

**DELVING INTO A NOVEL EXTREMOPHILIC BACTERIAL
LACCASE: PURIFICATION, CHARACTERIZATION, AND
ITS APPLICATIONS IN
SUSTAINABLE INDUSTRIAL PROCESSING**

VARSHA PANWAR



INDIAN INSTITUTE OF TECHNOLOGY DELHI
JUNE, 2022

**DELVING INTO A NOVEL EXTREMOPHILIC BACTERIAL
LACCASE: PURIFICATION, CHARACTERIZATION, AND
ITS APPLICATIONS IN
SUSTAINABLE INDUSTRIAL PROCESSING**

Submitted by

VARSHA PANWAR

DEPARTMENT OF CHEMISTRY

*in fulfillment of the requirements of the degree of Doctor of Philosophy
to the*



INDIAN INSTITUTE OF TECHNOLOGY DELHI

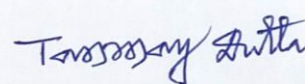
JUNE, 2022

CERTIFICATE

This is to certify that the thesis entitled “*Delving into a novel extremophilic bacterial laccase: Purification, characterization and its applications in sustainable industrial processing*” being submitted by **Ms. VARSHA PANWAR** to the Indian Institute of Technology Delhi for the award of the degree of *Doctor of Philosophy* in chemistry is a record of bonafide research work carried out by her. Ms. Varsha Panwar has worked under my guidance and supervision and has fulfilled the requirements for the submission of the thesis, which, to my knowledge, has reached the requisite standard. The results contained in this dissertation have not been submitted in part or full to any other university or institute for the award of any degree or diploma.

Date: 2nd June, 2023

Place: Delhi



Dr. Tanmay Dutta

Professor (Biochemistry)

Department of Chemistry

Indian Institute of Technology Delhi

Dr. TANMAY DUTTA
Associate Professor
Department of Chemistry
Indian Institute of Technology Delhi
Hauz Khas, New Delhi-110016, India

Acknowledgements

The success of any work is not solely dependent on an individual but the associated personalities play a greater role by continuous suggestions and evaluation. I take this opportunity to express my thanks to all the people who helped me in completion of this thesis.

*First and foremost, I express my sincere gratitude to my Ph.D. supervisor **Prof. Tanmay Dutta**.*

I am indeed fortunate to get an opportunity to work under his guidance. I am very thankful for his insightful ideas, encouragements, exemplary teaching, and unwavering support.

*I am indebted to my SRC members, **Prof. S.K. Khare**, **Prof. P. K. Chowdhury** and **Prof. Bhattacharyya** for critically evaluating my work and providing suggestions. I am also thankful to **Prof. Siddharth Pandey**, Head, department of Chemistry for providing facilities. I am very grateful to **Prof. Javed Sheikh** for providing important suggestions.*

*I am very thankful all the staff members of the **instrumentation lab**, **chemistry office**, **B.Tech Lab**, **M.Tech lab**, **M.Sc. lab** of the **Department of Chemistry, IIT Delhi** for their support and all possible help. I also thank **Central Research Facility** and **Nanoscale Research Facility**, IIT Delhi for providing crucial assistance in my experimental work. I acknowledge the **University Grants Commission** for my research scholarship and **Council of Scientific and Industrial Research, India** for funding my research project.*

I convey my sincere thanks to all my seniors and juniors for their constant support. I am delighted to acknowledge my dear friends for all possible help. My thesis would not have completed without the blessings of my parents, grandparents, and family. They motivated me through all the ways of my life. I adore them with my all respects.

Varsha Panwar
Delhi

Abstract

The popularity of enzymes in the industrial applications is growing due to their environment-friendly catalysis. Specifically, laccases are becoming more important because of their broad substrate specificity. Laccases are multicopper oxidases that can oxidise various types of phenolic and non-phenolic compounds. Currently, industrial operations are dominated by fungal laccases by virtue of their high redox potential, but fungal laccases become non-functional under many industrial processes which require extreme reaction conditions like high temperature and pressure, extreme pHs, presence of metal ions, organic solvents, salts, etc. In view of these limitations, new studies are exploring bacterial laccases owing to their extremotolerant properties for industrial deployment.

