



Indian Institute of
Technology Delhi



THE UNIVERSITY
OF QUEENSLAND
AUSTRALIA

**Lignocellulosic Biomass Valorisation using Deep Eutectic
Solvents for Sustainable Biorefineries**

by

VALLARI R. CHOURASIA

Submitted

in fulfilment of the requirements for the joint degree of

DOCTOR OF PHILOSOPHY

to the

**INDIAN INSTITUTE OF TECHNOLOGY DELHI
&
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JULY 2023



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This thesis is dedicated to

my dearest APPA

(My Grandfather- Nilkanth Kashinath Joshi)

Whose love has been the beacon of light in every era of darkness.

Who has been my supreme pillar of strength, inspiration, and encouragement.

Whose memory will forever be a source of solace and blessing.

Even though you never got to see this, you're in every page!

Certificate

This is to certify that the thesis entitled “**Lignocellulosic Biomass Valorisation with Deep Eutectic Solvents for Sustainable Biorefineries**” being submitted by **Ms. Vallari R. Chourasia** to the Indian Institute of Technology Delhi and University of Queensland for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her. **Ms. Vallari R. Chourasia** has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard. The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.



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Abstract

Concerns regarding the depletion of fossil fuel reserves and the environmental consequences of their use have motivated the development of renewable fuels. This has created a demand for alternative fuel resources derived from non-food substrates with low net carbon emission and has inspired the need to identify sustainable, inexpensive sources of biomass for an economically sustainable biorefinery proposition. Lignocellulosic biomass (LBM) represents a renewable, widespread, and low-cost source which can potentially be converted into high value products including biofuels with zero net CO₂ emissions. This research aimed to develop a sustainable bioprocess to utilise Sugarcane Bagasse (SB), an abundant lignocellulosic feedstock, from different sugarcane varieties. The study utilised advanced ionic liquids such as deep eutectic solvents (DESs) for increasing the pre-treatment efficiency by selective extraction of lignin and subsequently enhancing the digestibility of SB for further enzymatic saccharification and conversion to bioethanol. Physico-chemical characterizations performed using FE-SEM, FTIR, TGA and XRD analysis consistently indicated decomposition of bagasse structure after DES pre-treatment. Glucose recovery was predominantly influenced by the glucan content, as high sugar SB variety demonstrated the highest recovery of 92.8% for choline chloride: glycerol (ChCl:GLY) DES pre-treatment. However, maximum delignification of 81.2% with an enzymatic digestibility of 98.5% was achieved using choline chloride: lactic acid (ChCl:LA) DES. A one-pot pre-treatment and saccharification process in which DESs, and enzymes are utilised concurrently, resulted in the elimination of many intermediate and washing steps and a bioconversion method that is both cost-effective and energy-efficient. The biocompatibility of DESs was demonstrated with 90% of the stability of enzymes being retained in ChCl:GLY (10% w/v) after 24 h and a maximum glucan conversion of 72.6%, in a one-pot process. The one-pot process parameters were optimised for maximum saccharification yield using response surface methodology (RSM). The model suggested that

