EXPLORATION OF THE *PARTHENIUM HYSTEROPHORUS* BIOMASS FOR PRODUCTION OF LACCASE AND BIOETHANOL

ANURUP ADAK

CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

ANURUP ADAK

Centre for Rural Development and Technology

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DEDICATED

TO

THE ALMIGHTY

&

MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled **"Exploration of the *Parthenium hysterophorus* Biomass for Production of Laccase and Bioethanol"** submitted by **Mr. Anurup Adak** for the award of the degree **Doctor of Philosophy** has been prepared under our guidance with the rules and regulations of Indian Institute of Technology Delhi, India. The research report results presented in this thesis have not been submitted for any degree or diploma in any other institute or university.

(Dr. Satyawati Sharma)
Professor
Centre for Rural Development and Technology
Indian Institute of Technology Delhi
Hauz Khas, New Delhi – 110016, INDIA

(Dr. Lata)
Principal Scientist
Division of Microbiology
ICAR-Indian Agricultural Research Institute
Pusa, New Delhi – 110012, INDIA

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ABSTRACT

Industrialization and world population growth have continually increased the global energy demand leading to energy crisis because of depletion of traditional fossil fuel resources. Elevating problems regarding global warming and crude oil prices over recent year reignited public interest in alternative fuels and chemicals derived from renewable sources like lignocellulosic biomass. Amongst different type of lignocellulosic substrates, weedy biomass as a feedstock for biofuels, chemicals and biocatalyst is less investigated. *Parthenium hysterophorus* listed among world's seven most devastating and noxious herbaceous weed, is abundantly available in several parts of the world. This weed have became a threat to environment and agricultural productivity, so its management is necessary to prevent future problems. In view of this, the present research work is aimed to explore the potential of carrot grass (*P. hysterophorus*) for effective utilization as resource for laccase and bioethanol production.

Several fungal isolates were screened for biological pretreatment and coproduction of lignolytic enzymes by using *Parthenium* biomass as substrate. Of the eighteen isolates, one efficient white-rot fungus *Pseudolagarobasidium acaciicola* LA1 was selected for biological delignification and laccase production using *Parthenium* biomass. The statistically optimized medium resulted in laccase production of 56125.68 ± 418.15 IU/g dry substrate by *P. acaciicola* LA1. The crude enzyme displayed good stability over a wide range of pH and temperature and was active in presence of certain concentration of heavy metal ions and different organic solvents. Molecular analysis confirmed the presence of single laccase of 62.8 kDa. This is probably the first report of laccase production by *P. acaciicola* LA1 using *Parthenium* biomass in solid state fermentation.

Prior to saccharification of this biomass, physical and physicochemical pretreatments were compared with biological pretreatment by *P. acaciicola* LA1. Sodium hydroxide pretreatment of *Parthenium* biomass substantially increased the lignin removal and enhanced the cellulose digestibility as well as enzymatic accessibility. This was confirmed by structural and chemical analysis through SEM, XRD and FTIR of the raw and pretreated biomass. Statistical optimization of the alkali treatment based on RSM, resulted in 54.2 % delignification with maximum reducing sugar yield of 622.58 mg/gds. Saccharification optimization of the thermo-alkaline pretreated *Parthenium* biomass, can boost the reducing sugar yield of 685.44 ± 11.25 mg/gds after 48 h of hydrolysis with the saccharification rate of 14.30 mg/g/h. The co-fermentation of enzymatic hydrolysate with *S. cerevisiae* LN and *P. stipitis* NCIM 3498 resulted in maximum ethanol production of 18.92 ± 0.45 g/L with the fermentation efficiency of 70.70 % and ethanol yield of 0.42 g/g at 48 h of incubation. These results clearly demonstrate that *Parthenium* biomass represents as a cheaper, renewable and sustainable feedstock for laccase and ethanol production. Overall the present research is a comprehensive investigation for better management and utilization of *P. hysterophorus* biomass for economic production of laccase and bioethanol.

Keywords: Weedy biomass; *Parthenium hysterophorus*; White-rot fungus; Laccase; Pretreatment; Response surface methodology; Bioethanol

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Abbreviations

ABTS	2,2′-azino-bis-(3- ethyl-benzothizoline-6-sulphonic acid)
DNS	3,5-Dinitrosalicylic Acid
AFEX	Ammonia Fibre Expansion
ANOVA	Analysis of Variance
CMC	Carboxy Methyl Cellulose
cm	Centimeter
CBP	Consolidated Bioprocessing
°C	Degree Centigrade
DMP	Dimethoxyphenol
Fig.	Figure
FPU	Filter Paper Unit
FTIR	Fourier Transform Infrared Spectroscopy
GC	Gas Chromatography
g	Gram
gds	Gram dry substrate
HPLC	High Performance Liquid Chromatography
h	Hour
IU	International Unit
kDa	Kilo Dalton
LME	Lignin Modifying Enzymes
LiP	Lignin peroxidase
LC	Lignocellulose
L	Litre

MnP	Manganese peroxidase
mg	Milligram
ml	Millilitre
mm	Millimetre
mM	Millimoles
min	Minutes
nm	Nanometer
%	Percentage
psi	Pressure per Square Inch
RB	Reactive Black
RBBR	Remazol Brilliant Blue R
RSM	Response Surface Methodology
rpm	Revolutions per minute
SEM	Scanning Electron Microscopy
SHF	Separate Hydrolysis and Fermentation
SSCF	Simultaneous Saccharification and Co-Fermentation
SSF	Solid State Fermentation
SmF	Submerged Fermentation
v/v	Volume by volume
w/v	Weight by volume
w/w	Weight by weight
XRD	X-ray Diffraction