UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Development of a Non-Derivatizing Solvent System for the Pretreatment of South African Corn Cob

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A dissertation submitted to the Faculty of Engineering and the Built Environment, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree of Master of Science in Engineering.

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March 2019

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Journal Publications and conference presentations

Journal publications

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Eterigho-Ikelegbe, O., Development of a Non-derivatizing Hydrate Salt Pretreatment Solvent for Pretreatment and Fractionation of Corn Cob. *Cogent Engineering* (Submitted)

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Optimisation of dissolution conditions during fractionation of corn-cob in $ZnCl_2.4H_2O$/Urea solvent system: A statistical approach. *The Open Chemical Engineering Journal* (Submitted)

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Kinetic and thermodynamic study of dissolution of corn cob in $ZnCl_2.4H_2O$/ Urea solvent system. *International Journal of Industrial Chemistry* (Submitted)

Conference Proceedings and conferences attended

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Development of a Non-Derivatizing molten hydrate salt pretreatment system for South African corn cob: A preliminary investigation. **Oral Presentation.** *International Science Without Borders (WISWB)–Indaba*, p.53. ISBN: 978-0-620-78656-0. *24 March 2017. University of Johannesburg, Johannesburg, South Africa.*

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Developing a non- derivatizing cellulose solvent for the pretreatment of South African corn cob. **Oral Presentation**. *Carbon energy and the environment national student colloquium 2018. 21 June 2018. North-West University, Northwest Province, South Africa.*

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Development of an environmentally benign non-derivatizing pretreatment solvent for fractionation of corn cob: Effect of pretreatment variables **Oral Presentation**. *DST- UNESCO national engineering student conference. 17 September 2018. Cape Peninsula University of Technology, Symphony Way Bellville, Cape Town*. (2^{nd} Prize best oral presentation).

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Using a non-derivatising solvent system to improve the pretreatment efficiency of waste corn cob. **Poster Presentation**. *9th cross faculty postgraduate symposium. 29-30 October 2018, University of the Witwatersrand, Johannesburg, South Africa* (1st Prize faculty best poster, 2nd Prize cross faculty best poster).

Ejekwu, O., Daramola, M.O. and Ayeni, A.O., Development of an environmentally benign non-derivatizing pretreatment solvent for fractionation of corn cob: Effect of pretreatment variables. **Oral Presentation**. *4th National Conference on Global Climate Change, GCC. 3-6 December 2018. Bolivia lodge, Polokwane, Limpopo province, South Africa.*

Acknowledgements

I would like to give all the glory to God, for the abundant blessings, strength and the will to persevere in times of need.

I would like to thank my supervisor, Prof Michael Daramola for his guidance, patience and support during the last 2 years. Your exceptional work ethic and willingness to teach made the journey much easier.

To my co-supervisor, Dr. Augustine Ayeni. Thank you for your assistance, advice, very valuable insights and contributions to this body of work. Thank you for the many words of encouragement, they were most appreciated.

Thank you to the National Research Foundation of South Africa, Department of Science and Technology, TATA and University of the Witwatersrand postgraduate merit award for the financial support whilst completing my Masters.

To my parents (Mr. Bududburisi Ejekwu and Mrs Salome Ejekwu) and brothers (Igenda Ejekwu and Kwesi Ejekwu), thank you for never giving up on me. Thank you for the constant support and prayers all through my academic career, always giving me the encouragement to achieve my dreams over the years and always encouraging me to work towards my goals.

To all the members of the research group; Sustainable Energy and Environment Research Unit (SEERU) and my colleagues, Ayotunde Awosusi, Michael Nnanna, Patrick Sekoai, Kelvin Yoro, Tsireledzo Mudau, Ezinne Igbokwe, Maxwell Ozonoh, Chinwe Unuigbe, Fortunate Farirai, Orevaoghene Eterigho-Ikelegbe, Luisa Agostinho, Tiemele Aka, Mbinzi Ngw. Thank you for the constructive criticism on every presentation and your contribution to this body of work. You all made the journey so much more fun.

I extend my appreciation to Mr Bruce Mothibeli, Mr Motlatsi, Mrs. Janet Smith for all the equipment, training and providing lab space. I would also like to thank the administrative staff in the School of Chemical and Metallurgical engineering.

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Abbreviations

AFEX: Ammonia fibre expansion

AIL: Acid insoluble lignin

ANOVA: Analysis of variance

ASL: Acid Soluble Lignin

CCD: Central composite design

CrI: Crystallinity Index

DNS: Dinitrosalicyclic acid

Ea: Activation energy

FTIR: Fourier transmission infra-red

HMF: 5-hydroxymethyl furfural

IL: Ionic liquid

MHS: Molten Hydrate Salts

NREL: National Renewable Energy Laboratory (Golden, CO, USA)

RSM: Response Surface Methodology

SEM: Scanning electron microscopy

XRD: X-ray diffraction

Abstract

Depleting fossil fuels and the increasing energy demand has necessitated the move to alternative renewable forms of energy. Lignocellulosic biomass is a renewable and sustainable source for highly valuable bio-based chemicals and material production in a biorefinery system. The effective fractionation of the main components of lignocellulosic biomass (cellulose, hemicellulose and lignin) into usable forms is a crucial step in unlocking an economically viable, high-value product producing biorefinery. The main concern associated with the conversion of lignocellulose is overcoming biomass recalcitrance using pretreatment while still maintaining a green, cost-effective and energy efficient process. Over the last decade, molten hydrate salts have been used for isolated cellulose dissolution, however very few studies have been done to check their ability in lignocellulosic biomass pretreatment.

The aim of the study was to compare seven molten hydrate salt solvent systems including unary, binary and ternary mixtures of $ZnCl_2.4H_2O$, $LiClO_4.3H_2O$ and Urea for the effective pretreatment of corncob in terms of physicochemical properties and pretreatment efficiencies and to optimise these efficiencies. The molten salt hydrate pretreatment systems used in this study are aimed at fractionating the corn cobs biomass into a solid fraction which mostly contains cellulose and lignin as the major components, while the liquid fraction contains hemicellulose as the main component.

The pretreatment experiments were carried out at 70 °C for 60 minutes at a biomass: solvent ratio of 1:10. Physicochemical change after pretreatment was checked by FTIR, XRD and SEM. The most efficient solvent mixture was identified by gravimetric analysis for its ability to fractionate the biomass into a cellulose and lignin rich solid fraction and a hemicellulose-rich liquid fraction. The effect of solvent pretreatment operating variables (temperature, time and solvent concentration) was investigated to maximize cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery from the biomass by response surface methodology (RSM) approach using a central composite design (CCD).

Physicochemical analysis showed a decrease in crystallinity and an increase in surface area after the pretreatment in all the MHS solvents tested. This work has successfully shown the use of $ZnCl_2.4H_2O$/ Urea, to pre-treat and fractionate corn cob with high recovery of cellulose (100%), low recovery of hemicellulose (42%) and lignin (44%) when compared to the other proposed systems. Through the RSM approach, optimum pretreatment conditions obtained

were: 90 min, 120 ^{o}C and concentration of 71.32%/28.68 (w/w) $ZnCl_2.4H_2O$/ Urea. At these conditions, the predicted recovery for cellulose, hemicellulose and lignin 99.03%, 27.18% and 72.43% respectively with a desirability of 0.902. The actual recovery was 91%, 29% and 68% for cellulose, hemicellulose and lignin respectively at the same conditions.

For a better understanding of the dissolution kinetics and thermodynamics of cellulose, hemicellulose and lignin dissolution in $ZnCl_2.4H_2O$/ Urea solvent system, a kinetic study was carried out. The results reveal the dissolution to be a 1st order kinetics and the obtained activation energy for cellulose, hemicellulose and lignin dissolution were 14.10 kJ.mol^{-1}, 11.29 kJ.mol^{-1} and 7.606 kJ.mol^{-1} ,respectively. The positive values for ΔH and ΔG indicate that the dissolution process for all three components are endothermic and endergonic. The less negative ΔS for hemicellulose dissolution (-0.190; -0.195 kJ.mol^{-1}) showed that the process of dissolution of hemicellulose occurred more rapidly and produced more stable products. It was concluded that $ZnCl_2.4H_2O$/ Urea pretreatment provided a potential way to fractionate lignocellulosic biomass which can improve the effective utilization of all feedstock fractions.

CHAPTER ONE

1. Introduction

1.1 Motivation and background

Due to the world's increased energy and petrochemical demand, the insecurity in energy supply, the increase fossil fuel price as well as increased environmental pollution, alternative sources of energy which can replace conventional fossil fuels need to be investigated. These alternative sources include proposed biorefinery solutions which seek to convert waste lignocellulosic biomass to fuels, materials and platform chemicals currently produced from oil refinery (Ragauskas et al., 2006).

Lignocellulosic biomass is the most abundant plant material on the planet (Wilson, 1988). It has been proven to give promising yields in processes designed for the production of fuels, chemicals and materials. Lignocellulosic biomass is composed primarily of cellulose, hemicellulose and lignin. These polymers are interlinked in a hetero-matrix (Cowling and Kirk, 1976). These three components make up approximately 90% of the dry matter of lignocellulosic biomass. The remainder of the material is ash and extractives. These three main components (i.e., cellulose, hemicellulose and lignin) are required for the conversion of the biomass to high value added products for profitable utilisation of lignocellulose. In addition to bioethanol production from cellulose, the remaining components in the lignocellulosic biomass can be economical sources of value added products, materials and platform chemicals (Clark, 2007).

Lignocellulosic biomass can be derived from diverse, inexpensive and widespread resources, e.g., forestry resources or residues, agricultural residues, energy crops, food scraps and municipal solid waste. Corncob waste is a major renewable agricultural residue in corn production which is produced in large amounts all over the world. In South Africa, 14.5 million tons of corn cob waste is produced annually according to the National Agricultural Marketing Council of South Africa NAMC (National Agricultural Marketing Council, 2012). This makes them the second highest produced agricultural wastes. Corn cobs can be burned as fuel, used as animal feed or recycled as fertilizer in soil (Inglett, 1970). Recently, more attention is being paid to the potential applications of corn cob in the production of bio-based energy and chemicals due to the development of the biorefinery industry. Corn cob waste,

being highly rich in cellulose and hemicellulose components, can be applied in biofuels and biobased chemical as a renewable and inexpensive carbon source.

The bioconversion of biomass is hindered by its recalcitrance which is caused by the inherent association of cellulose, hemicelluloses and lignin in the lignocellulosic biomass. In order to overcome this recalcitrance, pretreatment must be done. There are many contributing factors to the recalcitrance of lignocellulose (Chaturvedi and Verma, 2013). These factors include inaccessible surface area, cellulose crystallinity, cellulose sheathing by hemicellulose and protection by lignin. Pretreatment of lignocellulosic biomass is required to overcome recalcitrance as well as increase the accessibility of enzymes to cellulose to maximise product recovery for improved economics of lignocellulosic bio-refineries (Sindhu et al., 2016). Pretreatment is a crucial step and arguably the most expensive processing step in the processing of lignocellulosic biomass as it accounts for approximately 40% of the total costs of processing (Sindhu et al., 2016). To date, a variety of fractionation and pretreatment processes including chemicals, physical and biological pre-treatment methods are being explored in academia and industry. Examples of pretreatment methods include; oxidative delignification, acid and alkali hydrolysis (Jacobsen and Wyman, 2000) ionic liquid (Zhu, 2008), hydrothermal (Garrote et al., 2002), inorganic salt immersion, steam explosion (Liu et al., 2013), microbial (Wan and Li, 2011). These methods are able to remove lignin, separate hemicellulose and cellulose effectively or change other structural features of the biomass such as decreasing the degree of polymerisation or cellulose crystallinity, increase of the surface area of the biomass which results in an increase in the ability of enzymes or chemical catalysts to bind and hydrolyse cellulose. However, these pretreatment methods still encounter a number of sustainability problems.