at optimal conditions, a maximum saccharification yield of 70.4% can be obtained and the glucose conversion was mainly impacted by enzyme concentration, followed by biomass loading and finally time. To analyse the kinetics of enzyme concentration on reaction rate, a modified Michaelis-Menten kinetics model was applied that demonstrated high agreement with experimental data. Additionally, to further improve the process dynamics, the DES pre-treatment process was integrated with microwave heating for delignification of SB. Moreover, the conventional DESs used were incorporated with Lewis acids ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) in an effort to provide the acidity essential for fractionation while preserving the maximum biocompatibility of DESs. Furthermore, the effect of water addition to these DESs was investigated to enhance their efficacy. In this context, choline chloride: ethylene glycol: $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (ChCl:EG:NI) at a molar ratio 1:2:0.016 with 20 w% water as a co-solvent produced the most promising results, with 84% delignification and 99% enzyme digestibility. A combined experimental and kinetic investigation of the delignification process utilising traditional binary ChCl:EG DES and the novel ternary ChCl:EG:NI DES in a microwave-assisted pre-treatment using Py-GC/MS and TGA studies was conducted. Py-GC/MS analysis of lignin demonstrated that DES pre-treatment of SB with ChCl:EG:NI resulted in the lowest release of phenolic compounds, indicating the highest delignification efficiency. Furthermore, the kinetic studies confirmed higher delignification efficiency of the ChCl:EG:NI DES, resulting in activation energy of 115.70 ± 1.83 and 129.36 ± 1.18 kJ/mol using the NL-INT and NL-DIF models respectively, as compared to the 126.89 ± 2.5 and 140.35 ± 1.65 kJ/mol for the untreated SB. Further, a comparative study between multi-unit operations and one-pot integrated process for mass balance, economic and environmental analysis was established. The one-pot integrated process led to the production of 43.6 g/L of ethanol with 8.7 g ethanol per 100 g dry SB. In this case, 50.6% maximum theoretical yield of ethanol was attained. Moreover, a preliminary cost analysis revealed that the net selling price of ethanol reduced by

30% in the integrated approach with an estimated net selling price of 9.75 \$/gal. Additionally, the life cycle assessment demonstrated the environmental effect of both processes, indicating that the multi-unit process had a 10% greater impact on global warming and a 4% greater impact on water depletion than the one-pot technique. Thus, this research illustrates the potential of biocompatible DESs, and provides new opportunities for developing an effective and sustainable biomass conversion process.

सारांश

जीवाश्म ईंधन भंडार की कमी और उनके उपयोग के पर्यावरणीय परिणामों के बारे में चिंताओं ने नवीकरणीय ईंधन के विकास को प्रेरित किया है। इसने कम शुद्ध कार्बन उत्सर्जन के साथ गैर-खाद्य स्रोत से प्राप्त वैकल्पिक ईंधन संसाधनों की मांग पैदा की है और आर्थिक रूप से धारणीय बायोरिफाइनरी प्रस्ताव के लिए जैवभार के धारणीय, सस्ते स्रोतों की पहचान करने की आवश्यकता को प्रेरित किया है। लिग्नेसेलुलॉसिक जैवभार (एलबीएम) एक नवीकरणीय, व्यापक और कम लागत वाले स्रोत का प्रतिनिधित्व करता है जिसे संभावित रूप से शून्य शुद्ध कार्बन डाईऑक्साइड उत्सर्जन के साथ जैव ईंधन सहित उच्च मूल्य वाले उत्पादों में परिवर्तित किया जा सकता है। इस शोध का उद्देश्य विभिन्न गन्ने की किस्मों से प्रचुर मात्रा में लिग्नेसेलुलॉसिक फीडस्टॉक, “गन्ना” (एसबी) का उपयोग करने के लिए एक स्थायी जैव प्रक्रिया विकसित करना था। अध्ययन में लिग्निन के चयनात्मक निष्कर्षण द्वारा पूर्व-उपचार दक्षता बढ़ाने के लिए मंद्र गलनक्रांतिक विलायक (DES) जैसे उन्नत आयनिक तरल पदार्थ का उपयोग किया गया और बाद में आगे एंजाइमेटिक सैकरिफिकेशन और बायोएथेनॉल में रूपांतरण के लिए एसबी की पाचन क्षमता को बढ़ाया गया। FE-SEM, TGA और XRD विश्लेषण का उपयोग करके किए गए भौतिक-रासायनिक लक्षण वर्णन ने लगातार DES पूर्व-उपचार के बाद संरचना के अपघटन का संकेत दिया। ग्लूकोज रिकवरी मुख्य रूप से ग्लूकन सामग्री से प्रभावित थी, क्योंकि उच्च चीनी एसबी किस्म ने कोलीन क्लोराइड के लिए 92.8% की उच्चतम पुनः प्राप्ति का अध्ययन किया: ग्लिसॉल (CHCl: GLY) DES पूर्व-उपचार। हालांकि, कोलीन क्लोराइड: लैक्टिक एसिड (CHCl: LA) DES का उपयोग करके 98.5% की एंजाइमेटिक पाचनशक्ति के साथ 81.2% का अधिकतम परिसीमन हासिल किया गया। एकपक्षीय पूर्व-उपचार और सैकरिफिकेशन प्रक्रिया जिसमें DES, और एंजाइमों का समवर्ती रूप से उपयोग किया जाता है, के परिणामस्वरूप कई मध्यवर्ती और धोने के चरणों और एक जैव रूपांतरण विधि का उन्मूलन हुआ जो लागत प्रभावी और ऊर्जा-कुशल दोनों है।

