

TABLE OF CONTENTS

Description	Page No.
Declaration	ii
Certificate	iii
Abstract	v
Acknowledgement	vii
Preface	ix
List of Tables	xvi
List of Figures	xvii
List of Appendices	xix

Chapter	Description	Page Number
Chapter 1		
General Introduction and Review		
1.1.	General Introduction	3
1.2.	Objectives	5
1.3.	Review of literature	6
1.3.1	Agricultural wastes and agroresidues	6
1.3.2	Composition of agroresidues	7
1.3.3	Enzymatic deconstruction of lignocellulose	10
1.3.3.1	Cellulolytic enzymes	11
1.3.3.2	Hemicellulolytic enzyme	12
1.3.3.3	Ligninolytic enzyme	13
1.3.4	Natural lignocellulolytic environments	14
1.3.4.1	Insect/termite gut	14
1.3.4.2	Rumen microbiota	15
1.3.4.3	Soil microbiota	15

1.3.4.4	Gastropod molluscs	15
1.3.4.5	Marine microbiome	16
1.3.4.6	Decaying wood	16
1.3.4.7	Microbial consortia-based approach for production of glycoside hydrolases	16
1.3.4.8	Thermophiles and thermoactive GHs as potent tools in valorization of agroresidues.	17
1.3.5	Pretreatment and saccharification of agroresidue	18
1.3.5.1	Physical pretreatment	19
1.3.5.2	Chemical pretreatment	21
1.3.5.3	Physico-chemical methods	22
1.3.5.4	Biological pretreatment	23
1.3.6	Microbial enzymes based saccharification of lignocellulose	24
1.3.7	Application of lignocellulolytic microbes in valorisation of agro residues.	25

Chapter 2

Development of thermophilic lignocellulose depolymerizing microbial consortia and analysis of production and activity of thermophilic glycoside hydrolases (GHs)

2.1	Introduction	28
2.2	Materials and methods	28
2.2.1	Materials	28
2.2.2	Generation of lignocellulose degrading thermophilic consortia	29
2.2.3	Determination of bacterial growth, total reducing sugar and extracellular protein content	29
2.2.4	Enzyme extraction and precipitation	30
2.2.5	Agarose well diffusion assay of endoglucanase and endoxylanase	30

2.2.6	Enzyme assays	30
2.2.7	SDS-PAGE and zymogram analysis of endo-xylanase, endo-glucanase and β -glucosidase	31
2.2.8	Characterization of cellulases and xylanases produced by GR-RS	31
2.2.8.1	Temperature optima and stability	31
2.2.8.2	pH optima and stability	31
2.2.8.3	Effect of additives on cellulases and xylanases	32
2.3	Results	32
2.3.1	Development of thermoactive cellulose degrading microbial consortium	32
2.3.2	Preliminary analysis of bacterial growth, release of reducing sugars and protein content	32
2.3.3	Detection of depolymerisation of xylan and cellulose by agarose well diffusion assay	33
2.3.4	Dynamics of extracellular Glycoside Hydrolases (GHs) production by FS-WB and GR-RS	35
2.3.5	Enzyme profiling by zymogram analysis	38
2.3.6	Characterisation of cellulolytic component of GR-RS	40
2.3.6.1	Temperature optima and stability	40
2.3.6.2	pH optima and stability	41
2.3.6.3	Effect of additives	41
2.3.7	Characterisation of xylanolytic potential of GR-RS	42
2.3.7.1	Determination of temperature optima and stability	42
2.3.7.2	Determination of pH optima and stability	42
2.3.7.3	Effect of metals and additives on activity	43
2.4	Discussion	44

Chapter 3

Comparative Metagenomic and Metatranscriptomic analysis of GR-RS consortium developed under mesophilic and thermophilic conditions.

3.1	Introduction	49
3.2	Materials and methods	50
3.2.1	Materials	50
3.2.2	Generation of lignocellulose degrading mesoactive and thermoactive consortia from goat rumen contents	50
3.2.3	Sample preparation for metagenomic and metatranscriptomic analysis	51
3.2.4	Metagenomic analysis	51
3.2.4.1	Metagenomic sequencing	51
3.2.4.2	De novo metagenome assembly	51
3.2.4.3	Taxonomic profiling of metagenome data	51
3.2.5	Metatranscriptome analysis	51
3.2.5.1	RNA sequencing and de novo metatranscriptome assembly	51
3.2.5.2	Functional annotation	52
3.2.5.3	Abundance estimation	52
3.2.5.4	Differential analysis	52
3.3	Results	53
3.1	Taxonomic profiling of GR-RS-T and GR-RS-M	53
3.1.1	Relative abundance of different phyla in GR-RS-T and GR-RS-M	53
3.1.2	Relative abundance of bacteria in GR-RS-T and GR-RS-M at genus level	55
3.2	Gene Ontology profile analysis	57

3.3	Abundance of cazyme transcripts in GR-RS-T and GR-RS-M	58
3.4	Differential transcriptional expression of cellulolytic and xylanolytic enzymes	59
3.5	Discussion	63

Chapter 4

Studies on saccharification potential of GR-RS-T derived ligninocellulolytic enzymes, and a comparison with commercial cellulase blend.

4.1	Introduction	69
4.2	Materials and Methods	70
4.2.1	Materials	70
4.2.2	Production of consortium enzyme preparation (CEP) from goat rumen contents based consortium bred on rice straw (GR-RS-T)	71
4.2.3	Preliminary screening for saccharification potential of CEP from GR-RS-T	71
4.2.4	Biological pretreatment of rice straw and production of spent rice straw	71
4.2.5	Protein Estimation	71
4.2.6	Characterization of consortium enzyme preparation (CEP) and commercial cellulase blend (CCB) for cellulolytic and xylanolytic potential	72
4.2.7	Comparative High Temperature-Small Scale Saccharification (HTSS)	72
4.2.8	Scanning electron microscopy (SEM)	72
4.2.9	Metabolomic analysis of saccharified product by LC-MS analysis	73
4.3	Results	73

4.3.1	Preliminary screening for saccharification potential of CEP from GR-RS on agro-residues	73
4.3.2	Characterization of cellulolytic and xylanolytic potential of CEP and CCB	74
4.3.3	Biological pretreatment of rice straw	74
4.3.4	Comparative analysis of saccharification potential of CEP /CCB on URS/SRS	75
4.3.5	SEM analysis	76
4.3.6	Metabolomic analysis of saccharification filtrate of SRS treated with CCB/ CEP by LC-MS analysis.	76
4.4	Discussion	78
5	Summary and Conclusion	83
6	Bibliography	87
7	Appendices	107