In order to be sustainable, biomass utilisation will depend heavily on the successful deployment of innovative-green chemistry. Green chemistry is defined as the efficient utilisation of raw materials (preferably renewable), the elimination of waste and avoidance of toxic or hazardous solvents and reagents in chemical processes (Clark, 2007). Examples of some green solvents include cheap, eco-friendly and recyclable systems are molten hydrate salts (MHS) solvent systems. Molten hydrate salts are aqueous solutions of inorganic salts at extraordinarily high concentration, which varies for each salt, depending on the coordination number of the salt cation (Sen et al., 2013). MHSs have cations that are highly hydrated (coordinated with water), while the anion is coordination-free in the system. This nature gives molten salt hydrates some unique properties such as high boiling point, low vapour pressure

and liquid status at moderate temperature, MHS are even able to swell and dissolve cellulose and biomass in a non-derivatising manner. Molten salt hydrates have been used as solvent and reaction medium in various chemical reactions and processes including biomass conversion. Because of similar properties and performance with ionic liquids, molten hydrate salts are often known as inorganic ionic liquids. However, molten hydrate salts usually have lower cost, lower viscosity and lower toxicity than organic ionic liquids. Some examples of MHSs are $LiClO_4 \cdot 3H_2O$, $LiSCN \cdot 2H_2O$, $LiI_2 \cdot 2H_2O$, $ZnCl_2 \cdot 4H_2O$ and their mixtures which have been proven to enable the dissolution of cellulose at relatively mild conditions (Sen et al., 2013).

The establishment of sustainable production of biofuels, biochemical and biomaterials in the future depends on the integration of green chemistry and low environmental impact technologies into biorefinery. Green chemistry is a set of principles which govern the manufacture and application of products with the aim of eliminating the use and generation of environmentally hazardous chemicals (Capolupo and Faraco, 2016). The development of processes technologies which are both innovative and sustainable is mandatory in achieving the shift towards bio-based production of fuels, chemicals and materials.

This study therefore seeks to compare the efficiency of seven different molten hydrate salt solvent systems on the pretreatment of South African corn cobs and to propose a new solvent system for lignocellulose fractionation. This solvent was optimised and the efficiency was assessed as the ability for the pretreatment to recover polysaccharide fractions and lignin post pretreatment. This was done in order to determine a pretreatment process which is less energy intensive, generates less waste and is both environmentally and economically more attractive than conventional routes.

1.2 Problem statement

Corn cob waste is discarded in large quantities annually in South Africa. The waste often causes environmental pollution due to lack of its effective utilisation or disposal. There is a large number of environmental and economic challenges related to the use of fossil fuels. The use of waste biomass can potentially eradicate these challenges. In the past, most attempts at addressing fossil fuel related problems using biomass have been focused on biofuels production mostly while biomaterials and biochemical production are not at widely researched. This causes the biomass to be underutilized. A possible solution to these problems is biorefinery. In the production of biofuels from lignocellulose, which has been

extensively studied, the main objective of pretreatment has been the recovery of cellulose based sugar monomers with minimal degradation (inhibitory) products, but not focusing on the quality of the other components (hemicellulose and lignin). In biorefinery, there is a focus on the utilisation of other lignocellulose components. The pretreatment/fractionation methods need to be reviewed in order to isolate and separate all components in good quality in addition to the production of cellulose-based products. Recently, cellulose solvents and green solvent technologies are being developed to solve this problem. Ionic liquids have been used as solvents for biomass green conversion and are known for their ability to directly dissolve cellulose, hemicellulose, lignin and even raw lignocellulose at low temperature. However, ionic liquids are still expensive and their recovery and reuse is still a challenge. Inorganic molten salt hydrates, are a kind of green solvent ionic liquid-like system with similar physicochemical properties to ionic liquids. Molten hydrate salts are known to have excellent ability to dissolve polysaccharides, such as cellulose and hemicellulose, in an inexpensive and recyclable manner. However, their use in the pretreatment and fractionation of lignocellulose is still currently being researched at infancy stage, there are limited accounts in the scientific literature of dissolution of whole lignocellulosic biomass using molten hydrate salts in relation to biomass fractionation.

1.3 Research aim

The thesis aims to improve the potential economic use of lignocellulosic biomass (e.g corn cob) by developing and optimising an efficient molten hydrate salt solvent system for effective pretreatment and fractionation of corn cob.

1.4 Research objectives

1. Investigate the effect of molten hydrate salt solvent systems on the pretreatment of corn cobs on the physical and chemical characteristics of corn cobs.

2. Identify the most efficient solvent system with respect to the recovery of cellulose, hemicellulose and from the biomass.

3. Investigate the effect of dissolution parameters such as solvent concentration, reaction time and temperature on the pretreatment efficiency of the solvent systems.

4. Optimise the pretreatment conditions of the pretreatment process to maximize cellulose recovery, hemicellulose recovery in the liquid fraction and delignification of the biomass.

5. Develop a kinetic model to explain the dissolution behaviour of corncob the recovery of cellulose, recovery in the liquid fraction of hemicellulose and delignification of corn cobs.

1.5 Thesis organisation

The first chapter is a general introduction that introduces the background, the motivation behind using molten hydrate salts for pretreatment of lignocellulosic biomass and includes the thesis aim and objectives, problem statement and a description of the thesis organisation.

Chapter 2 reviews the relevant literature that motivated the study and establishes a background for the results from the study. It covers the motivation behind using lignocellulose for production of fuels, chemicals and materials with special emphasis on platform chemicals that can be derived from the biomass. It also provides a detailed review of the structure and chemistry of lignocellulose and brief look into the major pretreatment step required to access the biomass for conversion. It provides a critical review of the various pretreatment mechanisms present including advantages and disadvantages of each.

Chapters 3-5 follow the objectives of the research. Chapter 3 focuses on addressing the first and second objective of this thesis by outlining the development of a facile and inexpensive method for the pretreatment of corn cob by investigating the effect of the different molten hydrate salt systems on the physical and chemical characteristics and identifying the most efficient solvent system. Chapter 4 aims to optimise the most efficient solvent system identified in Chapter 3 using response surface methodology. Chapter 5 presents a detailed study of the dissolution kinetics of each component of the lignocellulosic biomass in the optimised molten hydrate salt solvent system. It presents the reaction kinetics as well as thermodynamic parameters involved.

Chapter 6 gives the conclusion and future work of the project. In this chapter the findings and conclusions presented throughout the thesis as they relate to the specific research objectives from Chapter 1 are outlined and future research recommendations are discussed.

CHAPTER TWO

2. Literature review

2.1 Bioenergy

There is currently a heavy reliance on fossil resources. About 82% of the world's energy needs are currently being met by fossil resources (Schierbaum, 2009). Unexpected disruptions in oil supplies usually lead to severe economic problems. In addition, other environmental problems such as greenhouse gas emissions, air pollution and acid rain arise from the use of fossil fuels. Several alternative energy resources have been extensively investigated such as solar, geothermal, solar, hydropower and biomass. Among the alternative energy resources, one of the fastest growing forms is the energy from biomass.

Bio energy is energy which is derived from organic sources such as lignocellulosic materials, agricultural crops and wastes. Presently, the use of biomass in its traditional form continues to be the main source of energy in many developing nations (Dermibas, 2004). The traditional way of using biomass is by direct combustion: it is responsible for over 97% of the world's bioenergy production (Dermibas, 2004). In many countries, the traditional use of biomass by direct combustion is challenging in terms of processing and logistics, and the overall environmental benefits are questionable. In addition, direct combustion of biomass results in a lost opportunity with regards to the co-production of additional chemicals and energy (Mando, 2013). It is thus more sustainable to develop efficient and clean processes for the biomass conversion in order to generate a wide variety of products.

There are multiple non-traditional methods for the conversion of biomass energy. They can be categorised into thermochemical conversion and biochemical processes. Thermochemical processes are based on the action of heat which allows the chemical reactions that are necessary to transform the material into energy to take place (Bridgwater, 1996). These include gasification and torrefaction. Gasification is a high-temperature process (between 750 and 1800 °C) which involved the degradation of biomass to produce energy-rich gases. Pyrolysis is a medium- to high-temperature process (400–750 °C) which causes the degradation of biomass and results in mostly liquid products for energy generation. Torrefaction is a low-temperature treatment (below 300 °C) of biomass which produces a highly energy dense solid fuel (Bridgwater, 1996). These processes typically have commercialization challenges as the set-up and processing costs are usually high, the

products in comparison to fossil fuels are typically of poorer quality. In order to produce valuable chemicals and materials, these types of conversions depolymerise lignocellulose directly without distinguishing the components and the biomass is regarded as a mixture of C, H and O elements. The degraded products are also so complex that upgrade is usually required (Dermibas, 2009). Biochemical conversion processes allow the exploitation of energy obtained through chemical reactions, by the use of enzymes, fungi and micro-organisms that take place in the biomass under particular conditions. This process allows for the fractionation of lignocellulose to the main components of cellulose, hemicellulose and lignin followed by their conversion to the desired chemicals or materials. The major obstacle of thins process is the efficient disassembly of the recalcitrant complex (Lee, 1997). Biomass conversion into products by fermentation is a very flexible process which could lead to a wide range of value added products including biofuels, biochemicals, or biomaterials (Waldron, 2010). Biochemical processing technologies likely offers more value-added specialty chemical product options than thermochemical technologies and also has the added benefit of being less energy intensive. It however has the challenge of being too slow and labour intensive.

2.2 Biochemical conversion of lignocellulosic biomass

There a three main steps involved in the bioconversion of lignocellulosic biomass to fuels and chemicals. These main steps being pretreatment, hydrolysis and fermentation. A schematic of the biological conversion process is represented in Figure 2.1. Firstly, the feedstock handling stage includes size reduction, washing and drying. The biomass then undergoes pretreatment by chemical/mechanical/biological or a combination thereof to open up the biomass structure. Hydrolysis of the components of lignocellulose to fermentable reducing sugars through chemical or biological methods then occurs (Lee, 1997). The hydrolysed sugars are then fermented to products. The hydrolysis process is catalysed either by acids or enzymes while the fermentation process is carried out by yeasts or bacteria. After the hydrolysis and fermentation stages, purification of the fermented products occurs by distillation, dehydration and other separation processes. The residual compounds can be recovered and used as solid fuel or undergo conversion to value added co-products (Yang and Wyman, 2008; Zheng et al., 2009).

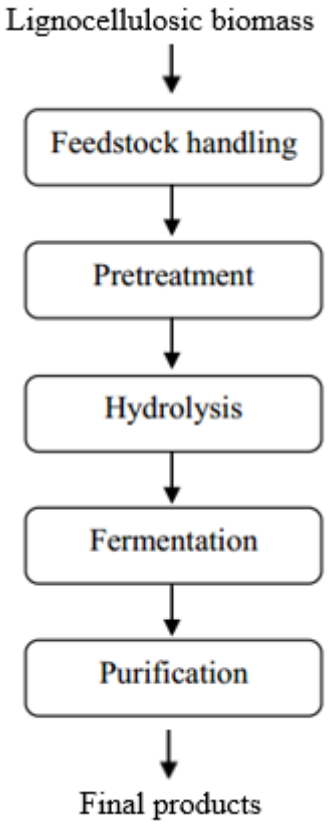


Figure 2.1 Schematic representation of lignocellulosic biomass conversion process.

2.3 Bio-based Chemicals

The petrochemical industry is based on a small number of base chemicals which are derived from crude oil components. By comparison, the bio-based industry is also based on a small number of building blocks known as platform molecules which are derived from biomass components. These molecules have a variety of functional groups which can be transformed into a number of commercially relevant value-added chemicals. These platform chemicals when compared to the oil derived chemicals may either be the same or different, but possessing similar functions (Werpy et al., 2004)

In the sight of environmental concerns, rising energy costs, depletion of natural resources and greenhouse gas emissions, there is a need for the production of platform chemicals from renewable resources. The term ‘platform chemical’ is used to describe chemical that is versatile starting material for making specialty chemicals. The US Department of Energy (DOE) reported a list of twelve such sugar derivable platform chemicals, which can be transformed into new groups of useful chemicals and products (Werpy et al., 2004). The chemical structures of these twelve chemicals are shown in Figure 2.2. These sugar derived

platform chemicals include 1,4-diacids (succinic acid, fumaric acid, malic acid), 2,5-furan dicarboxylic acid (2,5-FDCA), 3-hydroxy-propionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, levulinic acid, 3- hydroxybutyrolactone, glycerol, sorbitol and xylitol/arabitol. Following the report, 5-hydroxymethyl furfural, ethanol, acetone as well as lactic acid have also been considered as platform chemicals. However, only the production of bio-ethanol as well as the fermentation of lactic acid from biomass feedstock is well established in the market and commercially available (Cherubini and Strømman, 2011; Altaf et al., 2007). Therefore, more research is required on the processing and production of bio-based chemicals from biomass due to the fact that biomass, in particular lignocellulosic biomass, has been promoted as being a major source of renewable resources for the future production of bio-based chemicals.