DES की जैव-रासायनिकता का अध्ययन किया गया, जिसमें एंजाइमों की 90% स्थिरता को 24 घंटे के बाद CHCl:GLY (10% w/v) में बनाए रखा गया और एक-पॉट प्रक्रिया में 72.6% का अधिकतम ग्लूकन रूपांतरण किया गया। प्रतिक्रिया सतह पद्धति (RSM) का उपयोग करके अधिकतम सैकरिफिकेशन उपज के लिए वन-पॉट प्रक्रिया मापदंडों को अनुकूलित किया गया। मॉडल ने सुझाव दिया कि इष्टतम परिस्थितियों में, 70.4% की अधिकतम सैकरिफिकेशन उपज प्राप्त की जा सकती है और ग्लूकोज रूपांतरण मुख्य रूप से एंजाइम एकाग्रता से प्रभावित होता है, इसके बाद जैवभार लोडिंग और अंत में समय होता है। प्रतिक्रिया दर पर एंजाइम एकाग्रता के गतिज अध्ययन का विश्लेषण करने के लिए, एक संशोधित माइकलिस-मेंटेन गतिज अध्ययन मॉडल लागू किया गया जिसने प्रयोगात्मक डेटा के साथ उच्च अनुबंध का अध्ययन किया।

इसके अतिरिक्त, प्रक्रिया की गतिशीलता को और बेहतर बनाने के लिए, DES पूर्व-उपचार प्रक्रिया को एसबी के अपघटन के लिए माइक्रोवेव हीटिंग के साथ एकीकृत किया गया। इसके अलावा, उपयोग किए गए पारंपरिक DES को लुईस एसिड ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) के साथ शामिल किया गया था ताकि DES की अधिकतम जैव-रासायनिकता को संरक्षित करते हुए फ्रैक्शनेशन के लिए आवश्यक अम्लता प्रदान की जा सके। इसके अलावा, उनकी प्रभावकारिता को बढ़ाने के लिए इन DES में पानी के जोड़ के प्रभाव की जांच की गई थी। इस संदर्भ में, कोलीन क्लोराइड: एथिलीन ग्लाइकोल: $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (CHCl: EG: NI) एक सह-विलायक के रूप में 20 w% पानी के साथ दाढ़ अनुपात 1: 2: 0.016 पर सबसे आशाजनक परिणाम उत्पन्न करता है, जिसमें 84% डेलिग्नेफिकेशन और 99% एंजाइम पाचनशक्ति होती है। Py-GC / MS और TGA अध्ययनों का उपयोग करके माइक्रोवेव-असिस्टेड पूर्व-उपचार में पारंपरिक बाइनरी CHCl:EG DES और नोबेल टर्नेरी CHCl: EG: NI DES का उपयोग करके डेलिग्नेफिकेशन प्रक्रिया की एक संयुक्त प्रयोगात्मक और गतिज जांच अभिप्रमाणित गई।

