at 150 rpm	
5.6	Percentage removal of 100% leachate BOD ₅ , COD, NH ₃ -N and pH changes by coculture mycelia of immobilized <i>G. australe</i> and <i>T. menziesii</i> after treatment for 28 days, incubated at room temperature, shaking at 150 rpm.	95
6.1	Profiling of enzymes produced by <i>G. australe</i> in Medium A incubated at room temperature, agitated at 150 rpm	116
6.2	Profiling of enzymes produced by <i>T.menziesii</i> in Medium A incubated at room temperature, agitated at 150 rpm	116
6.3	Enzymes profile of enzyme extract from <i>G. australe</i> in Medium B incubated at room temperature, agitated at 150 rpm	118
6.4	Enzymes profile of enzyme extract from <i>T.menziesii</i> in Medium B incubated at room temperature, agitated at 150 rpm	118
6.5	Activity of enzymes in extract and crude enzyme of <i>G. australe</i> incubated at room temperature, agitated at 150 rpm for four days	121
6.6	The oxidative reaction of lignin peroxidase	128
6.7	The oxidative pathway for catalytic action of laccase and manganese peroxidase on lignin	129

LIST OF TABLES

Tables	Page
2.1 Waste generation in Malaysia	12
2.2 Characteristics of young and older leachate	16
2.3 Bioremediation treatment technologies	32
3.1 General characteristics of the Municipal Solid Wastes (MSW) landfills included in the study	45
3.2 Characteristics of landfill leachates with respect to general chemical parameters	53
3.3 Comparison of leachate characteristics from active and closed landfills based on selected pollution parameters	57
5.1 Percentage removal of leachate BOD_5 , COD, NH_3-N and pH changes by free-cell mycelia of <i>G. australe</i> after 30 days incubation in submerged cultures at various treatments	86
5.2 Percentage removal of leachate BOD_5 , COD, NH_3-N and pH changes by free-cell mycelia of <i>T. menziesii</i> after 30 days incubation in submerged cultures at various treatments	87
5.3 Percentage removal of 100% and 50% leachate BOD_5 , COD, NH_3-N and pH changes by <i>G. australe</i> immobilized on Ecomat at weekly intervals for 28 days, incubated at room temperature, shaking at 150 rpm	86
5.4 Percentage removal of 100% leachate BOD_5 and COD by <i>Trametes menziesii</i> , <i>Ganoderma australe</i> and coculture mycelia of immobilized <i>G. australe</i> and <i>T. menziesii</i> after treatment for 28 days, incubated at room temperature, shaking at 150 rpm	97

5.5	Percentage removal of BOD ₅ , COD, NH ₃ -N and pH changes in raw leachate (100%) after treatment by Ecomat-immobilized <i>G. australe</i> in column packing at room temperature	99
5.6	Percentage removal of BOD ₅ , COD, NH ₃ -N and pH changes in diluted leachate (50%) after treatment by Ecomat-immobilized <i>G. australe</i> in column packing at room temperature	101
5.7	Application of fungi in waste treatment	103
6.1	Percentage removal of BOD ₅ , COD, NH ₃ -N and pH changes in 100% leachate after treatment with crude enzymes of <i>G. australe</i> , incubated on an orbital shaker at 80 rpm with different concentrations of crude enzyme for 24 hours	124
6.2	Percentage removal of BOD ₅ , COD, NH ₃ -N and pH changes in 100% leachate after treatment with 10 U/ml crude enzyme of <i>G. australe</i> , incubated on an orbital shaker at 80 rpm at different time exposure	126
6.3	Percentage removal of BOD ₅ , COD, NH ₃ -N and pH changes after incubated with immobilized <i>G. australe</i> on Ecomat at room temperature for 7 days, shaking at 150 rpm and with 10 U/ml crude enzyme at 4 hours time exposure on an orbital shaker at 80 rpm	130
6.4	Summarize result of leachate treatment based on percentage removal of BOD ₅ , COD, and NH ₃ -N removal and pH changes using concentrated (100%) leachate at various conditions	134

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	Polycyclic aromatic hydrocarbons
Fe	Iron
Ca	Calcium
Mg	Magnesium
Cd	Cadmium
As	Arsenic
EQA	Environment Quality Act
AOP	Advanced oxidation processes
H ₂ O 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 ₂ O 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 ₂ PO ₄	Monopotassium phosphate
K ₂ HPO ₄	Dipotassium phosphate
NH ₄ CL	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 ₂ O 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