In this study, we isolated a novel thermostable bacterial laccase (LacT) from a hot-sulphur spring water isolate *Brevibacillus agri*. LacT was characterized in terms of optimum pH and temperature, pH and thermo-stability. The enzymatic activity and stability of LacT were examined in the presence of various organic solvents, metal ions and inhibitors. Also, the halotolerance ability of LacT was evaluated in the presence of salts like NaCl and KCl. Different types of natural and synthetic substrates of laccase were used to investigate the substrate specificity of LacT.

The industrial relevance of LacT was scrutinized by employing it in the bleaching and dye decolourization processes. In the textile industries, fabric bleaching and dyeing are considered as the major polluting steps. Commercial bleaching uses pernicious chemicals like chlorine, sodium hypochlorite, etc., which are highly detrimental to environment and human health. In our study, we have performed LacT-mediated bio-bleaching that eliminates the requirement of hazardous bleaching agents. On the other hand, dyes are recalcitrant complexes that are highly resistant to decomposition. They are well-proven mutagens or carcinogens. The

potential of LacT to decolourize hazardous textile dyes viz. Malachite Green, Reactive Black 5, Bromophenol Blue and Direct Red dye was comprehensively studied.

The high input cost for the production and purification of enzymes forestalls their usage in the industrial applications. In order to produce low-cost purified LacT on the larger scale, we optimised both the media for LacT production and its purification strategy. Moreover, the extracellular nature of LacT made its purification fast and less costly. Purified LacT was characterized through biophysical studies such as fluorescence spectroscopy and circular dichroism spectroscopy. Thermo-gravimetric analysis of LacT and a fungal laccase from *Trametes versicolor* was conducted to understand their thermal denaturation profiles at high temperatures.

The final part of the thesis covers the application of LacT in sustainable dyeing. In the commercial dyeing, harmful aromatic amines like phenylenediamines are employed as dye precursors. The American Contact Dermatitis Society classified these precursors as severe allergens. Additionally, harmful organic solvents and highly reactive chemical bleaching agents namely H_2O_2 , KMnO_4 are used as oxidants. They may cause eczema, allergy, hypersensitivity and dermatitis. In this study, we have developed an alternative laccase-based sustainable dyeing. LacT-mediated dyes/colourants were developed by using natural plant phenols namely, gallic acid, ferulic acid, syringaldehyde and catechol as dye precursors. Moreover, there was no use of precarious organic solvents and strong bleaching agents especially H_2O_2 in LacT-driven dyeing.

औद्योगिक अनुप्रयोगों में एंजाइमों की लोकप्रियता उनके पर्यावरण के अनुकूल कटैलिसीस के कारण बढ़ रही है। विशेष रूप से, उनकी व्यापक सबस्ट्रेट विशिष्टता के कारण लैककेस अधिक महत्वपूर्ण होते जा रहे हैं। लैककेस मल्टीकॉपर ऑक्सीडेज हैं जो विभिन्न प्रकार के फेनोलिक और गैर-फेनोलिक यौगिकों का ऑक्सीकरण कर सकते हैं। वर्तमान में, औद्योगिक संचालन में उनकी उच्च रेडॉक्स क्षमता के आधार पर फंगल लैकेस का प्रभुत्व है, लेकिन कई औद्योगिक प्रक्रियाओं के तहत फंगल लैकेस गैर-कार्यात्मक हो जाते हैं, जिसके लिए उच्च तापमान और दबाव, चरम पीएच, धातु आयनों की उपस्थिति, कार्बनिक सॉल्वेंट्स जैसी अत्यधिक प्रतिक्रिया की स्थिति की आवश्यकता होती है। इन सीमाओं को ध्यान में रखते हुए, नए अध्ययन औद्योगिक तैनाती के लिए उनके चरम-सहिष्णु गुणों के कारण बैक्टीरिया की कमी की खोज कर रहे हैं।