लिमिन के Py-GC/MS विश्लेषण से पता चला है कि CHCl: EG: NI के साथ एसबी के DES पूर्व-उपचार के परिणामस्वरूप फेनोलिक यौगिकों की सबसे कम रिहाई हुई, जो उच्चतम डेलिग्नेफिकेशन दक्षता का संकेत देती है। इसके अलावा, गतिज अध्ययनों ने CHCl: EG: NI DES की उच्च डिलिग्नेफिकेशन दक्षता की पुष्टि की, जिसके परिणामस्वरूप में क्रमशः NL-INT और NL-DIF का उपयोग करके $115.70 \pm 1.18 \text{ Kg/mol}$ और $129.36 \pm 1.18 \text{ Kg/mol}$ की सक्रियण ऊर्जा प्राप्त हुई जबकि अनुपचारित एसबी के लिए सक्रियण ऊर्जा 126.89 ± 2.5 और $140.35 \pm 1.65 \text{ Kg/mol}$ प्राप्त हुई। इसके अलावा, बड़े पैमाने पर संतुलन, आर्थिक और पर्यावरणीय विश्लेषण के लिए बहु-इकाई संचालन और वन-पॉट एकीकृत प्रक्रिया के बीच एक तुलनात्मक अध्ययन स्थापित किया गया। एक-पॉट एकीकृत प्रक्रिया ने प्रति 100 ग्राम सूखे एसबी में 8.7 ग्राम इथेनॉल के साथ 43.6 ग्राम / लीटर इथेनॉल का उत्पादन किया। इस मामले में, इथेनॉल की 50.6% अधिकतम सैद्धांतिक उपज प्राप्त की गई। इसके अलावा, एक प्रारंभिक लागत विश्लेषण से पता चला है कि इथेनॉल का शुद्ध बिक्री मूल्य 9.75 डॉलर / गैल के अनुमानित शुद्ध बिक्री मूल्य के साथ एकीकृत दृष्टिकोण में 30% कम हो गया है। इसके अतिरिक्त, जीवन चक्र मूल्यांकन ने दोनों प्रक्रियाओं के पर्यावरणीय प्रभाव का अध्ययन, यह दर्शाता है कि मल्टी-यूनिट प्रक्रिया का ग्लोबल वार्मिंग पर 10% अधिक प्रभाव पड़ा और वन-पॉट तकनीक की तुलना में पानी की कमी पर 4% अधिक प्रभाव पड़ा। इस प्रकार, यह शोध जैव संगत DES की क्षमता को दर्शाता है, और एक प्रभावी और धारणीय जैवभार जैवभार रूपांतरण प्रक्रिया विकसित करने के लिए नए अवसर प्रदान करता है।

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support, and any other original research work used or reported in my thesis.

The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the policy and procedures of The University of Queensland, the thesis be made available for research and study in accordance with the Copyright Act 1968 unless a period of embargo has been approved by the Dean of the Graduate School.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis and have sought permission from co-authors for any jointly authored works included in the thesis.

Publications included in this thesis

1. **Chourasia, V.R.**, Pandey, A., Pant, K.K., Henry, R.J. 2021. Improving enzymatic digestibility of sugarcane bagasse from different varieties of sugarcane using deep eutectic solvent pre-treatment. *Bioresource Technology*, 337, 125480.
2. **Chourasia, V.R.**, Bisht, M., Pant, K.K., Henry, R.J. 2022. Unveiling the potential of water as a co-solvent in microwave-assisted delignification of sugarcane bagasse using ternary deep eutectic solvents. *Bioresource Technology*, 351, 127005.
3. Bohre, A., Modak, A., **Chourasia, V.R.**, Jadhao, P.R., Sharma, K., Pant, K.K. 2022. Recent advances in supported ionic liquid catalysts for sustainable biomass valorisation to high-value chemicals and fuels. *Chemical Engineering Journal*, 450, 138032.