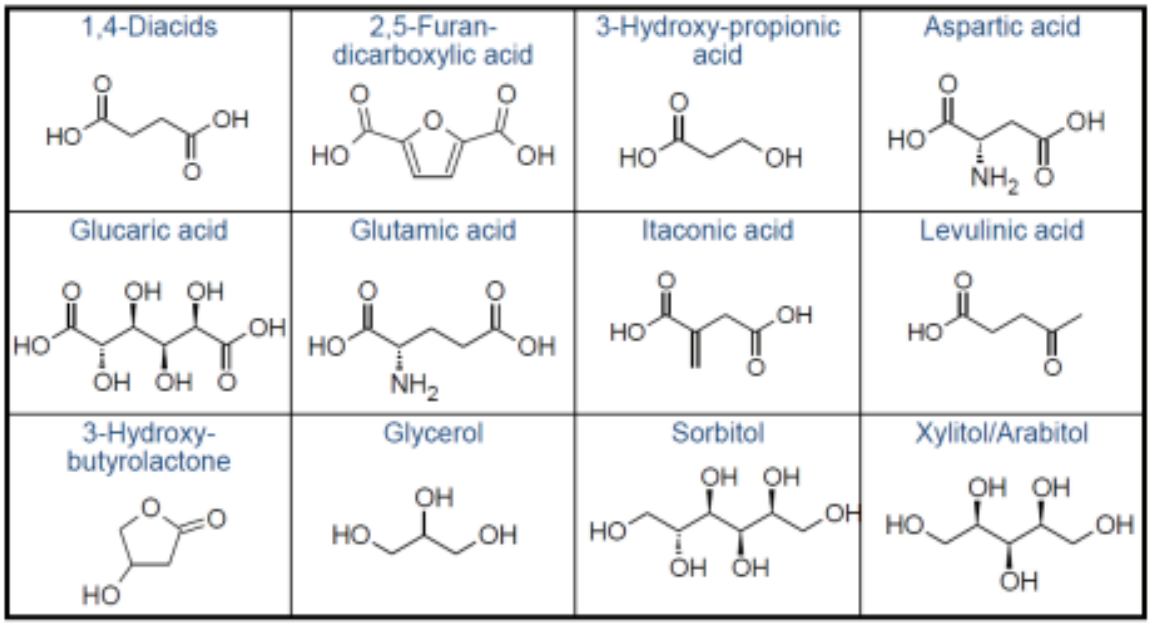


Figure 2.2 Top 12 potential bio based chemicals that can be produced from biomass (Werpy et al., 2004)

2.4 The challenge of waste

Waste is a major problem globally and is becoming increasingly significant in developing countries. According to the World Bank, world cities generate approximately 1.3 billion tons of solid waste annually and the waste generation is expected to increase to 2.2 billion tons by 2025 (Hoornweg and Perinaz, 2012). The cost of managing this solid waste is also estimated to increase from the current $200 billion annually to about $375 billion annually in 2025. Low-income countries are expected to have the most severe cost increases (more than five-

fold increases) and lower–middle income countries (more than four-fold increases) (Hoornweg and Perinaz, 2012). It is imperative to put programmes in place to reduce, reuse, recycle and even beneficiate as much waste as possible before burning it (and recovering the energy) or otherwise disposing of it. Due to the increasing costs of traditional fossil fuels along with insecurity in its supply, there has been a realisation that the traditional linear economy model which is extract-process-consume-dispose is unsustainable. There must rather be a shift towards a circular economy whereby there will be continued use of resources when they no longer serve any purpose in their current form. This is known as *waste beneficiation*.

To fully utilise these biomass waste resources, new technologies need to be developed that will make use of biomass for materials production in addition to current biofuels. Development of efficient techniques for the fractionation of lignocellulosic biomass into its core components (cellulose, hemicellulose and lignin) will facilitate research on the production of specific biomass derived sugars as well as platform chemicals and eventually, value-added commodity chemicals. This will still preserve the concept of the biorefinery by promoting effective utilisation of all fractions from the feedstock.

In South Africa, residues generated across the entire value-chain of agriculture and forestry product processing accounts for over 6% of the waste generated in the country. Experts value the globally-recognized, secondary resource potentials of these waste to be upwards of ZAR15 billion annually (Baloyi et al., 2015). Agro-wastes such as corn cobs, wheat husks are very promising resources for beneficiation as they are generated extensively in South Africa

2.5 Corn cob

Corn cob is also one of the most abundant raw materials for biocompatible products along with bagasse, rice straw, wheat straw and other lignocellulosic substrates. Corn cob is the residue left from removing the maize grains and is what remains of the ear on which the kernels grow. Cobs make up about 20 wt. % of the yield of the corn residue. Corn cob residues are sufficiently available in South Africa and are produced by the maize industry in large quantities of approximately 14.5 million tons annually (National Agricultural Marketing Council, 2012).

The use of corn cob would eliminate the dependence on a food source, corn, as a feedstock for biofuel as corn cob is the identical alternative to corn grain. Carvalho et al. (2002) investigated the use of corn cob to produce ethanol. They recorded 84% and 94% percentage

yields of ethanol respectively. Many other reports on bio-products (ethanol, lactic acid) from the enzymatic hydrolysis of corn cob such as ethanol, lactic acid are well documented (Carvalho Lima et al., 2002; Miura et al., 2004). This makes corn cob an attractive option for biofuel and biochemical production.

Corn cob contains approximately 70% carbohydrate and 20% lignin. There needs to be substantial energy content in the lignocellulosic materials in order to consider its use as an energy feedstock (YaNing et al., 2012). Energy content is measured in energy per unit volume or weight. In an energy production process, these measurements are necessary when considering the volume of feedstock that needs to be utilised, harvested, stored and transported. The more energy dense the material, the less volume of feedstock needed for the production of energy (Zych, 2008). A comparison of the energy content of feedstock, on a dry basis, is given in Table 2.1. The energy content of corn cob is less than that of fossil fuels and coal, however, it is similar and in some cases, higher compared to that of other biomass feedstock.

Table 2.1 Energy content of biomass feedstock versus fossil fuels (Zych, 2008).

	Corn cob	Corn stover	Switchgrass	Wood pellets	Bituminous coal	Fuel oil
Energy content (MJ/kg)	18.25-19.18	17	18	19	25.5	43.5

The energy content of corn cob is about 18.5 MJ/kg which is slightly higher than that of corn stover (17 MJ/kg) which is the residue of a corn plant after grain harvest, including the leaves, stalks, husks and cobs and switchgrass (18 MJ/kg). The energy density of corn cob in addition is 5,000 MJ/m^3, which is significantly higher than that of corn stover (850 MJ/m^3) (Preto, 2007) and switchgrass (2,160 MJ/m^3) (Lam et al., 2008), a significant advantage over these feedstock in the reduction of transportation cost in biofuel and biochemical production.

2.6 Lignocellulosic biomass

Lignocellulosic biomass materials are agricultural residues, such as corn cobs and corn stover as well as quickly grown grasses or plants, such as switchgrass. These kinds of biomass are abundant, do not interfere with food crops and require less energy input (Lee, 1997).

Lignocellulosic biomass is composed primarily of cellulose, hemicellulose and lignin. The lignocellulosic biomass cell wall is a combination of crystalline cellulose fibrils joined together by amorphous hemicellulose which is cross-linked with lignin (Figure 2.3) (Wyman, 1994). The amount of these components in the biomass depends on the maturity, tissues and species of the plant cell wall as well as environmental conditions (Barakat and Rouau, 2014). These structures in the biomass and their configuration in the cell wall affects the enzymatic hydrolysis of the carbohydrates to sugars. It is therefore critical to understand each component when utilising lignocellulosic biomass.

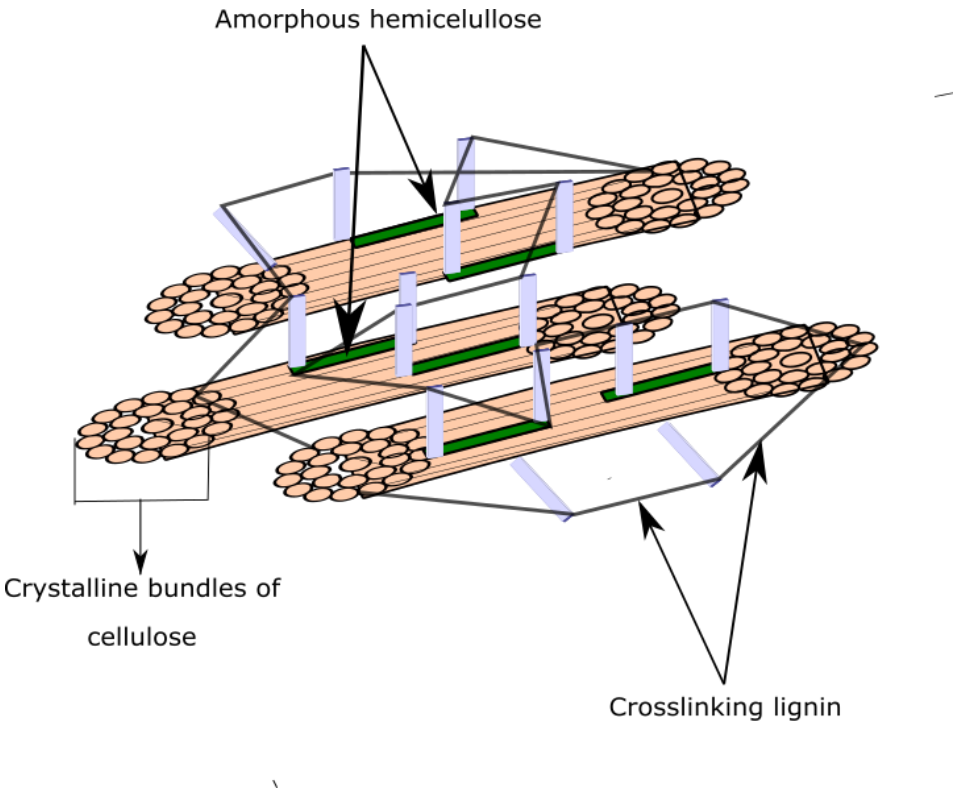


Figure 2.3 The general structure of lignocellulosic biomass (Adapted from Murphy and McCarthy, 2005)

Cellulose is the major component of lignocellulosic biomass. It is a chemically stable and extremely insoluble molecule that interacts with the rest of the cell wall matrix mainly through hydrogen bonding with hemicellulose (Sjöström, 1993). Cellulose is a polymer formed by β-D-glucose monomers in a linear chain joined by β-1,4 glycosidic linkages (Figure 2.4). The chains are linked together by strong intra and inter molecular hydrogen bonds. The bundles of cellulose molecules form of micro fibrils, where highly ordered crystalline domains and less ordered amorphous regions alternate (Sjöström, 1993). The cellulose molecules form extremely ordered crystalline regions through parallel orientation,

but are also well-connected to the amorphous regions of more disordered structure (Lynd et al., 2002). The cellulose structure is connected laterally by intermolecular hydrogen bonds. The bonds are responsible for the high tensile strength of cellulose, insoluble of cellulose in most solvents and the resistance to enzymatic degradation (Moore and Jung, 2001). Cellulose conversion into fuels and chemicals is of great importance as approximately half of the organic carbon found in the environment is found in the form of cellulose.

