

# VU Research Portal

## **Biodegradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin by a ligninolytic fungus and its laccases**

Thi Ngoc Dao, Anh

2022

### **document version**

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

### **citation for published version (APA)**

Thi Ngoc Dao, A. (2022). *Biodegradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin by a ligninolytic fungus and its laccases*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam]. s.n.

### **General rights**

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

### **Take down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

### **E-mail address:**

[vuresearchportal.ub@vu.nl](mailto:vuresearchportal.ub@vu.nl)

# Contents

List of abbreviations .....	9
<b>Chapter 1. General Introduction.....</b>	<b>11</b>
1. General background .....	12
2. White rot fungi in bioremediation .....	13
3. Lignin-modifying enzymes.....	16
3.1 Laccase.....	16
3.2 Lignin peroxidases.....	17
3.3 Manganese peroxidase .....	18
4. Fungal laccase .....	18
4.1 Production .....	18
4.2 Characteristics of fungal laccases .....	19
4.3 Laccase mediator system.....	20
5. Biodegradation of 2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin .....	21
5.1. 2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin.....	21
5.2 Dioxin degradation by White-rot fungi and their lignin-modifying enzymes .....	22
6. Bioanalysis of 2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin and related dioxins.....	23
7. Aim, objectives and outline of the thesis .....	25
8. VN-Basic: an international collaboration between Vietnam Academy of Science & Technology (VAST) and the BE-Basic Foundation .....	26
References.....	26
<b>Chapter 2. Screening white-rot fungi for bioremediation potential of 2,3,7,8-tetrachlorodibenzo-<i>p</i>-dioxin.....</b>	<b>33</b>
Abstract.....	34
1. Introduction .....	35
2. Materials and Methods .....	36
2.1 Fungal strains .....	36
2.2 Chemicals .....	36
2.3 Fungal enzyme assays.....	36
2.4 LMEs screening and laccase production .....	37
2.5 Fungal classification and molecular typing .....	38
2.6 TCDD analysis by DR CALUX® .....	38

2.7 Experimental setup for TCDD degradation .....	38
3. Results and Discussion .....	39
3.1 Fungal screening for LME production .....	39
3.2 Optimization of LME production .....	41
3.3 Fungal Classification.....	43
3.4 Mycodegradation of 2,3,7,8-tetrachlorodibenzo-p-dioxin .....	45
Acknowledgements.....	48
References.....	48
Supplemental information .....	50
<b>Chapter 3. Characterization of 2,3,7,8-tetrachlorodibenzo-p-dioxin biodegradation by extracellular lignin-modifying enzymes from ligninolytic fungus.....</b>	<b>53</b>
Abstract.....	54
1. Introduction .....	55
2. Materials and Methods.....	57
2.1 Rigidoporus sp. FMD21, culture conditions and its crude extracellular enzymes	57
2.2 Fungal laccase and MnP assays.....	57
2.3 Experimental setup.....	57
2.4 Chemical analysis.....	59
2.5 Calculation and statistical analysis.....	60
3. Results and Discussions.....	61
3.1 Degradation of 2,3,7,8-TCDD by the ligninolytic fungus Rigidoporus sp. FMD21...	61
3.2 Degradation of 2,3,7,8-TCDD by fungal extracellular enzymes from Rigidoporus sp. FMD21 .....	62
3.3 2,3,7,8-TCDD degradation metabolite.....	65
3.4 The role of laccase-mediators during enzymatic degradation of 2,3,7,8-TCDD ...	68
Acknowledgements.....	71
References.....	72
Supplemental information .....	76
<b>Chapter 4. Elucidating fungal <i>Rigidoporus</i> species FMD21 lignin-modifying enzyme genes and 2,3,7,8-tetrachlorodibenzo-p-dioxin degradation by laccase isozymes .....</b>	<b>81</b>
Abstract.....	82
1. Introduction .....	83
2. Materials and Methods.....	84

2.1 Biological materials .....	84
2.2 Chemicals .....	85
2.3 Genome sequencing and analysis.....	85
2.4 Laccase heterologous expression in <i>Pichia pastoris</i> .....	86
2.5 2,3,7,8-TCDD degradation experimental setup .....	87
2.6 Laccase assay and protein concentration assay .....	88
2.7 2,3,7,8-TCDD extraction and analysis .....	88
3. Results and discussion.....	89
3.1 <i>Rigidoporus</i> sp. FMD21 genome sequencing and laccase genes .....	89
3.2 Heterologous expression of laccases .....	93
3.3 Degradation of 2,3,7,8-TCDD by <i>Rigidoporus</i> sp. FMD21 laccase isozymes .....	96
Acknowledgments.....	99
Conflict of Interest Statement.....	99
References.....	99
Supplemental information .....	103
<b>Chapter 5. Diversity and characteristics of laccase isoforms from a single fungal strain</b>	
<b><i>Rigidoporus</i> sp.....</b>	<b>105</b>
Abstract .....	106
1. Introduction .....	107
2. Materials and Methods .....	109
2.1 Microorganisms and cultivation conditions.....	109
2.2 Chemicals and reagents .....	109
2.3 Laccase isoforms isolation .....	109
2.4 SDS-PAGE and zymography.....	110
2.5 Kinetics of laccase isoforms .....	110
2.6 Effect of physicochemical factors on laccase isoforms.....	111
2.7 Effect of water-miscible solvents and solvent tolerance .....	112
2.8 Laccase assay .....	112
2.9 Protein measurement .....	112
2.10 Statistical analysis .....	113
3. Results and discussion.....	113
3.1 <i>Rigidoporus</i> sp. FMD21 laccase isoform profile.....	113
3.2 Extracellular laccase isoform isolation.....	116

3.3 Kinetic properties of isolated laccases.....	118
3.4 Physicochemical factors affecting laccase isoform activity .....	121
3.5 Effect of solvents on laccase activity and stability.....	127
Conclusions .....	129
Acknowledgments.....	129
Conflict of Interest Statement.....	129
References.....	129
Supplemental information .....	133
<b>Chapter 6. General discussion and future perspective.....</b>	<b>135</b>
1. Select lignin-modifying enzyme (LME) producing fungi as potential dioxin degrading fungal candidates .....	137
2. Determine the participation of laccase in 2,3,7,8-TCDD degradation process .....	139
3. Investigate the dioxin degrading fungus FMD21 at genomic level to reveal possible genes involved in dioxin degradation. ....	141
4. Characterize the properties of laccase to get better insight in its future biotechnological application possibilities .....	142
5. Future perspectives.....	143
References.....	145
<b>Summary.....</b>	<b>151</b>
<b>Tóm tắt .....</b>	<b>155</b>
<b>Samenvatting .....</b>	<b>159</b>
<b>List of publications .....</b>	<b>163</b>
<b>Acknowledgments.....</b>	<b>165</b>