Submitted manuscripts included in this thesis

1. **Chourasia, V.R.**, Bisht, M., Pant, K.K., Henry, R.J. Response surface optimization and kinetic assessment of Deep eutectic solvent mediated one-pot pre-treatment and saccharification of sugarcane bagasse (*Submitted to Biotechnology for Biofuels and Bioproducts*)
2. **Chourasia, V.R.**, Sahoo, A., Mohan, M., Bhaskar, T., Pant, K.K., Singh, S., Henry, R.J. Disintegration of Lignocellulosic biomass using Deep eutectic solvents: Degradation kinetics and Py-GCMS study (*Submitted to Green Chemistry*)
3. **Chourasia, V.R.**, Bisht, M., Pant, K.K., Henry, R.J. Investigating biocompatibility of ternary DESs using one-pot integrated process for biofuel production using sugarcane bagasse (*Submitted to Biotechnology to ACS Sustainable chemistry and engineering*)

Conferences and Workshops

1. Poster presentation for “Application of Deep Eutectic Solvents for the pre-treatment of sugarcane bagasse to improve biomass digestibility” during the *International Conference on Biotechnology for Sustainable Agriculture, Environment and Health (BSAEH 2021)*, MNIT Jaipur, India, 2021.
2. Poster presentation on “Kinetic evaluation of One Pot fractionation and saccharification of mixed Sugarcane Bagasse varieties using Deep Eutectic Solvents” during the *43rd Symposium on Biomaterials, Fuels and Chemicals, USA, 2021*.
3. Flash talk and presentation on “Microwave-assisted one-pot bioconversion of sugarcane bagasse using metal-based ternary deep eutectic solvent” during the *International conference on BREEECH 2021 organized by BRSI at CSIR-IIP, Dehradun*.
4. Oral presentation on “Microwave facilitated one-pot bioconversion of sugarcane bagasse using novel ternary deep eutectic solvent” during the *International symposium on green chemistry (ISGC), La Rochelle, France, 2022*.
5. Oral presentation on “Understanding the role of water as a cosolvent in microwave facilitated delignification of sugarcane bagasse using ternary Deep Eutectic Solvents” during the *3rd Advances in Green Chemistry Conference (AGChem2022), Poznan, Poland, 2022*.
6. Poster presentation for “Investigating biocompatible Deep Eutectic Solvents for one pot integrated Bioethanol production from sugarcane bagasse” during the *Key Technologies in the Bioeconomy 2022, Straubing, Germany, 2022*.
7. Research Networking Workshop by Sugarcane Research Australia (SRA) held at The University of Queensland on 8th July 2020.

Contributions by others to the thesis

All chapters

Editorial suggestions were provided by the candidate's supervisors, Prof. Robert Henry, Prof. K. K. Pant, and PhD committee, Prof. Anurag Rathore, Prof. Sunil Kumar Khare and Prof. Greg Birkett.

Chapter 3

Dr. Ashish Pandey and Dr. Meena Bisht assisted in conceptualisation and synthesis of deep eutectic solvents.

Chapter 4

Dr. Katrina Hodgson-Kratky provided the sugarcane bagasse samples for all the Australian varieties. Dr. Virginie Perlo provided the compositional data for all the sugarcane genotypes used in the study. Dr. Agnelo Furtado assisted in the experimental set up and characterisation.

Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research involving human or animal subjects

No animal or human subjects were involved in this research.

Acknowledgement

“To succeed, you need to find something to hold on, something to motivate you, something to inspire you.” - Tony Dorsett

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TABLE OF CONTENTS

Abstract	i
सारांश (Abstract in Hindi)	iv
Declaration by author	vi
Publications included in this thesis	vii
Submitted manuscripts included in this thesis	vii
Conferences and Workshops	viii
Contributions by others to the thesis	ix
Statement of parts of the thesis submitted to qualify for the award of another degree	ix
Research involving human or animal subjects	ix
Acknowledgements	x
Financial support	xii
Keywords	xii
Australian and New Zealand standard research classifications (ANZSRC)	xii
Fields of research classification	xii
Table of Contents	xiii
List of figures	xvii
List of tables	xx
List of abbreviations	xxi
Chapter 1: Introduction	1
1.1 CURRENT GLOBAL ENERGY SCENARIO AND THE ROLE OF RENEWABLE ENERGY	1
1.2 SIGNIFICANCE AND ADVANCEMENT OF BIOENERGY	6
1.3 LIGNOCELLULOSIC BIOMASS (LBM) AS A FEEDSTOCK FOR BIOFUELS	10
1.3.1 Cellulose	14
1.3.2 Hemicellulose	15
1.3.3 Lignin	17
1.4 LBM CONVERSION TO PLATFORM CHEMICALS	17
1.5 RESEARCH OBJECTIVES	20