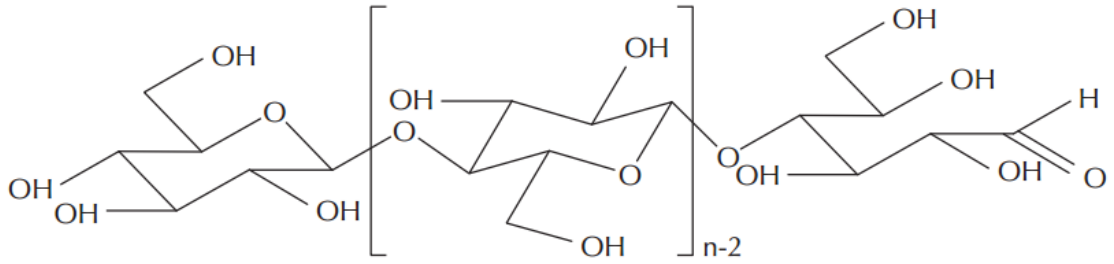


Figure 2.4 Chemical structure of cellulose chain (Adapted from Harmsen et al., 2010; Chang, 2007)

Hemicellulose is a heteropolymer consisting of pentose sugar monomers (D-xylose and L-arabinose) as well as hexose sugars (D-mannose, D-glucose and D-galactose) where xylose is the most abundant. Hemicellulose also contains small amounts of organic acids such as uronic acid which occurs in the form of glucuronic acid (and its 4-*O*-methyl ether) and galacturonic acid (Saha and Bothast, 1997). Xylan is the main heteropolymer of hemicellulose and is made up of a xylose residue backbone which are joined by β-1, 4-glycosidic linkages (Figure 2.5). In addition, xylan is covalently bonded to lignin and inter- and intra-molecular hydrogen bonded to cellulose which aids in protecting the cellulose fibres and further increases the resistance of lignocellulose against degradation. Hemicellulose is hydrophillic and amorphous; and is easily hydrolysed by enzymatic and chemical methods (Moore and Hatfield, 1994)

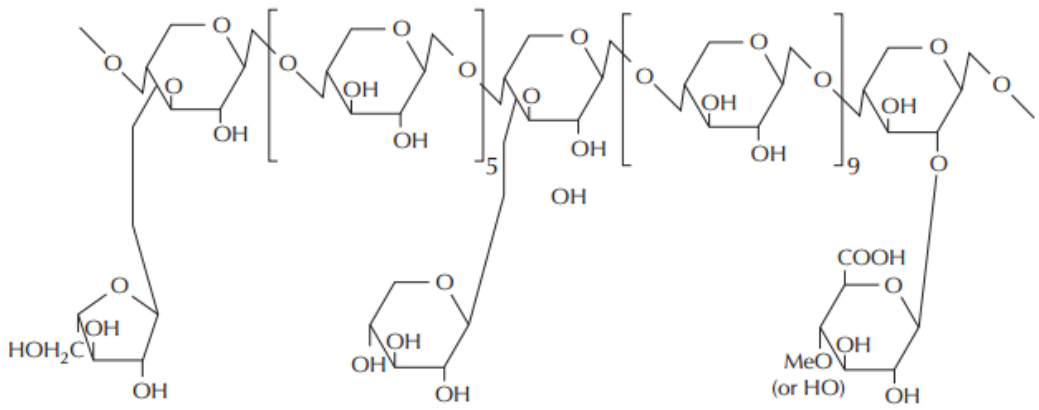


Figure 2.5 Chemical structure of hemicellulose chain (Adapted from Harmsen et al., 2010)

Lignin is a heavily cross-linked amorphous aromatic hetero-polymer which consists of phenyl propane monomers p-coumaryl alcohol, sinapyl alcohol and coniferyl alcohol linked by C−C bonds and C−O−C (Figure 2.6). Higher amounts of lignin in the lignocellulosic biomass are directly proportional to the resistance of the biomass to degradation by chemical and enzymatic methods. Lignin limits hydrolysis by cellulose surface shielding and enzyme inactivation (Taherzadeh and Karimi, 2008). Lignin is insoluble in water and most often resistant in acidic conditions however, the removal of lignin from the heteromatrix of the cell wall can be achieved by sulphite pulping, carbon dioxide explosion treatment, alkaline treatment or pulping, acid treatment, biological pretreatment (enzymes), organosolv treatment, ammonia fibre explosion, ionic liquid treatment, oxidative delignification and hydrothermal aquasolvent (Ramos, 2003; Whetten et al., 1998). Lignin acts in the form of a glue, filling up gaps around and between cellulose. It causes the impermeability of the cell wall and aids in protecting the plants against fungal and microbial attacks (Ramos, 2003; Whetten et al., 1998). Lignin associates with carbohydrates, especially hemicellulose by covalent bonds at the α and C-4 carbon sites in the benzene ring. This interaction is known as the lignin carbohydrate complex (LCC).

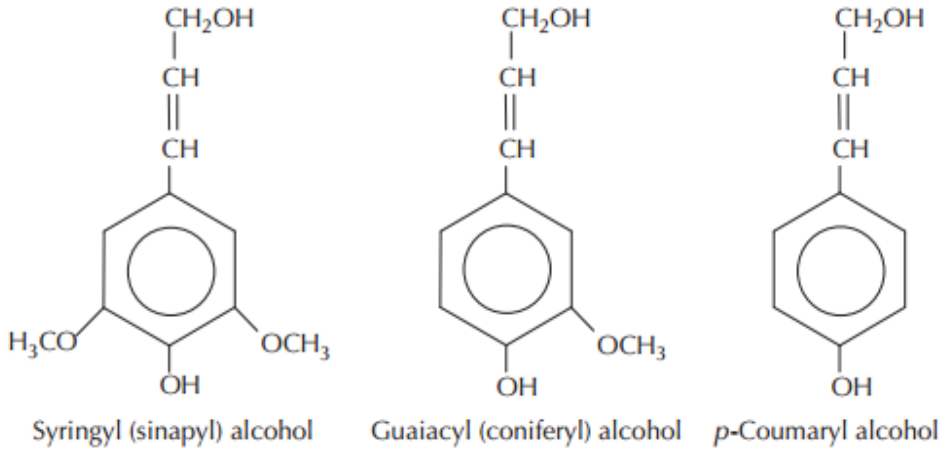


Figure 2.6: The three primary lignin monomers (Adapted from Chang, 2007)

Although the three components mentioned above are the main constituents of lignocellulosic biomass, a small fraction of other components known as extractives are present. These components include waxes, chlorophyll, resin acids, fats, phenolic substances, terpenoids, and gums (Sluiter et al., 2008; Sjöström, 1993). Other extractable inorganic materials regarded as ash content are also present in biomass. These consist of major elements Si, Na, K, Mg and Ca and minor elements Al, Fe, Mn, P and S (Vassilev et al., 2010). The ash is removed by washing or extracting.

2.7 Lignocellulosic Biorefinery

A biorefinery is the integration of biomass conversion processes for the production of a range of chemicals, materials and fuels from biomass. Biorefineries are promising alternatives to petroleum refineries in the future as they utilise broad ranges of feedstock (which are abundant and can include waste) and processes. Lignocellulosic feedstock consists of three major chemical constituents: cellulose, hemicellulose and lignin. Figure 2.7 shows an overview of the potential products from each major component of the lignocellulosic feedstock biorefinery (Mabee et al., 2005). Fractionation, or separation, of petroleum into its component constituents has been the key methodology to develop high value petrochemical end products. In the same way, in order to increase the efficiency of the biorefinery, the utilization of all the main components of the biomass needs to be improved and this is done primarily by fractionation (Pu et al., 2008).

Cellulose is mainly hydrolysed to glucose monomers which are then fermented to products such as acetone, succinic acid, ethanol and lactic acid. Both cellulose and hemicellulose can be hydrolysed to hydroxymethylfurfural (HMF) and furfural by further conversion processes

(Werpy et al., 2004). HMF is used in softeners, lubricants and a variety of other chemicals. Additionally, hemicellulose can be hydrolysed to fuels, chemicals and other applications in the food industry (Isikgor and Becer, 2015). Hemicellulose can also be hydrolysed to glucose as well as the other five membered sugars (xylose, arabinose) and six membered sugars (mannose, galactose, rhamnose). Xylose, the main component of hemicellulose can be converted to fuels such as hydrogen and ethanol, chemicals such as furfurals (Waldron, 2010), mentioned previously as well as succinic acid (Akhtar et al., 2014). In addition, Xylose can be converted into xylitol (Sánchez et al., 1998). Xylitol is a polyalcohol and has high value in pharmaceutical, dental care and food industries due to its unique properties. This includes the fact that its sweetness is metabolically independent of insulin and anti-carcinogenic properties (Sánchez et al., 1998). Arabinose, a minor pentose sugar in hemicellulose can be converted to chemicals such as astaxanthin (Yang et al., 2011) and furfural (Yoo et al., 2016). Although Lignin cannot be hydrolysed to sugars, its conversion is also quite important. Lignin has a unique structure and chemical properties which allows for the production of a wide variety of chemicals in particular, aromatic compounds chemicals (vanillin, cresols, catechol, resorcinol, guaiacol). Lignin is thus considered as the major aromatic resource of the bio-based industry. Furthermore some current uses of lignin include solid fuels for combustion, oil well drilling fluids as well as cement and concrete, concrete admixture in asphalt, animal feed binders, phenolic resins, fertilisers and other agrochemicals, adhesives, biomaterials (bio plastics, foams, polymers, etc.) (Isikgor and Becer, 2015).

In order to optimise the potential use of the pentose sugars for fermentation to produce commercially valuable products, it is important to investigate sugar recovery. However, the recalcitrance of biomass hinders the hydrolysis of the biomass components to sugars. Enzyme and microbial accessibility to the sugars is limited due to structural features of the biomass. Lignocellulosic biorefinery involves a fractionation or pretreatment step. These pretreatment methods need to be optimised for different feedstocks, solvents, chemicals and energy efficiencies (Alvira et al., 2010; Dermibas, 2004; FitzPatrick et al., 2010). More research is required into the pretreatment and fractionation technologies to ensure the production of high yield and purity of lignocellulosic components in the biorefinery.

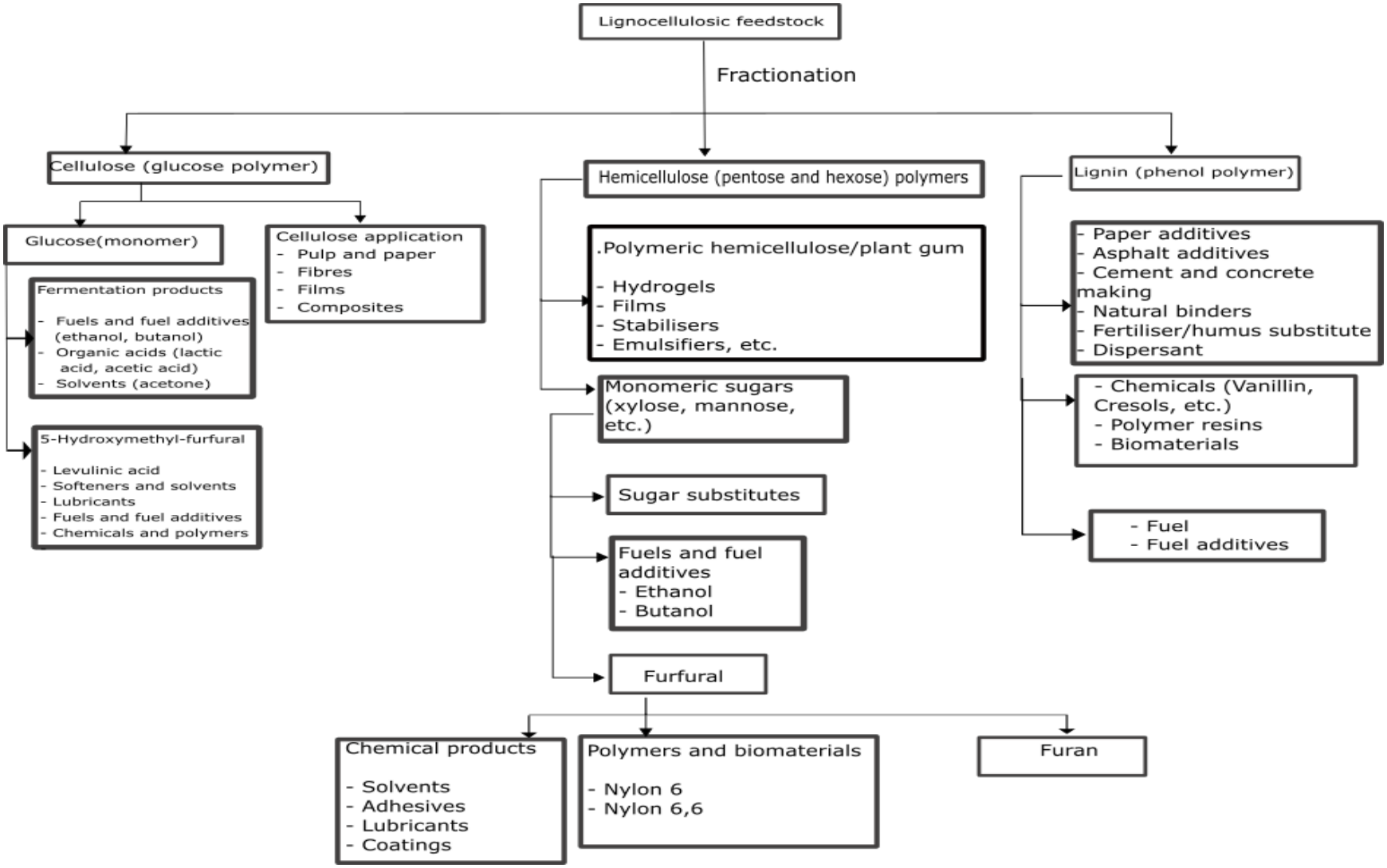


Figure 2.7 Lignocellulose feedstock biorefinery (Adapted from Kamm and Kamm, 2004; Stark, 2010)

2.8 Pretreatment and fractionation

There are many aspects which affect the hydrolysis of cellulose and hemicellulose such as cellulose degree of polymerization (DP), crystallinity, substrate accessibility, porosity, lignin content and particle size. Amongst these factors, the most limiting determined was substrate accessibility (Waldron, 2010). One of the major barriers for the optimal use of lignocellulosic biomass is the inability to separate (fractionate) and recover the main fractions efficiently and in usable forms. Pretreatment is the most crucial step employed in the conversion of lignocellulosic biomass to fuel as it determines the efficiency of the downstream processes (Lee, 1997). The main purpose of the pretreatment step is to break down and fractionate the matrix of hemicellulose and lignin in order to expose the cellulose and hemicellulose to enzymatic degradation thereby increasing substrate accessibility as illustrated in Figure 2.8 (Wildschut et al., 2013). The search for an effective and economically feasible lignocellulose pretreatment technique is one of the key areas of research in biorefinery. The development of effective pretreatment techniques and discovering the optimum process conditions of these techniques can be used to overcome the cost barrier associated with the use of biomass. Pretreatment is able to influence downstream costs as it determines fermentation toxicity, enzymatic hydrolysis rate, as well as enzyme loading (FitzPatrick et al., 2010).