इस अध्ययन में, हमने एक गर्म-सल्फर स्प्रिंग वाटर आइसोलेट ब्रेविबैसिलस एग्री से एक उपन्यास थर्मोस्टेबल बैक्टीरियल लैकेस (LacT) को अलग किया। इष्टतम पीएच और तापमान, पीएच और थर्मो-स्थिरता के संदर्भ में एलएसीटी की विशेषता थी। विभिन्न कार्बनिक सॉल्वेंट्स, धातु आयनों और अवरोधकों की उपस्थिति में लैक्ट की एंजाइमैटिक गतिविधि और स्थिरता की जांच की गई। साथ ही, सोडियम क्लोराइड और पोटेशियम क्लोराइड जैसे लवणों की उपस्थिति में LacT की हेलोटोलेरेंस क्षमता का मूल्यांकन किया गया। लैकेस के विभिन्न प्रकार के प्राकृतिक और सिंथेटिक सबस्ट्रेट्स का उपयोग लैक्ट की सबस्ट्रेट विशिष्टता की जांच के लिए किया गया था।

एलएसीटी की औद्योगिक प्रासंगिकता को ब्लीचिंग और डाई डीकोलराइजेशन प्रक्रियाओं में नियोजित करके इसकी जांच की गई। कपड़ा उद्योगों में कपड़े की ब्लीचिंग और रंगाई को प्रमुख प्रदूषणकारी कदम माना जाता है। व्यावसायिक विरंजन में क्लोरीन, सोडियम हाइपोक्लोराइट आदि हानिकारक रसायनों का उपयोग होता है, जो पर्यावरण और मानव स्वास्थ्य के लिए अत्यधिक हानिकारक हैं। हमारे अध्ययन में,

हमने एलएसीटी-मध्यस्थता वाले जैव-विरंजन का प्रदर्शन किया है जो खतरनाक विरंजन एजेंटों की आवश्यकता को समाप्त करता है। दूसरी ओर, रंजक पुनर्गठित परिसर होते हैं जो अपघटन के लिए अत्यधिक प्रतिरोधी होते हैं। वे अच्छी तरह से सिद्ध उत्परिवर्तन या कार्सिनोजेन्स हैं। एलएसीटी की खतरनाक कपड़ा रंगों को रंगहीन करने की क्षमता। मैलाकाइट ग्रीन, रिएक्टिव ब्लैक 5, ब्रोमोफेनोल ब्लू और डायरेक्ट रेड डार्क का व्यापक अध्ययन किया गया।

एंजाइमों के उत्पादन और शुद्धिकरण के लिए उच्च निवेश लागत औद्योगिक अनुप्रयोगों में उनके उपयोग को रोकता है। बड़े पैमाने पर कम लागत वाले शुद्ध LacT का उत्पादन करने के लिए, हमने LacT उत्पादन और इसकी शुद्धि रणनीति दोनों के लिए मीडिया को अनुकूलित किया। इसके अलावा, LacT की बाह्य प्रकृति ने इसके शुद्धिकरण को तेज और कम खर्चीला बना दिया। प्यूरीफाइड लैकटी को फ्लोरेसेंस स्पेक्ट्रोस्कोपी और सर्कुलर डाइक्रोइज्म स्पेक्ट्रोस्कोपी जैसे बायोफिजिकल अध्ययनों के माध्यम से चित्रित किया गया था। उच्च तापमान पर उनके थर्मल विकृतीकरण प्रोफाइल को समझने के लिए एलएसीटी का थर्मो-ग्रेविमेट्रिक विश्लेषण और ट्रामेट्स वर्सीकोलर से एक फंगल लैकेस आयोजित किया गया था।

थीसिस के अंतिम भाग में टिकाऊ रंगाई में एलएसीटी के आवेदन को शामिल किया गया है। वाणिज्यिक रंगाई में, फेनिलीनडायमाइन्स जैसे हानिकारक सुगंधित अमाइन डार्क अग्रदूतों के रूप में कार्यरत हैं। अमेरिकन कॉन्टैक्ट डर्मेटाइटिस सोसाइटी ने इन अग्रदूतों को गंभीर एलर्जी के रूप में वर्गीकृत किया है। इसके अतिरिक्त, हानिकारक कार्बनिक सॉल्वेंट्स और अत्यधिक प्रतिक्रियाशील रासायनिक विरंजन एजेंट जैसे H_2O_2 , $KMnO_4$ का उपयोग ऑक्सीडेंट के रूप में किया जाता है। वे एक्जिमा, एलर्जी, अतिसंवेदनशीलता और जिल्द की सूजन का कारण बन सकते हैं। इस अध्ययन में, हमने एक वैकल्पिक लैकेस-आधारित टिकाऊ रंगाई विकसित की है। एलएसीटी-मध्यस्थ रंगों/रंगकों को प्राकृतिक पौधों के फिनोल नामतः गैलिक एसिड, फेरुलिक एसिड, सीरिजल्लिहाइड और कैटेकोल को डार्क अग्रदूतों के रूप में उपयोग करके विकसित किया गया था। इसके अलावा, लैकटी-संचालित रंगाई में अनिश्चित कार्बनिक सॉल्वेंट्स और मजबूत विरंजन एजेंटों विशेष रूप से H_2O_2 का कोई उपयोग नहीं था।

