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
&
UNIVERSITY OF QUEENSLAND

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 CO_2 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 delginification 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 ($MgCl_2.6H_20$, $NiCl_2.6H_20$) 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: $NiCl_2.6H_2O$ (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। लिग्नोसेलुलोसिक 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 लिग्नोसेलुलोसिक 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 अध्यन्न किया: ग्लिसरॉल (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 अध्यन्न 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 अध्यन्न 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 ($MgCl_2.6H_20$, $NiCl_2.6H_20$) 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: $NiCl_2.6H_2O$ (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 नोवेल टर्नेरी CHCl: EG: NI DES का उपयोग करके डेलिगनेफिकेशन प्रक्रिया की एक संयुक्त प्रयोगात्मक और गतिज जांच अभिप्रमाणित गई।

लिग्निन 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 डेलिगनेफिकेशन 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 ± 1.18 Kg/mol और 129.36 ± 1.18 Kg/mol की सक्रियण ऊर्जा प्राप्त हुई जबकि अनुपचारित एसबी के लिए सक्रियण ऊर्जा 126.89 ± 2.5 और 140.35 ± 1.65 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 अध्यन्न, 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

There are a number of people without whom this work seemed to be impossible, and I am grateful for this opportunity to thank each one of them for their contributions.

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Sugarcane bagasse, Lignocellulosic biomass, pre-treatment, deep eutectic solvents, enzymatic hydrolysis, bioethanol

Australian and New Zealand standard research classifications (ANZSRC)

ANZSRC code: 070304 Crop and Pasture Biomass and Bioproducts, 30%

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Fields of research classification

FoR code: 0703 Crop and Pasture Production, 30%

FoR code: 0904 Chemical Engineering, 35%

FoR code: 1003 Industrial Biotechnology, 35%

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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	$NiCl_2.6H_2O$
MG	$MgCl_2.6H_2O$
SSF	Simultaneous saccharification and fermentation
SHF	Separate hydrolysis and fermentation
CBP	Consolidated bioprocess
MESP	Minimum ethanol selling price
GHG	Greenhouse gases