An ideal pretreatment will retain nearly all the cellulose present in the original material so that high theoretical yields are obtained from enzymatic hydrolysis or fermentation (Mosier et al., 2005). Other desirable pretreatment feature include: (1) Generating high holocellulose (hemicellulose and cellulose) yields. Holocellulose content in biomass is high therefore the potential yield from this fraction significantly affects process efficiency and economy; (2) enhance sugar formation as well as successive sugar formation by enzymatic hydrolysis; (3) avoid the formation of by-products which inhibit the downstream hydrolysis and fermentation processes; and (4) be cost-effective; (5) avoid destruction of hemicellulose and cellulose fractions of biomass; (6) minimizes waste generation; (7) enable lignin recovery for conversion to value added co-products in a simplified downstream process; (8) utilise cost effective chemicals. Pretreatment methods for lignocellulosic biomass generally fall into 3 basic categories namely; physical, chemical and biological (Kumar et al., 2009). Combinations of these have also been employed. Every pretreatment process has a unique effect on the cellulose, hemicellulose and lignin components of the biomass as described in Table 2.2. However, to enhance the overall utilisation of lignocellulosic biomass, the

recovery yield and the purity of each component after upstream processing needs to be maximised.

Fractionation is a Pretreatment characteristic where the main components of the biomass (cellulose, hemicellulose, lignin) are separated in order for each component to be easily processed (Huang et al., 2008). This biomass fractionation concept is a crucial step in the development and optimisation of biorefining technologies. Fractionation is an important property of pretreatment as it increases the efficiency of the biorefining process as well as increasing the potential value added products which result. However, the development and application of technologies for fractionation of lignocellulosic biomass are still not economically or technically feasible. Therefore, more research is needed in this area (Huang et al., 2008).

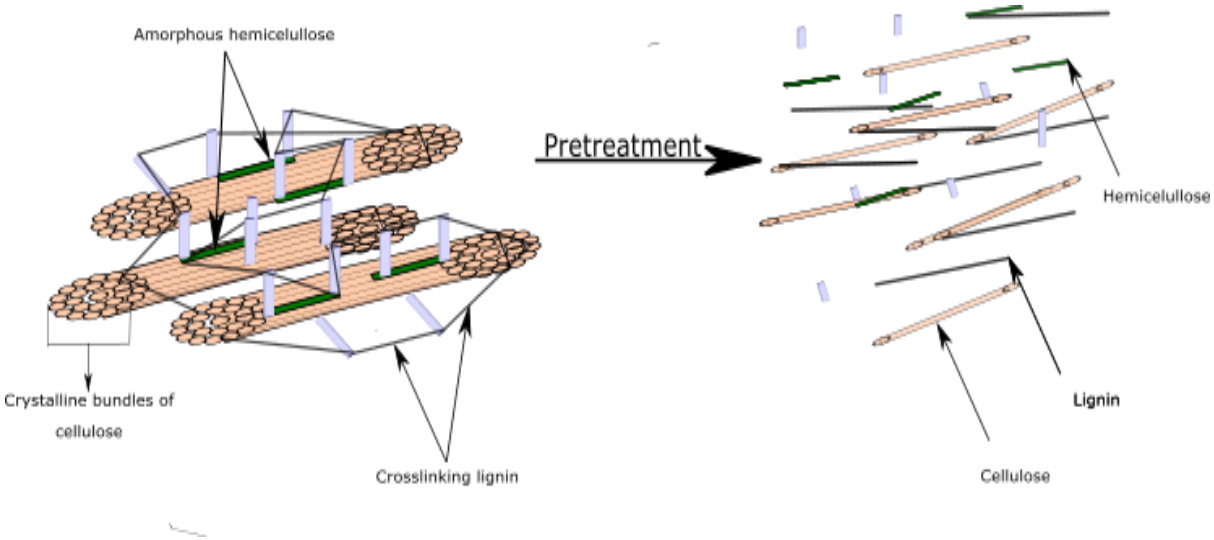


Figure 2.8 Schematic representation of the effect of pretreatment on lignocellulosic biomass in which the pretreatment separated hemicellulose and lignin from cellulose enabling effective enzymatic digestibility (Adapted from Mosier et al., 2005)

2.8.1 Physical Pretreatment

Physical pretreatment is one of the most basic forms of lignocellulosic pretreatment. It involves the mechanical reduction of the particle size in order to increase the surface area of the particle and reduce both the cellulose crystallinity and degree of polymerization (DP). To date, a variety of physical pretreatment processes have been developed. These include grinding, milling, irradiation (microwaves, electron beam, gamma rays and ultrasounds) extrusion and (Sun and Cheng, 2002). Mechanical pretreatment is often done prior to further processing steps in order to improve process efficiency. Factors such as capital costs,

operating costs, scale-up possibilities and depreciation of equipment are very important. Mechanical pretreatment are high energy processes. The energy demand is dependent on biomass type and particle size. The pretreatment becomes economically unfeasible above a certain particle size. (Kumar et al., 2009). An additional major drawback of physical pretreatment is that the chemical composition remains the same, thus the shielding effect from lignin and hemicellulose is still present.

2.8.2 Biological Pretreatment

In this type of pretreatment, microorganisms such as brown, white and soft rot-fungi are used as they are able to degrade hemicellulose and lignin by synthesizing potent cellulolytic enzymes during hydrolysis. White rot-fungi are efficient organisms for pretreatment as it produces enzymes which degrade lignin such as lignin peroxidase and manganese peroxidase (Bak et al., 2009). Biological pretreatment are advantageous as they require low energy and mild operation conditions. Therefore biological pretreatment of lignocellulosic biomass is considered as an eco-friendly, cheap and efficient alternative. However, several issues such as the carbohydrate loss to effectively sustain microbial growth, process control as well as reactor scaling are major problems (Malherbe and Cloete, 2002). According to Bak et al. (2009), 64.9% glucose was obtained after the pretreatment of rice straws using *Phanerochaete chryosporiom* for 15 days and using *Cyathus stercoreus*. Keller et al. (2003) obtained 35.7% digestibility after 29 days. This further illustrates the lengthy pretreatment times associated with biological pretreatment. These drawbacks can be avoided by the development of more aggressive microbial strains leading to the future development of low scale plants.

2.8.3 Physicochemical Pretreatment

The most commonly used physicochemical method for the pretreatment of lignocellulosic materials is steam explosion. It utilises both physical and chemical methods to break apart the lignocellulosic material. this method involves the treatment of biomass using high temperatures and pressure for a short period (Kumar et al., 2009). Explosive decompression of the biomass material is then achieved by dropping the pressure suddenly. The remarkable autohydrolysis and explosive significantly increase bioconversion, the digestibility of substrate as well as its reactivity toward other catalytic reactions. The sudden decompression that follows reduces the temperature, which quenches the process. The major drawback of steam explosion pretreatment is that toxic derivatives are generated which inhibits the

downstream hydrolysis and fermentation stages (Zheng et al., 2009).These inhibitors may include furan derivatives, such as 5–hydroxymethyl–2-furaldehyde, furaldehyde and phenolic compounds (derived from lignin depolymerization) (Isikgor and Becer, 2015).

Another well-known physicochemical pretreatment process is Ammonia fiber explosion. This process is similar to steam explosion. In this pretreatment, biomass undergoes exposure to liquid ammonia (concentrated (>70%) or anhydrous), under high pressure and temperatures. The pressure is then released quickly, this vapourizes the ammonia which can then be recovered and recycled (Bals et al., 2010). The combination of ammonia and the depressurization solubilise hemicellulose, remove lignin, de-crystalize cellulose and expand the cell wall of the treated biomass (Mosier et al., 2005). Residence time and ammonia loading have the highest effect on the economics of the process. A severe limit of this process to large scale applications is the cost of the recovery and recycling of the ammonia after the pretreatment. In addition, the energy demand of this process affects the total variable operating costs of the biorefinery (FitzPatrick et al., 2010).

Carbon dioxide explosion is a physicochemical pretreatment which involves the use of CO_2 as asupercritical fluid (SC–CO_2) (Zheng et al., 2009). This process differs from other treatments in this category as it occurs under lower temperatures compared to steam explosion and AFEX and has reduced costs compared to AFEX. In aqueous solutions, carbonic acid is formed from CO_2 which favours the hydrolysis of biomass (Sun and Cheng, 2002). CO_2 molecules are in the same size range with water and ammonia molecules which means they can penetrate the small pores of lignocellulosic biomass in the same way as water and ammonia. This mechanism is a high pressure system (Sun & Cheng, 2002). After the explosive release of CO_2 pressure, cellulose and hemicellulose components are disrupted and this increases the amount of accessible surface area for enzymatic degradation. Disruption of cellulose and hemicellulose structure occurs and the accessible surface area for enzymatic attack subsequently increases. The major disadvantage of this pretreatment is the high capital cost of high–pressure equipment which may inhibit the commercial feasibility of this lignocellulosic pretreatment (Bridgwater, 1996).

In summary, although both the biological and physical processes possess some essential advantages, they are overall not economically efficient compared to chemical methods.

Relatively, the chemical pretreatment methods are the more efficient processes in the disruption of recalcitrant structures of lignocellulosic biomass.

2.8.4 Chemical Pretreatment

Chemical pretreatment is the pretreatment of lignocellulosic biomass with chemical substances. Some chemicals reagents include acids, alkali, organic solvents, cellulose solvents and ionic liquids. Among them, the most commonly used pretreatment methods are alkali and acid pretreatment (Bensah and Mensah, 2013).

Alkali pretreatments utilise alkaline solvents, such as calcium hydroxide, ammonia and sodium hydroxide for the pretreatment of biomass for lignin removal and dissolution of hemicellulose to efficiently increase cellulose accessibility. When looking at the chemical reactions, the alkali pretreatment causes the ester and glycosidic side chains to degrade which results in swelling and partial decrystallisation of cellulose, deconstruction of lignin and partial hemicellulose solvation (Kim et al., 2016). Lime pretreatment of biomass is characterised as an alkali pretreatment and occurs under mild conditions. However, in order to remove lignin, longer reaction times are used (Xu et al., 2010). In addition, oxidative conditions can be achieved using air to enhance the removal of lignin. Under these oxidative conditions, lignin removal can reach up to 88% compared to non-oxidative conditions which reaches a 50% lignin removal (Kim et al., 2009). Xylose yield can vary between 70% and 80% (Hu et al., 2008). Alkali pretreatment with NaOH is optimal at high temperatures and long reaction time. Delignification ranges between 33% and 72% depending on reaction conditions while xylose yield ranges between 90% and 99% (Vancov and McIntosh, 2011) xylan remain in solids. Up to 86% lignin removal was observed 121 °C for 15 min and 0.75% NaOH (Wang et al., 2010). Soaking in aqueous ammonia (SAA) at mild temperatures results in approximately 50% lignin removal, however all the cellulose and xylan remain in the solid form. The solid form in combination with the low lignin removal causes low glucose digestibility by enzymes as the presence of lignin as well as the solid xylans blocks access of the hydrolytic enzymes to the substrate (Kang et al., 2012).