1.6	ORGANIZATION OF THESIS	20
Chapter 2: Literature Review		23
2.1	CONCEPT OF BIOREFINERY	23
2.1.1	Petrochemical refinery v/s Biorefinery	26
2.1.2	Lignocellulosic Biorefinery (LCB)	28
2.2	SUGARCANE BAGASSE AS A RICH SOURCE OF LBM	32
2.3	BIOMASS CONVERSION PROCESSES	34
2.4	CELLULOSIC ETHANOL PRODUCTION PROCESS	39
2.5	INFLUENCE OF BIOCHEMICAL COMPOSITION OF BIOMASS ON ITS CONVERSION PROCESS	42
2.6	CURRENT PRE-TREATMENT TECHNOLOGIES	44
2.6.1	Physical pre-treatment	50
2.6.2	Chemical pre-treatment	54
2.6.3	Physico-chemical pre-treatment	65
2.6.4	Biological pre-treatment	71
2.7	DEEP EUTECTIC SOLVENTS (DESS): GREEN SOLVENTS FOR BIOMASS CONVERSION	74
2.7.1	Bibliometric analysis	79
2.7.2	Physicochemical properties of DESs	81
2.7.3	DES as a Pre-treatment solvent for biomass	85
2.7.4	Recyclability of DESs	87
2.8	BARRIERS TO SUSTAINABILITY OF LIGNOCELLULOSIC BIOREFINERY	88
2.9	RESEARCH GAPS	89
Chapter 3: DES pre-treatment for improved SB digestibility		93
3.1	INTRODUCTION	94
3.2	MATERIALS AND METHODS	95
3.2.1	Feedstock and chemicals	95
3.2.2	DES preparation and pre-treatment	96
3.2.3	Compositional Analysis	97
3.2.4	Characterization of untreated and pre-treated SB	99
3.2.5	Enzyme saccharification	100
3.3	RESULTS AND DISCUSSION	101
3.3.1	Compositional analysis	101
3.3.2	Effect of DES	104
3.3.3	Relation between lignin removal and enzyme amenability	106
3.3.4	Scanning Electron Microscope (SEM) Analysis	108
3.3.5	Fourier Transform Infra-red (FTIR) Analysis	110
3.3.6	X-ray diffraction (XRD) Analysis	111

3.3.7	Thermogravimetric Analysis (TGA)	112
3.4	CONCLUSION	114
Chapter 4: One pot DES pre-treatment and saccharification: statistical optimization and enzyme kinetic study		116
4.1	INTRODUCTION	117
4.2	MATERIALS AND METHODS	118
4.2.1	Feedstocks and chemicals	118
4.2.2	DES preparation	119
4.2.3	Enzyme activity and stability in the presence of DES	119
4.2.4	One-pot pre-treatment and saccharification process	120
4.2.5	Experimental design and optimization (DOE)	121
4.2.6	Statistical analysis	122
4.2.7	Kinetic model	123
4.3	RESULTS AND DISCUSSION	124
4.3.1	Glucan conversion after One-pot process	124
4.3.2	Enzyme activity and stability in DES environment	126
4.3.3	Optimization of One-Pot process conditions	129
4.3.4	Effect of process parameters on saccharification yield	133
4.3.5	Kinetic assessment	136
4.4	CONCLUSION	139
Chapter 5: Microwave-assisted pre-treatment using novel ternary DESs		142
5.1	INTRODUCTION	143
5.2	MATERIALS AND METHODS	146
5.2.1	Feedstock and chemicals	146
5.2.2	Synthesis of ternary DESs	146
5.2.3	Solvatochromic parameters and viscosity measurement of DESs	147
5.2.4	Microwave-assisted DES pre-treatment and DES recovery	148
5.2.5	Enzymatic saccharification	149
5.2.6	Compositional analysis	150
5.2.7	Physicochemical analysis	150
5.3	RESULT AND DISCUSSION	151
5.3.1	Effect of addition of water on DES properties	151
5.3.2	Impact of water as a co-solvent for SB fractionation	157
5.3.3	Lignin precipitation and DES recovery	163
5.3.4	Enzyme digestibility measurement	166

