

## TABLE OF CONTENTS

	Page
Executive Summary.....	i
Objectives .....	v
Key Project Findings .....	vi
Recommendations.....	ix
Table of Contents .....	x
List of figures.....	xiv
List of tables .....	xvi
Acknowledgements .....	xvii
Project Outputs.....	xviii
<b>Chapter 1: Background and review of literature .....</b>	<b>1</b>
1.1 Introduction.....	1
1.2.1 The white rot fungus <i>Trametes versicolor</i> .....	2
1.2.2 The effect of nutrition and culture conditions on enzyme production in <i>T. versicolor</i> .....	4
1.3.1 Laccase enzymes.....	5
1.3.2 Production of laccases .....	7
1.3.3 Regulation of laccase production.....	8
1.3.4 Protein secretion in filamentous organisms .....	10
1.4 Substrates susceptible to degradation by <i>T. versicolor</i> .....	10
1.4.1 Phenols .....	10
1.4.2 Chlorophenols .....	11
1.4.3 Polychlorinated biphenyls .....	12
1.4.4 Polycyclic aromatic hydrocarbons (PAHs) .....	13
1.4.5 BTEX.....	14
1.4.6 Dyes.....	14
1.4.7 Nitro-Substituted Compounds.....	15
1.4.8 Xylenols.....	15
1.4.9 Kraft pulp bleaching and delignification.....	15
1.5 Bioreactors for the application of <i>Trametes</i> systems.....	17
1.6.1 Immobilised biofilm reactors .....	18
1.6.2 Fluidised bed reactors .....	19
1.6.3 Airlift loop reactors (ALR).....	20
1.7.1 Project objectives .....	23
1.7.2 Specific objectives.....	24
<b>Chapter 2: Development of culture methodologies for <i>Trametes</i> strains (Part 1) Medium Development .....</b>	<b>25</b>
2.1 Introduction.....	25
2.2 Methods .....	26
2.2.1 Strain preservation .....	26
2.2.2 Valuation of growth media .....	26
2.2.3 Medium development .....	26
2.2.4 Inoculation.....	27

2.2.5	Biomass determination.....	27
2.2.6	Comparison of growth media.....	27
2.2.7	Determination of laccase enzyme activity.....	28
2.3	Results.....	28
2.3.1	Comparison of media.....	28
2.3.2	Development of TDM.....	30
2.3.3	Growth and enzyme activity of <i>T. pubescens</i> .....	34
2.3.4	Effect of inducers.....	36
2.4	Conclusions.....	39
<b>Chapter 3:</b>	<b>Development of culture methodologies for <i>Trametes</i> strains</b>	
	<b>(Part 2) Factors influencing growth and laccase production in</b>	
	<b>flask culture.....</b>	<b>42</b>
3.1	Introduction.....	42
3.2	Method.....	44
3.2.1	Strain preservation.....	44
3.2.2	Liquid Culture.....	44
3.2.3	Inoculation.....	44
3.2.4	Biomass determination.....	44
3.2.5	Laccase preparation for inhibition study.....	45
3.2.6	Measurement of laccase enzyme activity.....	45
3.2.7	Measurement of total microbial activity.....	45
3.2.8	Effect of phenolic effluent on physiology and enzyme production.....	46
3.2.9	Effect of culture age prior to cresylic effluent addition on growth and enzyme production.....	46
3.2.10	HPLC analysis.....	46
3.2.11	Electron microscopy.....	47
3.3	Results.....	47
3.3.1	Enzyme production in flasks.....	47
3.3.2	Morphological effects of phenolic effluents on biomass.....	48
3.3.3	Optimisation of enzyme production.....	49
3.3.4	Changes in microbial activity of <i>T. versicolor</i> cultures exposed to effluent compounds.....	54
3.3.5	Effects of effluent addition on metabolic activity of <i>T. versicolor</i> .....	56
3.3.6	Inhibition of the ABTS laccase assay.....	58
3.3.7	The oxygen utilisation rate (OUR) assay for laccase.....	60
3.3.8	Utilisation of the new strain <i>T. pubescens</i> .....	63
3.4	Conclusions.....	64
<b>Chapter 4:</b>	<b>Development of bioreactor systems (Part 1): Immobilised biofilm</b>	
	<b>reactors for cultivation of <i>T. versicolor</i>.....</b>	<b>67</b>
4.1	Introduction.....	67
4.1.1	Transverse hollow fibre bioreactor (TFHFBR).....	67
4.1.2	Trickle bed bioreactor (TBR).....	68
4.2	Methods.....	69
4.2.1	Transverse flow hollow fibre bioreactor (TFHFBR) set-up.....	69
4.2.2	TFHFBR system configuration.....	70
4.2.3	TFHFBR operation.....	71
4.2.4	TBR set-up.....	72