Organosolv pretreatment method uses organic solvents or aqueous organic solvents, in the presence or absence of catalysts, at high temperatures (as high as 210 °C) and pressure (as high as 30 bar) for the treatment of lignocellulose (Harmsen et al., 2010; Wildschut et al., 2013). Poplar wood chips pretreated with steam and followed by organosolv treatment was done to separate hemicellulose, lignin and cellulose components. Lignin extraction was found

to increase to 66%, while cellulose recovery was 98% and 88% of cellulose was hydrolysed to glucose after 72 h (Sathitsuksanoh et al., 2012).

Dilute acid, especially sulfuric acid, is a chemical pretreatment process which is effective in hydrolysing hemicellulose in the lignocellulosic biomass. Dilute sulfuric acid pretreatment is a widely studied pretreatment technique for a wide variety of lignocellulosic biomass (Yang and Wyman, 2008; Hu and Ragauskas, 2012). This pretreatment resulted in high hemicellulosic sugar recovery in the pretreatment liquid and in cellulose recovery in the solid fraction with enhanced enzymatic convertibility. The temperature of the reaction ranges from 140-200°C, while the acid concentration is usually lower than 4% (w/w) and the reaction time is between 4 minutes up to one hour (Galbe and Zacchi, 2007). The digestibility of the lignocellulosic material increases after dilute acid pretreatment with up to 90% recovery of hemicellulose in the liquid fraction (Schell et al., 2003). Major disadvantages of dilute acid pretreatment include equipment corrosion problems and the generation of fermentation inhibitors (Jönsson and Martín, 2016).

Attempts have previously been made to dissolve single components of the biomass in order to fractionate lignocellulose. These efforts have led to the discovery of various cellulose solvents which can separate cellulose preferentially from the biomass.

Table 2.2 The comparison of chemical pre-treatment methods.

Pre-treatment	Operating conditions	Effects	Advantages	Disadvantages	References
Dilute acid	Temp: ~140–2000°C Pressure: Not reported Time: 4– 60 min Chemicals: H_2SO [~0.5–4.0%]	• Hemicellulose removal • Lignin transformation and removal	• Lignin removal is achieved • Widely studied for many years. • Most species perform well.	• Corrosion: requires expensive materials. • Inhibitors (furfural and aldehydes).	(Ishizawa et al., 2009)
Wet oxidation	Temp: 180-200°C Time: 5-15 min Chemicals: H_2O_2 (1-5%)	• Lignin removal • Hemicellulose dissolution • Cellulose decrystallisation	• Low fermentation inhibitors • Good removal of lignin	• High equipment costs • Not yet proven at pilot scale	(Harmsen et al., 2010)
AFEX	Temp: ~60–100°C Pressure: 250–300psig Time: ~5 min Chemicals: Ammonia	• Lignin removal • Minimum hemicellulose dissolution Cellulose decrystallisation	• Most ammonia can be recovered. Considerable lignin removal • Minimum degradation products • Moderate temperature • Short residence time	• Costly solvent (ammonia) • High environmental impact	(Bradshaw et al., 2007)
Steam explosion	Temp: 180-210°C Time: 1-10 min Chemicals: acetic acid	• Hemicellulose removal • Lignin transformation	• Low residence time • Limited use of chemicals • Low environmental impact	• Use of high temperature and pressures • Low lignin removal	(Ruiz et al., 2008)
Lime	Temp: ~50–160°C Pressure: 1 atm (air) or 300psi (oxygen) Time: ~2 h to 8 weeks Chemicals: lime	• Removal of lignin	• Preserve sugars. • Limit the formation of degradation products. • Minimize energy demand. • Low capital costs (long-term).	• Longer time • Irrecoverable salts • Biomass size sensitive	(Kim and Holtzapple, 2005)
Organosolv	Temp: 100-250°C Time: 30-60 min Chemicals: Alcohol (40-60%)/ organic acids (HCOOH, peracids)	• Considerable lignin removal • Hemicellulose removal	• Sulphur-free high purity and quality lignin • Solvent recyclable	• Some toxicity • Extra washing process • Solvent cost	(Harmsen et al., 2010)
Ionic liquid	Chemicals: Ionic liquids	• Dissolution of biomass	• Solvents are thermally stable, highly polar and have negligible vapour pressure	• Solvent cost • Need for solvent recovery and reuse	(Qiu et al., 2012)

2.8.5 Cellulose solvents

Certain compounds that are capable of dissolving cellulose are used in the pretreatment of lignocellulosic biomass. Solvents that dissolve cellulose in tall fescue, bagasse, orchard grass and cornstalks have previously resulted in cellulose to glucose conversion of 90% (Ladisch et al., 1978) and showed enhancement of enzyme hydrolysis as a result of biomass disruption before hydrolysis. The past decade has seen the development of a variety of solvents for cellulose swelling and dissolution with various physical and chemical properties. The amorphous cellulose which results has a highly increased surface area and is thus more accessible than before pretreatment, which affects the hydrolysis rate.

These solvents can be classified as derivatizing or non-derivatizing, single or multi component mixtures, as well as aqueous and organic shown in Figure 2.9. Derivatizing solvent groups dissolve cellulose through covalent modification giving an unstable ether, ester or acetyl intermediate. Formic acid, protic acids, trifluoroacetic acid, dichloroacetic acid and paraformaldehyde/DMSO have all been investigated as derivatizing solvents (Fischer et al., 2002). Unfortunately, derivatizing solvents can undergo side reactions resulting in unknown products, which reduces reproducibility and may obstruct downstream processing. Among the protic acids, phosphoric acid (85%) is regarded as the most efficient dissolving cellulose. Dissolution occurs by an esterification reaction to form cellulose phosphate (Fischer et al., 2002). However, due to limitations such as high toxicity, high cost and difficulty in solvent recovery or extreme processing conditions, the use of this solvent is not feasible. A cellulose solvent-based lignocellulose (CSLF) was presented by Sathitsuksanoh et al. (2012). The technique was done by using a cellulose solvent, volatile organic solvent and water to fractionate the lignocellulosic biomass at relatively low pressures (50 °C and atmospheric) and temperatures. The process achieved this fractionation by exploiting the differences in solubility of cellulose, lignin and hemicellulose in the cellulose solvent, water and organic solvent, respectively (Sathitsuksanoh et al., 2012).

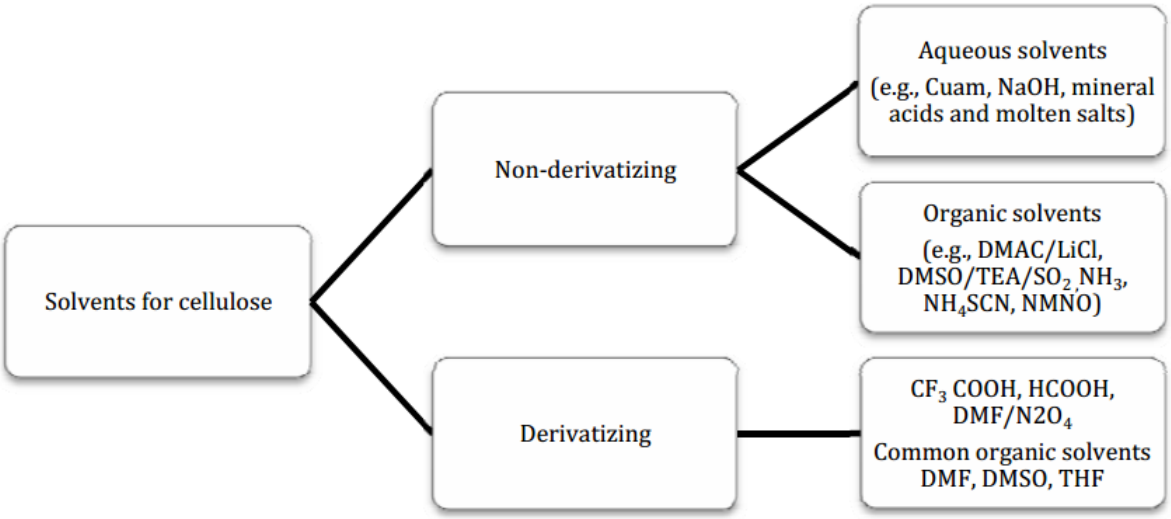


Figure 2.9 Characterisation of cellulose solvents (Adapted from Hagiwara and Ito, 2000)

Non-derivatizing solvents are capable of dissolving solvents through physical intermolecular interactions. Non-derivatizing solvents have the ability to fractionate the biomass into the various constituents as they leave the cellulose in the oligomeric form. These solvent systems include ionic liquids, aqueous alkali solutions, organic solvents in the presence of an inorganic salt, amine oxides, aqueous complex solutions and inorganic molten salt hydrates (MHS). Hydrates of molten inorganic salts are a significant class of cellulose solvents which have received considerable attention over the years. These MHS systems use a water to salt molar ratio which is close to the coordination number of the strongest hydrated cation with the water molecules being tightly bound to the inner coordination sphere of the cation in order to dissolve cellulose (Figure 2.10) (Leipner et al., 2000). Nearly all of the molten hydrate dissolving systems, capable of dissolving cellulose contain Li^+ or Zn^{2+} ions. However, not all hydrated melts with these ions are successful solvents for cellulose. Molten salt hydrates such as $ZnCl_2 \cdot H_2O$ and $LiClO_4 \cdot H_2O$ (Figure 2.10) are able to solubilise cellulose by the interaction between hydroxyl groups and ionic species and breaking the network of hydrogen bonds (Figure 2.11). Furthermore, water is also an important factor in the dissolution (Fischer et al., 2003). This is evident in the ability of $LiCl \cdot 2H_2O$ to swell cellulose better than $LiCl \cdot 5H_2O$. Lu and Shen (2011) observed that $ZnCl_2 \cdot 4H_2O$ acts as a weak swelling agent for bacterial. Awosusi et al. (2017) studied the effect of water crystallisation on the dissolution efficiency of Zinc chloride MHS and found that $ZnCl_2 .4H_2O$ was effective in pre-treating corn cob and extracted 43% glucan and 95% of xylan.

Other important factors influencing the dissolution process include the hydration deficiency of the coordinating sphere as well as the acidity of the solvent system (Leipner et al., 2000).

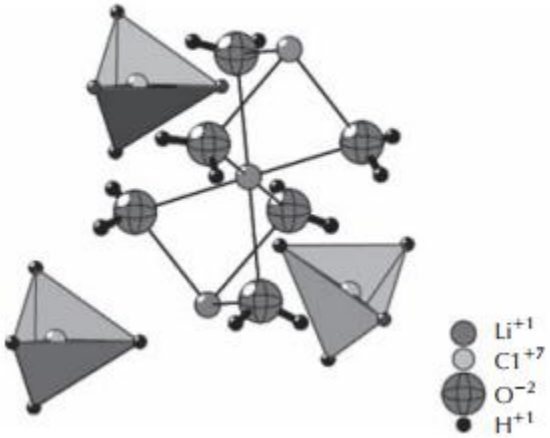


Figure 2.10 Crystal structure of $LiClO_4.3H_2O$ (Adapted from Leipner et al., 2000)

Several features are required in order for a cellulose solvent to be effective. These features include the ability of the solvent to dissolve cellulose at low temperatures, low cost of the solvent, high recyclability, thermo-stable and non-toxic to the subsequent microbial fermentation (Sathitsuksanoh et al., 2012). Solvent extraction does come with its own challenges in terms of toxicity and volatility of the solvents and the use of pressure vessels.

The advantages associated with the use of inorganic MHS over other existing solvent systems are many. Inorganic molten salt hydrates are easily prepared and are also more cost effective compared to other non-derivatizing cellulose solvents. In addition, MHS provides an environmentally friendly alternative to dissolving cellulose. The preparation MHS solvents do not require toxic or volatile organic (although not completely inert) compounds. There is also the added advantage of solvent recycling after pretreatment (Sen et al., 2013). The inorganic molten salt solutions can be recovered by low pressure distillation using anti solvents such as water and ethanol, which can be recycled for further use making it effective on a large scale. For these reasons, molten salt hydrates will be used as solvents for the pretreatment of lignocellulosic biomass in this study over existing solvent systems (Sanghamitra et al., 2013). It is however not enough for a solvent to only decrystallise cellulose, the solvent must also be able to overcome the interactions which keep the chains intertwined. Urea will be tested in the complexes as part of the solvent systems due to the fact

that urea hydrates function as hydrogen bond donors and receptors between the solvent molecules and prevent the re-association of cellulose (Xiong et al., 2014).