5.4	CONCLUSION	168
Chapter 6: One Pot Integrated Bioethanol Production		170
6.1	INTRODUCTION	171
6.2	MATERIALS AND METHODS	180
6.2.1	Proximate and Elemental analysis	180
6.2.2	Higher heating value (HHV)	181
6.2.3	Thermogravimetric analysis	181
6.2.4	Kinetic studies	181
6.2.5	Py-GC/MS analysis	183
6.2.6	One-pot integrated process	184
6.2.7	Cost analysis for bioethanol production	185
6.2.8	Environmental impact using lifecycle assessment	186
6.3	RESULTS	187
6.3.1	Proximate and elemental analysis	187
6.3.2	Higher heating value	189
6.3.3	Thermogravimetric analysis	190
6.3.4	Kinetic study of delignification	192
6.3.5	Py-GCMS analysis	193
6.3.6	Mass balance	195
6.3.7	Comparison of cost of ethanol production	198
6.3.8	Comparison of environmental impact by Life cycle assessment	200
6.4	CONCLUSION	202
Chapter 7: Conclusion and Future Prospects		206
7.1	CONCLUSIONS	206
7.2	SWOT ANALYSIS	207
7.3	FUTURE PROSPECTS	207
References		212
Appendix		233
Curriculum Vitae		239

List of Figures

Figure 1.1: Trend in Global Primary Energy Supply	2
Figure 1.2: Trend in Global Primary Energy Consumption	3
Figure 1.3: Trend in Total energy consumption by source.....	5
Figure 1.4: Global Biofuel Production (2021).....	8
Figure 1.5: Typical chemical composition of LBM.....	13
Figure 1.6: Cellulose structure.....	14
Figure 1.7: Hemicellulose structure.....	16
Figure 1.8: Structure of Lignin polymer.....	18
Figure 1.9: Typical routes for biomass conversion to biofuels and platform chemicals.....	19
Figure 2.1: Concept of Biorefinery.....	24
Figure 2.2: Framework of the Biorefinery classification system (de Jong et al., 2012).....	26
Figure 2.3: Typical routes for sugarcane bagasse valorisation (Chandel et al., 2012).....	33
Figure 2.4: Biomass Conversion Schemes in a Lignocellulose Biorefinery	34
Figure 2.5: Schematic representation of a Pre-treatment process	45
Figure 2.6: Classification of Pre-treatment methods and their possible effects	46
Figure 2.7 Schematic representation of formation of a deep eutectic solvent (DES)	78
Figure 2.8: Network map of co-occurrence of keywords.....	80
Figure 3.1: Effect of DES pre-treatment on composition of SB, lignin removal (%), glucose recovery (%)	106
Figure 3.2: Enzyme amenability before and after DES pre-treatment	108
Figure 3.3: SEM images of a. Untreated SB b. ChCl:GLY treated SB c. ChCl:LA treated SB d. ChCl:MA treated SB	109
Figure 3.4: FTIR spectra for (top to bottom) 1. ChCl:GLY pre-treated SB 2.ChCl:MA pre- treated SB 3. Untreated SB 4. ChCl:LA pre-treated SB.....	110