4.2.5	TBR operation .....	72
4.2.6	SEM preparation .....	73
4.3	Results .....	73
4.3.1	Growth in the TFHFBR .....	73
4.3.2	Growth in the TBR .....	78
4.4	Conclusions.....	81
<b>Chapter 5:</b>	<b>Development of bioreactor systems (Part 2): Airlift loop reactor (ALR) for laccase production and bioremediation by <i>Trametes</i> species .....</b>	<b>83</b>
5.1	Introduction.....	83
5.1.1	Bubble recirculation and gas holdup in ALRs.....	83
5.1.2	Effect of sparger aperture size (porosity).....	84
5.1.3	Chear forces in ALRs .....	85
5.1.4	Mass transfer in ALRs .....	86
5.2	Methods .....	88
5.2.1	Culture and growth conditions .....	88
5.2.2	Inoculum preparation.....	88
5.2.3	ALR operation .....	88
5.2.4	ALR sampling .....	88
5.2.5	Glucose determination.....	88
5.3	Results .....	89
5.3.1	Optimisation of air supply rate and oxygen transfer .....	89
5.3.2	Growth in an ALR – effects on fungal morphology .....	91
5.3.3	Laccase production during fermentation of <i>T. pubescens</i> .....	93
5.3.4	Effect of effluent addition on biomass morphology and laccase production .....	95
5.3.5	Optimisation of laccase production in an ALR.....	100
5.4	Conclusions.....	103
<b>Chapter 6:</b>	<b>Reaction characterisation and optimisation of the bioremediation process .....</b>	<b>104</b>
6.1	Introduction.....	104
6.2	Methods .....	105
6.2.1	Culture and growth conditions .....	105
6.2.2	Inoculum preparation.....	105
6.2.3	Determination of laccase enzyme activity .....	105
6.2.4	HPLC analysis of phenolics .....	105
6.2.5	ALR operation .....	106
6.2.6	ALR sampling .....	106
6.3	Results .....	106
6.3.1	Removal of phenols from effluents in flask reactions .....	106
6.3.2	Removal of phenols from solution in the ALR .....	108
6.3.3	Characterisation of the reaction products of bioremediation .....	111
6.4	Further development of the ALR bioremediation system.....	113
6.4.1	Investigation of physical design parameters (Pentech contribution).....	113
6.4.2	Measurements of a 160mm diameter reactor .....	115
6.5	Scale up of the ALR design .....	116
6.6	Inclusion of a membrane separation unit .....	116

6.7	Conclusions.....	118
<b>Chapter 7: General conclusions and recommendations .....</b>		<b>119</b>
7.1	Key findings.....	119
7.1.1	Physiology of <i>Trametes</i> strains with respect to bioremediation potential..	119
7.1.2	Bioreactors for application of <i>Trametes</i> strains in bioremediation .....	120
7.1.3	Usefulness of this bioremediation system.....	121
7.2	Implications of the research carried out in this study.....	121
7.3	Further development .....	123
7.3	Recommendations.....	123
<b>References .....</b>		<b>124</b>