Table of Contents

Certificate		i
Acknowledgements		ii
Abstract		iii
Table of Contents		vii
List of Figures		xi
List of Tables		xiii
List of Abbreviations		xiv
CHAPTER 1	INTRODUCTION	1-35
1.1	Introduction	2
1.2	Review of literature	3
1.2.1	<i>Laccases</i>	3
1.2.2	<i>Occurrence of laccases</i>	4
1.2.3	<i>Classification of laccases</i>	5
1.2.4	<i>Kinetic properties of laccases</i>	7
1.2.5.	<i>Structural properties of laccase</i>	8
1.2.6.	<i>Catalytic mechanism of laccase</i>	10
1.2.7.	<i>Laccase mediator system</i>	11
1.2.8.	<i>Production of laccases</i>	15
1.2.9.	<i>Bacterial laccases</i>	18
1.2.10.	<i>Industrial and biotechnological applications of laccases</i>	19
1.3	Objectives	31
1.4	Scope of the thesis	31
CHAPTER 2	SCREENING, ISOLATION, PRODUCTION AND CHARACTERIZATION OF A NOVEL EXTREMOPHILIC BACTERIAL LACCASE (LacT)	36-65
2.1	Introduction	37
2.2	Materials and Methods	39
2.2.1	<i>Materials</i>	39

2.2.2	<i>Sample collection and isolation of strain</i>	39
2.2.3	<i>Laccase production</i>	41
2.2.4	<i>Laccase assay</i>	42
2.2.5	<i>SDS-PAGE and zymogram analysis of laccase</i>	43
2.2.6	<i>Effect of pH on activity and stability of laccase</i>	43
2.2.7	<i>Effect of temperature on activity and stability of laccase</i>	44
2.2.8	<i>Effect of organic solvents on LacT activity</i>	44
2.2.9	<i>Effect of different types of metal ions on LacT activity</i>	45
2.2.10	<i>Effect of salts on LacT activity</i>	45
2.2.11	<i>Effect of inhibitors on LacT activity</i>	46
2.3	Results and Discussion	46
2.3.1	<i>Effect of pH on LacT activity</i>	49
2.3.2	<i>Effect of temperature on LacT activity</i>	54
2.3.3	<i>Substrate specificity of LacT</i>	56
2.3.4	<i>Effect of organic solvents on LacT activity</i>	59
2.3.5	<i>Effect of metal ions on LacT activity</i>	61
2.3.6	<i>Effect of salts on LacT activity</i>	62
2.3.7	<i>Effect of inhibitors on LacT activity</i>	64
2.4	Conclusions	65
CHAPTER 3 DECOLORISATION OF TEXTILE DYES AND		66-83
SUSTAINABLE BIO-BLEACHING OF DENIM BY LacT		
3.1	Introduction	66
3.2	Materials and Methods	70
3.2.1	<i>Materials</i>	70
3.2.2	<i>Microorganism and LacT production</i>	71
3.2.3	<i>Dye Decolourization by LacT</i>	71
3.2.4	<i>Denim bio-bleaching by LacT</i>	72
3.3	Results and Discussion	73
3.4	Conclusions	83