Figure 2.11 Dissolution mechanism of molten hydrate salts (Adapted from Fischer et al., 2003).

CHAPTER 3

3. Effect of MHS pretreatment on the chemical composition and structure of corn cob

3.1 Introduction

In view of environmental concerns, in global warming resulting from the over use of fossil fuels, there is a need for platform chemicals, materials and fuels from renewable lignocellulosic resources by biochemical conversion. A promising renewable source is lignocellulosic biomass which is composed primarily of cellulose, hemicellulose and lignin. The key to the efficient utilisation of lignocellulosic biomass is the pretreatment by a simple and mild technique and then the conversion of the resulting fractions to value added platform chemicals, intermediates or materials.

Fractionation of lignocellulosic biomass by pretreatment into cellulose, hemicelluloses and lignin components has been proposed as the first stage of refining biomass to high value-added products. Therefore a major characteristic of the pretreatment is the ability to fractionate the components. In this chapter, seven molten salt hydrate pretreatment systems classified into unary ($ZnCl_2.4H_2O$, $LiClO_4.3H_2O$, Urea), binary ($LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$, $ZnCl_2.4H_2O$/ Urea, $LiClO_4.3H_2O$/Urea) and ternary ($LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$ /Urea) were investigated for their abilities to pre-treat and fractionate biomass. This chapter describes the effect of different molten hydrate salts systems composing of $LiClO_4.3H_2O$, $ZnCl_2.4H_2O$ and Urea. The evaluation of the compositional changes in the major components of corncob namely, hemicellulose, lignin and cellulose; and to characterise multi-level structures of solid residues by a combination of methods, such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) to evaluate the efficiency of the different pretreatment with respect to cellulose, hemicellulose and lignin recovery and identifying the most efficient system. The results of the screening will form the basis for the selection of a molten hydrate salt solvent system for pretreatment of biomass with the highest potential for further optimisation of process parameters.

3.2 Materials and methods

3.2.1 Materials

Raw corn cobs of (*Zea mays*) were collected from domestic farming homes in Mukula Village, Limpopo South Africa. The dried samples were stored in capped plastic bottles tightly and were used shortly after collection. The cobs were cut to size using a band-saw (Mössner August, GmbH Co., Germany) and then shredded in a rotor mill (Retsch® SE-200, Germany) fitted with 640 μm - 4 mm s. Deionized water with resistivity of 18 MΩ·cm was produced from Milli-Q water (Millipore). $ZnCl_2.4H_2O$ (98%) was purchased from Rochelle Chemical Reagent Co. Ltd. In addition, analytical-grade H_2SO_4 (98%), $LiClO_4.3H_2O$ (99%), Urea (99%), Acetone (99%) and glucose (99%) were purchased from Sigma Aldrich Chemical Company.

3.2.2 Pretreatment of corn cob

Seven molten salt hydrate pretreatment systems classified into unary ($ZnCl_2.4H_2O$, $LiClO_4.3H_2O$ and Urea), binary ($LiClO_4.3H_2O/ZnCl_2.4H_2O$, $ZnCl_2.4H_2O$/Urea and LiClO4.3H2O/Urea) and ternary ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea) at a ratio of 1:1 and 1:1:1 for the binary and ternary complexes respectively were investigated for the treatment of corncob. The pretreatment was carried out in a shaking incubator at 70 °C for 60 minutes at a constant biomass to solvent ratio of 1:10 (5 g dry biomass will be added to 50 g of each solvent) at agitation speed of 270 rpm. The slurries obtained from each pretreatment method were recovered and separated into solid and liquid fractions by vacuum filtration.

3.2.3 Compositional analysis of the solid and liquid fractions of the corn cob

3.2.3.1 Solid fraction compositional analysis

The compositional analysis of untreated and MHS-treated biomass were analysed for hemicellulose, lignin, extractive and ash content. The analysis is summarised in Figure 3.1

Extractive Determination

To determine the extractive content of the corn cob, approximately 2.5 g of dry biomass was placed in an extraction thimble and into the Soxhlet apparatus. The weight of the dry biomass and the thimble were recorded. Approximately 150 mL of 100% acetone was placed in the receiving flask. The receiving flask was mounted in water bath on a heating block. The extraction was carried out at 70 °C for four hours. Sample was dried after the end of each

extraction cycle at 105 °C in a convection oven. The extractive weight percent was calculated as the difference in weight of the raw and extracted sample at 105 °C (Di Blasi et al., 1999; Li et al., 2004).

Hemicellulose determination

To determine the hemicellulose, one gram of the extracted sample was added to 150 mL of 0.5 mol/L of NaOH. The mixture was then be boiled in a water bath for 3.5 hours and vacuum filtered and washed until neutral pH. The residue was dried at 45 °C to constant weight, cooled in a desiccator and weighed. The difference between the weight before and after the treatment is the hemicellulose fraction. The difference between the sample weight before and after this treatment is the hemicellulose (Li et al., 2004).

Lignin determination

To determine the lignin content, the first hydrolysis step was performed on 0.3 g of the extracted dried biomass using 72% sulfuric acid. The goal of this acid hydrolysis was to hydrolyse the carbohydrates in the substrate with concentrated sulfuric acid and leave the residual solids as acid insoluble lignin. Hydrolysis was made to occur by keeping the slurry at room temperature for two hours with manual swirling every 30 min. At the end of the first hydrolysis step, the slurry was washed into a beaker with 84 mL distilled water in order to dilute the acid to a 4% w/w concentration, bringing the total volume (along with the sulfuric acid) to 86.73 mL and the second hydrolysis step was made to occur in an autoclave at 121 °C for one hour.

After the autoclave cycle was completed, samples were cooled to room temperature before separating the solids from the acid hydrolysed liquor through crucible filtration. A pre-weighed crucible was used to retain the solid fraction and a plastic sampling tube was used to collect the hydrolysed liquor for analysis dissolved lignin. The solids in the crucible were first dried overnight at 105 °C and the weight was recorded.

For the acid soluble lignin analysis, the autoclaved hydrolysis solution was vacuum filtered and the filtered hydrolysed solid sample was dried and weighed. The dried samples were then combusted at 575 °C for at least four hours in a muffle furnace to determine the ash content in the sample. The acid insoluble lignin was calculated by the difference between the weight of the crucible and the weight of the crucible plus the solid dried at 105 °C minus the weight of the ash.

The acid soluble lignin fraction was determined by measuring the absorbance of the acid hydrolysed samples at 320 nm and the absorptivity was 30 l/g cm. this wavelength recommended by NREL was selected as it minimizes interference from carbohydrate degradation products. Distilled water was used as blank and the acid hydrolysed liquor was diluted with distilled water into the absorbance range of 0.7-1.0 before recording the absorbance value.

The amount of acid soluble lignin was calculated by the following equation:

$$Acid\ soluble\ lignin\% = \frac{Abs \times Vf \times Df}{\varepsilon \times ODW \times cell} \times 100 \quad (3.1)$$

Where Abs is the absorbance value at 320 nm, *Vf* is the volume of filtrate liquor, 86.73 mL, *Df* represents dilution factor, ε = absorptivity, 30 L/g cm, *ODW* = Oven dry weight of sample (g), *cell* = cell path length, 1 cm

The cellulose content (% w/w) was calculated by difference, assuming that extractives, hemicellulose, lignin, ash and cellulose are the only components of the entire biomass (Di Blasi et al., 1999; Li et al., 2004).

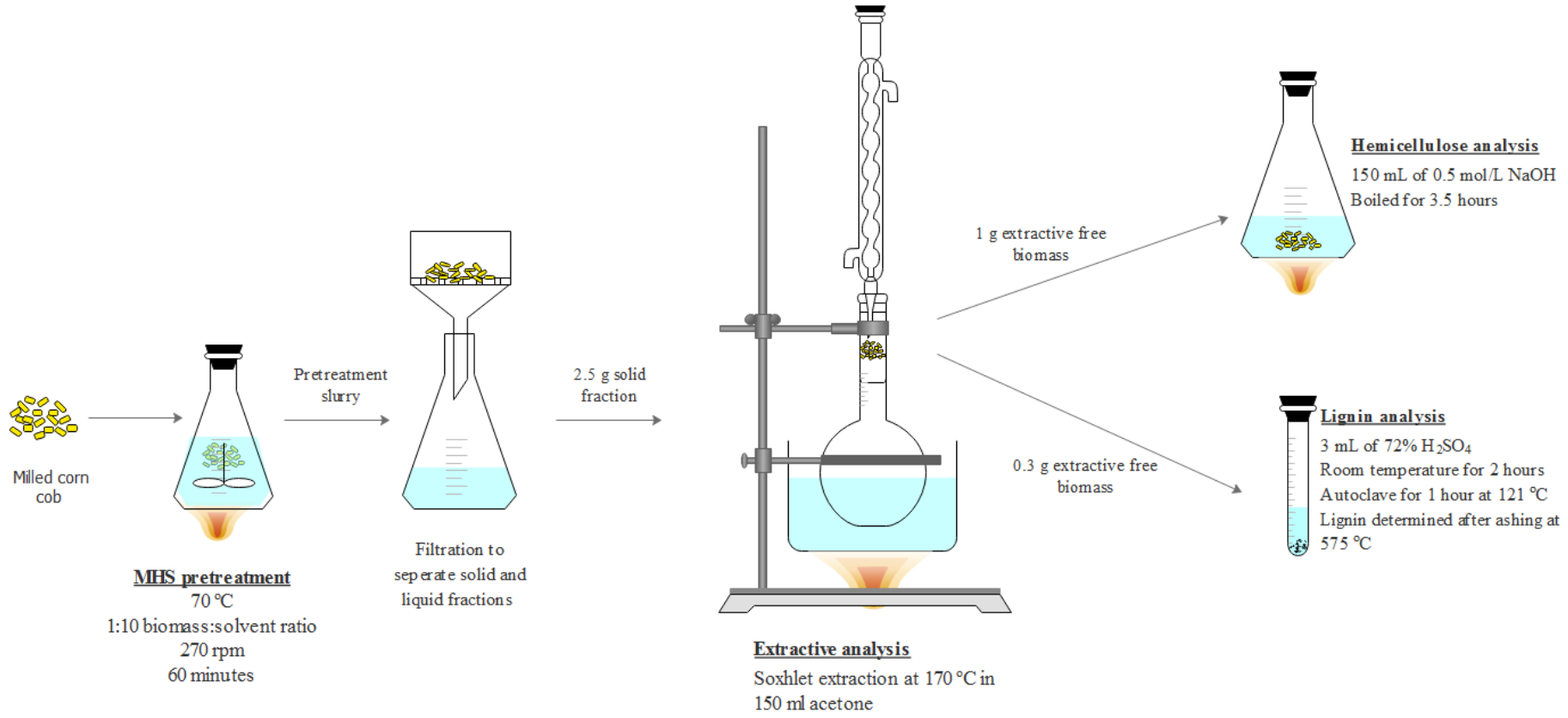


Figure 3.1 Method of compositional analysis for biomass.

(Cellulose% = 100%- Extractive% - Hemicellulose% - Lignin% - Ash%)

3.2.4 Quantitative evaluation of pretreatment to determine the efficiency of the solvents systems

In order to quantitatively evaluate the effects of various pretreatment methods on each component (cellulose, hemicellulose and lignin), recovery ratio (or yield) of each component was estimated. The efficiency of the pretreatment was evaluated based on the recovery/ yield of polysaccharides (cellulose and hemicellulose), the recovery in the liquid fraction of hemicellulose and lignin as adequate recovery of hemicellulose and cellulose sugars is important for an ideal pretreatment.

Biomass yield after pretreatment was calculated as follows (Varga, et al., 2003):

$$\text{Biomass yield}\left(\%\ \left[\frac{\text{w}}{\text{w}}\right]\right) = \frac{Dry\ biomass\ weight\ after\ pretreatment \times 100\%}{Dry\ weight\ of\ untreated\ biomass} \quad (3.2)$$

The recovery of each component was calculated as follows (Varga et al., 2003):

$$\text{Recovery of component}\left(\%\ \left[\frac{\text{w}}{\text{w}}\right]\right) = \frac{Component\ content\ in\ pretreated\ biomass \times biomass\ yield \times 100\%}{Total\ component\ in\ untreated\ biomass} \quad (3.3)$$

3.2.5 Total sugar determination of the liquid fraction

In this method, 3 mL of modified 3,5-Dinitrosalicylic acid (DNS) reagent was added to 1 mL of the liquid sample in a test tube with 1 mL of distilled water. The test tubes were then placed in a boiling water bath for 15 minutes and thereafter cooled for 15 minutes. The absorbance of the sample and DNS was then measured at 575 nm. Glucose standard curves were prepared and the values of total reducing sugar for the samples were then read from these glucose curve (Miller, 1959).