Figure 3.5: XRD patterns for (top to bottom) 1. ChCl:GLY pre-treated SB 2.ChCl:MA pre-treated SB 3. Untreated SB 4. ChCl:LA pre-treated SB.....	112
Figure 3.6: TGA patterns (top to bottom) 1. Untreated SB 2. ChCl:GLY pretreated SB 3. ChCl:MA pretreated SB 4. ChCl:LA pretreated SB.....	113
Figure 4.1: Schematic representation of the one-pot DES pre-treatment and saccharification of SB	118
Figure 4.2: Glucan conversion of all SB varieties with and without DESs.....	125
Figure 4.3: Enzyme activity at different concentrations of ChCl:GLY, ChCl:LA, ChCl:MA DESs after incubation of (a) 24h (b) 48h (c) 72h	127
Figure 4.4: Enzyme stability in presence of 10 w% DES using (a) Fluorescence spectroscopy (b) CD spectroscopy	129
Figure 4.5: Predicted vs Actual Saccharification yield of one pot process	131
Figure 4.6: Response surface plots for (a) effect of biomass loading and time (b) enzyme loading and time (c) biomass loading enzyme loading against saccharification yield	135
Figure 4.7: Desirability function for maximum saccharification yield (%)	136
Figure 4.8: Enzymic hydrolysis of SB at different enzyme concentrations	138
Figure 4.9: Effect of initial enzyme concentration on the initial hydrolysis rate of SB in the presence of DES. Experimental data is marked with dots and the continuous curve is obtained using the model	139
Figure 5.1: Schematic representation of a microwave-assisted DES pre-treatment using water as a co-solvent	144
Figure 5.2: Thermogravimetric analysis of DES mixtures and SB	155
Figure 5.3: FTIR analysis of DES mixtures	156
Figure 5.4: Compositional analysis of untreated and pre-treated SB	159

Figure 5.5: Delignification of SB after DES pre-treatment.....	159
Figure 5.6: XRD analysis of pre-treated SB.....	160
Figure 5.7: FTIR analysis of pre-treated SB.....	162
Figure 5.8: TGA of pre-treated SB.....	163
Figure 5.9: Enzymatic digestibility (%) after 72 h for untreated and DES pre-treated SB...	167
Figure 6.1: Schemes for one-pot integrated processing	174
Figure 6.2: Schematic representation of the one-pot integrated process for bioethanol production.....	179
Figure 6.3: LCA boundary for bioethanol production.....	187
Figure 6.4: TG and DTG degradation profile for SB	191
Figure 7.1: SWOT analysis for One-Pot Bio-ethanol production	207

List of Tables

Table 1.1: Biomass classification on the basis of their source material	11
Table 1.2: Lignocellulose biomass availability and chemical composition	12
Table 2.1: Comparison of the petrochemical refineries and biorefineries	28
Table 2.2: Estimated cost of dried biomass and its composition.....	34
Table 2.3: Summary of research studies on different pre-treatment methods using diverse feedstocks.....	47
Table 2.4: Classification of DESs based on their general formula.....	76
Table 2.5: Physicochemical properties of commonly used ChCl based DESs.....	85
Table 3.1: Composition of untreated and pre-treated SB	102
Table 4.1: Actual and coded levels of the experimental design variables.....	122
Table 4.2: Experimental and predicted responses of the three-level central composite design	131
Table 4.3: ANOVA of response surface model for optimization of the one pot process.....	132
Table 4.4: Regression analysis of one pot process and parameter estimates	132
Table 5.1: List of DESs used for SB pre-treatment ^a	147
Table 5.2: Solvatochromic parameters and Viscosity of DES mixtures	153
Table 5.3: Lignin yield (%) and DES recovery (%).....	165
Table 6.1: Physicochemical characteristics of petrol, diesel, and bioethanol	171
Table 6.2: Proximate and elemental analysis of SB	189
Table 6.3: Estimated average activation energy of SB via NL-INT and NL-DIF models....	192

List of Abbreviations

LBM	Lignocellulosic biomass
SB	Sugarcane bagasse
AIL	Acid Insoluble Lignin
ASL	Acid Soluble Lignin
IL	Ionic Liquid
DES	Deep Eutectic Solvent
ChCl	Choline chloride
EG	Ethylene glycol
GLY	Glycerol
LA	Lactic acid
MA	Malonic acid
NI	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$
MG	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$
SSF	Simultaneous saccharification and fermentation
SHF	Separate hydrolysis and fermentation
CBP	Consolidated bioprocess
MESP	Minimum ethanol selling price
GHG	Greenhouse gases