CHAPTER 4	PURIFICATION AND CHARACTERIZATION OF LacT	84-104
4.1	Introduction	85
4.2	Materials and Methods	86
4.2.1	<i>Materials</i>	86
4.2.2	<i>Microorganism</i>	87
4.2.3	<i>Media optimization and laccase production</i>	87
4.2.4	<i>LacT purification and assay</i>	88
4.2.5	<i>SDS-PAGE and zymogram analysis</i>	90
4.2.6	<i>MALDI-TOF/MS-MS for protein identification</i>	90
4.2.7	<i>Metal content of LacT</i>	90
4.2.8	<i>Fluorescence spectroscopy</i>	91
4.2.9	<i>Circular dichroism spectroscopy</i>	91
4.2.10	<i>Thermogravimetric analysis</i>	92
4.3	Results and Discussion	92
4.3.1	<i>Media optimization</i>	92
4.3.2	<i>LacT purification</i>	99
4.3.3	<i>Fluorescence spectroscopy of LacT</i>	100
4.3.4	<i>Circular dichroism spectroscopy</i>	107
4.3.5	<i>Thermogravimetric analysis</i>	102
4.4	Conclusions	104
CHAPTER 5	LacT-CATALYZED SYNTHESIS OF DYES FROM PLANT PHENOLS AND ITS APPLICATION IN SUSTAINABLE DYEING	105- 128
5.1	Introduction	106
5.2	Materials and Methods	107
5.2.1	<i>Materials</i>	107
5.2.2	<i>Synthesis of LacT-mediated dyes</i>	107
5.2.3	<i>In situ dyeing of hair fibres</i>	108
5.2.4	<i>Characterization of dyed hair fibres</i>	109
5.2.5	<i>Effect of washing/shampooing on colour permanence of dyes</i>	109

5.2.6	<i>Effect of pH on colour permanence of proposed dyes</i>	110
5.2.7	<i>Surface-morphological and anatomy studies of dyed hair fibres</i>	110
5.2.8	<i>Hair breakage studies</i>	111
5.2.9	<i>Energy dispersive X-ray (EDX) analysis</i>	111
5.3	Results and Discussion	112
5.3.1	<i>Dye synthesis and in situ dyeing of hair fibres</i>	112
5.3.2	<i>Quantification of color parameters and characterisation of dyed hair</i>	115
5.3.3	<i>Colour permanence of dyes to washing/shampooing</i>	119
5.3.4	<i>Retention of dyes under pH variations</i>	119
5.3.5	<i>Morphological and anatomical studies of dyed hair fibres</i>	121
5.3.6	<i>Hair breakage studies</i>	124
5.3.7	<i>Energy dispersive X-ray (EDX)</i>	125
5.4	Conclusions	128
SUMMARY & FUTURE PERSPECTIVES		129
REFERENCES		132
LIST OF PUBLICATIONS		149
BRIEF BIO-DATA		150

List of Figures

Figure No.	Description	Page No.
Fig. 1.1	Various industrial applications of laccase	8
Fig. 1.2	Active-site structure of laccase enzyme	10
Fig. 1.3	Catalytic mechanism of laccase enzyme	11
Fig. 1.4	Laccase-catalysed reaction by direct and indirect oxidation	13
Fig. 1.5	Oxidation of ABTS in the presence of laccase	15
Fig. 1.6	Chemical structures of synthetic laccase mediators	17
Fig. 1.7	Chemical structures of natural laccase mediators	18
Fig. 2.1.	16s RNA gene sequence of the identified strain (VP-1 strain)	49
Fig. 2.2	Phylogenetic tree analysis by using neighbour-joining method	51
Fig. 2.3	Plate assays showing zone of oxidation by <i>Brevibacillus agri</i>	52
Fig. 2.4	Zymogram analysis of LacT	53
Fig. 2.5	Effect of pH on LacT activity and LacT stability	54
Fig. 2.6	Effect of temperature on LacT catalytic activity and LacT stability	56
Fig. 2.7	Effect of organic solvents on LacT activity	61
Fig. 2.8	Effect of metal ions on LacT activity	64
Fig. 2.9	Effect of salts on the catalytic activity of LacT	64
Fig. 2.10	Effect of inhibitors on the catalytic activity of LacT	66
Fig. 3.1	Wavelength scans of the decolourization of Malachite Green dye	78
Fig. 3.2	Wavelength scans of the decolourization of Reactive Black 5 dye	79
Fig. 3.3	Wavelength scans of the decolourization of Bromophenol Blue	80
Fig. 3.4	Wavelength spectrum of Direct Scarlet Red dye after treatment with LacT	81
Fig. 3.5	Reflectance spectrums of denim fabric treated at pH 4.0 and pH 8.0.	83
Fig. 3.6	Percentage decrease in <i>K/S</i> of denim treated at (A) pH 4.0 (B) pH 8.0.	85
Fig 4.1	Effect of incubation time on LacT activity	101
Fig 4.2	Effect of carbon sources on catalytic activity of LacT	102
Fig 4.3	Effect of nitrogen sources on LacT activity	102
Fig 4.4	SDS-PAGE (10% w/v) gel and zymogram analysis of purified LacT	105
Fig 4.5	Intrinsic fluorescence emission of LacT at temperatures from 25-80 °C	108
Fig 4.6	Circular Dichroism spectra of LacT at temperatures ranging from 25-80 °C	109

Fig 4.7	Thermogravimetric analysis of bacterial laccase from <i>Brevibacillus agri</i> and fungal laccase from <i>Trametes versicolor</i> in temperature range of 30-110 °C.	111
Fig 5.1	Basic scheme of dyeing by LacT using natural plant phenols	121
Fig 5.2	LacT-mediated development of colours and hair dyeing	123
Fig 5.3	Colour distribution pattern of dyed hair samples	125
Fig 5.4	CIE L*a*b* color space diagram	126
Fig 5.5	Spectrum of Reflectance and K/S of dyed hair fibres	127
Fig 5.6	Dye retention of colored hair after shampooing	129
Fig 5.7	Effect of pH on the retainability of dyes	130
Fig 5.8	Optical microscopic images of longitudinal sections of undyed and dyed hair	132
Fig 5.9	SEM images of longitudinal cross-sections of undyed and dyed hair	134
Fig 5.10	Load vs Extension curve of dyed fibres of hair	135
Fig 5.11	EDX spectrums of undyed hair and dyed hair	137

List of Tables

Table No.	Description	Page No.
Table 1.1	Occurrence of different types of laccases in life forms	5
Table 1.2	Commercial applications of laccase	20
Table 2.1	List of reported bacterial laccases	38
Table 2.2	Screening media for laccase production	41
Table 2.3	Oxidation zone of laccase producing microbes isolated from hot spring water	48
Table 2.4	Nucleotide BLAST search analysis of strain VP-1 with nearest type strains	50
Table 2.5	LacT activities and specific activities in the presence of various natural and synthetic substrates.	58
Table 2.6	Comparison between LacT and other reported bacterial and fungal laccases	59
Table 3.1	Effect of LacT from <i>B. agri</i> on the decolorization of water-soluble dyes	81
Table 3.2	Change in colourimetric properties of denim after treatment with LacT	87
Table 4.1	Composition of growth medium for LacT production from <i>B. agri</i>	96
Table 4.2	Summary of LacT purification from <i>Brevibacillus agri</i>	105
Table 4.3	ICP-OES analysis of purified LacT	106
Table 4.4	Secondary structure content (%) of LacT after temperature treatments	110
Table 5.1	Oxidation activities of LacT for phenolic monomers	121
Table 5.2	Colour parameters of dyed-hair by LacT-mediated colouration reactions	124
Table 5.3	Tensile testing of LacT-mediated dyed hair fibres	136
Table 5.4	Elemental composition of dyed hair by EDX	138

List of Abbreviations

ABTS	2,2'-azino- <i>bis</i> (3-ethylbenzthiazoline-6-sulfonate)
BSA	Bovine Serum Albumin
BeStSel	Beta Structure Selection
BLAST	Basic Local Alignment Search Tool
CD	Circular Dichroism
DEAE	Diethylaminoethyl
DTT	Dithiothreitol
EDX	Energy Dispersive X-Ray
EDTA	Ethylenediaminetetraacetic acid
HBT	1-hydroxybenzotriazole
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
kDa	Kilo Dalton
KCl	Potassium chloride
LB	Luria-Bertani medium
LMS	Laccase Mediator System
MTCC	Microbial Type Culture Collection
NaCl	Sodium chloride
PAMPS	poly(2-acrylamido-2-methyl-1-propanesulfonic acid)
PPD	<i>para</i> -phenylenediamine
RPM	Revolutions Per Minute
SDS	Sodium Dodecyl Sulphate
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SEM	Scanning Electron Microscopy
SE	Standard error
SGZ	Syringaldehyde
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy
TEMED	(N, N, N', N'-tetramethyl ethylenediamine)
TGA	Thermogravimetric analysis
Tris	Tris(hydroxymethyl)aminomethane
UV	Ultra-Violet