3.2.6 Cellulose crystallinity determination using X-ray diffraction (XRD)

The corn cob sample crystallinity was analysed using a D2 advance diffractometer (Bruker, Germany) with a scan rate of 10 min^{-1} from 10° to 90° and Co Kα radiation (1.789 A°)

generated at 30 kV and 10 mA. In order to analyse the data, a method developed by Segal et al. (1959) will be used. The crystallinity indices (CrI) was calculated from the XRD patterns using the following equation:

$$CrI = \frac{I_{002} - I_{amp}}{I_{002}} \times 100\% \quad (3.4)$$

Where I_{002} is the intensity of 002 lattice diffraction (2θ = 22.6) and I_{amp} represents the intensity of amorphous section (2θ = 18).

3.2.7 Surface chemistry analysis using Fourier Transform infrared (FTIR)

Lignocellulosic materials contain alcohols, aromatics, alkanes, ketones, esters and other oxygen based functional groups which can be analysed using FTIR. Surface chemistry of the raw and pretreated biomass samples were evaluated using a PerkinElmer Frontier Fourier transform infrared spectrometer (PerkinElmer, USA) using the attenuated total reflectance (ATR) method. A diamond ATR plate with angle of incidence of 45° was used as the internal reflection element. Spectra was obtained from a range of 400 to 4000 cm^{-1} and obtained at a resolution of 4 cm^{-1}. The spectra was used to investigate the surface chemistry information of the samples while the crystallinity index (CrI) of biomass can also be measured using FTIR. There are two well-established indices which exist which are a measure of biomass crystallinity. The Lateral Order Index (LOI), also defined as the empirical CrI is a ratio of absorption band at 1430 cm^{-1} to absorption at 898 cm^{-1}. Total Crystallinity Index (TCI), was also determined based on the ratio of the absorption band at 1372 cm^{-1} and 2900 cm^{-1}.

Table 3.1 The FTIR bands' assignment (Adapted from Kline et al., 2010; Ren and Sun, 2010; Hu et al., 2012; Karatzos et al., 2012)

Band position (cm^{-1})	Assignment
770	Stretch vibration of D- glucopyranoyl
832	C-H bending (lignin)
895	β-glycosidic linkages between xylopyranose in xylan chains
974	C-O stretch vibration of arabinosyl side chain
1030	C-O-C pyranose ring stretch vibration (arabinoxylans)
1040-1050	C-O stretch in C-O-C glycosidic bonds
1100-1110	C-OH skeletal vibration (cellulose and hemicellulose)
1160	Arabinosyl side chains (arabinoxylans)
1240	C-O stretch vibration (lignin, xylan and ester groups)
1309	C-H bending (cellulose)
1370	C-H stretch (cellulose)
1420	C-H scissoring at C(6) in cellulose
1460	Asymmetric bending in CH_3 (lignin)
1510	C=C stretch of aromatic ring (lignin)
1590-1599	Uronic acid carboxylate vibration
1600	C=C stretch of aromatic ring (lignin)
1730	Unconjugated C=O stretch of acetyl, ester or carboxylic acid groups
2900	C-H stretch vibration
3380	O-H stretch vibration

3.2.8 Morphological analysis for evaluation of biomass topography

3.2.8.1 Scanning Electron Microscope (SEM)

SEM was used to check the surface morphology of the pretreated and untreated biomass. Using conductive tape, the dried samples were fixed onto aluminium stubs. The samples where then analysed by Carl Zeiss Sigma scanning electron microscope. This was followed by sputter coating using carbon and gold-palladium at five nanometre scale making the fibres conductive, avoiding degradation and charge build-up on the specimen surface. The SEM was operated under a vacuum and the images were captured.

3.3 Results and discussion

3.3.1 Biomass compositional analysis

3.3.1.1 Solid fraction

The raw material was characterised in terms of the composition of extractives, ash, cellulose, hemicelluloses and lignin as highlighted in Section 3.2.3. The characterisation is necessary in order to determine the extent of the chemical compositional changes resulting from the pretreatment. The composition of the raw and pretreated biomass samples are shown in Table 3.2. The total carbohydrate (cellulose and hemicelluloses i.e. the holocellulose) composition was 63.03% (w/w). The high saccharide content implies that analysed corn cob waste materials are promising feedstock with potentials for very high yields of valuable bio-based chemicals and products. Table 3.2 also shows the composition of the pretreated samples. However, the biomass recoveries were not shown, biomass recoveries are needed to analyse the effect of each pretreatment on the corn cob. The results in Table 3.2 for the pretreated samples do not include the fractions which are unrecovered in the solid fraction, as some mass was lost in the solid fraction during pretreatment. The recovery percentages are shown in Figure 3.2.

Table 3.2: Composition of raw and pretreated solid fraction (70 °C , 60 min, 100 mL total liquid volume, 5 g biomass loading) corn cob solid (%w/w)

		Raw	A $ZnCl_2.4H_2O$	B $LiClO_4.3H_2O$	C Urea	D $LiClO_4/ZnCl_2$	E $ZnCl_2$/Urea	F $LiClO_4$/Urea	G $LiClO_4/ZnCl_2$/Urea
Biomass yield		100.00	73.40	75.20	87.90	78.35	80.30	88.50	90.80
Extractives		2.80	16.3	6.50	4.80	17.80	14.40	6.40	16.08
Hemicellulose		40.34	34.46	40.00	38.00	29.00	40.84	28.00	30.00
Cellulose		22.69	27.89	24.61	19.60	31.21	25.9	25.93	18.63
Lignin	AIL	29.55	17.00	27.00	33.33	20.10	16.68	37.00	31.99
	ASL	1.88	3.75	1.35	2.74	1.85	1.64	1.11	1.65
Ash		2.56	0.60	0.54	1.53	0.35	0.56	1.56	1.74

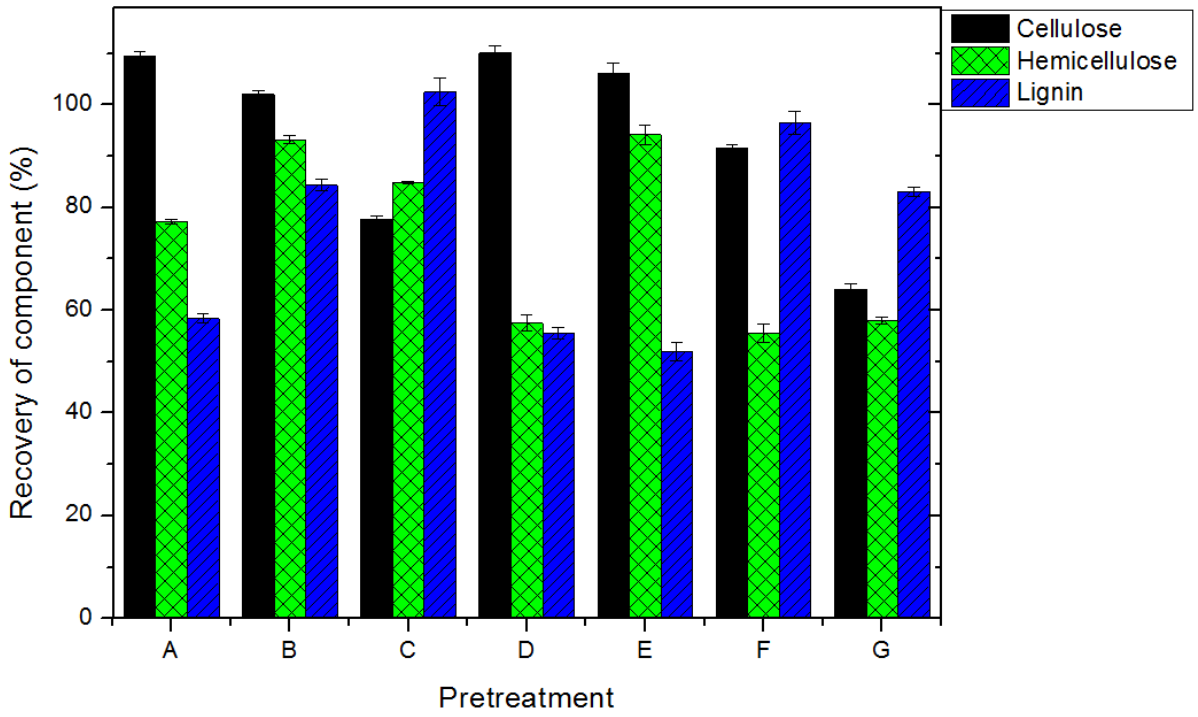


Figure 3.2 Recovery of polysaccharides cellulose, hemicellulose and lignin after pretreatment (error bars represent standard deviations of duplicates) (A): $ZnCl_2.4H_2O$. (B): $LiClO_4.3H_2O$. (C): Urea. (D): $ZnCl_2.4H_2O$/Urea. (E): $LiClO_4.3H_2O/ZnCl_2.4H_2O$ (F):$LiClO_4.3H_2O$/Urea. (G): $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea.

The recovery of each component was calculated using Equation (3.2) and Equation (3.3). The MHS solvent systems pretreatment was predicted to separate the lignocellulosic biomass into a liquid fraction which contains mostly solubilised hemicellulose as monomers and a solid fraction which is enriched with mostly oligomeric cellulose components. Therefore a high recovery of cellulose and a low recovery of hemicellulose and lignin are required (Fischer et al., 2003). Cellulose recovery observed in Figure 3.2 in the solid fraction was over 75% for all the tested biomass samples except pretreatment G ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea). The highest recovery of cellulose was approximately 109% and was recovered by pretreatment D ($ZnCl_2.4H_2O$/ Urea). Martin et al. (2007), in comparison, treated sugarcane bagasse biomass using wet oxidation at alkaline pH, 195 ^oC and 15 min and was able to achieve 70% cellulose recovery. Hemicellulose recovery was within the range of 57-93% with the lowest being D ($ZnCl_2.4H_2O$/ Urea), F ($LiClO_4.3H_2O$/Urea) and G ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea) with 99%, 55%, 66% recovery respectively. Georgieva et al. (2008) recovered 93% of cellulose

and 72% of hemicellulose from wet explosion pretreatment of wheat straw at 35% (v/v) hydrogen peroxide and 185 ^{o}C for 15 min

Lignin recovery was lowest for Pretreatment A, D and E with 66%, 77%, 88% recovery respectively. The high recovery of cellulose (109%) and low recovery of hemicellulose (58%) and lignin (56%) in the biomass samples pretreatment in sample D ($ZnCl_2.4H_2O$/ Urea) gave an indication that this is the most efficient solvent of the seven tested for the fractionation of corn cob . These results are in good agreement with formic acid pretreatment by Zhang et al. (2010) who under mild reaction conditions removed a relatively high ratio of hemicellulose (85%) and lignin (70%), while obtaining almost all the cellulose in the solid residue. This type of pretreatment produces toxic by products such as acetic acid and furfural which are usually unavoidable in pretreatment using acid solvents (Wyman, 1994).

The pretreatment in this study using MHSs occurred at a constant temperature of 70 ^{o}C and the results from the solid and liquid recovery reveal an efficient separation of the hemicellulose from cellulose as well as a low degradation of cellulose to by-products such as HMF and acetic acid during the pretreatment process. This is due to the non-derivatizing nature of the solvents. It has been observed that $ZnCl_2.4H_2O$/ Urea is an excellent solvent for biomass pretreatment due to its fractionation abilities. This is as a result of the interaction of the chloride anion (Cl^-) and the zinc cation (Zn^{2+}) of the solvent with the hydrogen atoms (H) and oxygen atoms (O) of the cellulose hydroxyl groups, respectively and the hydrogen bond formation with cellulose (Fischer et al., 2002). The relatively high lignin recovery in the solid fraction shows that only a small fraction of lignin was solubilised under the low pretreatment temperature conditions which caused minimal formation of low molecular weight lignin compounds. This gives some insight into the mechanism of the MHS pretreatment which shows that although delignification occurs, it is not as prominent in this type of pretreatment.

3.3.1.2 Liquid fraction

Depending on the pretreatment solvents, some of the cellulose and hemicellulose fractions will form sugar monomers in the liquid fractions. Figure 3.3 shows the yield of total sugars after pretreatment.

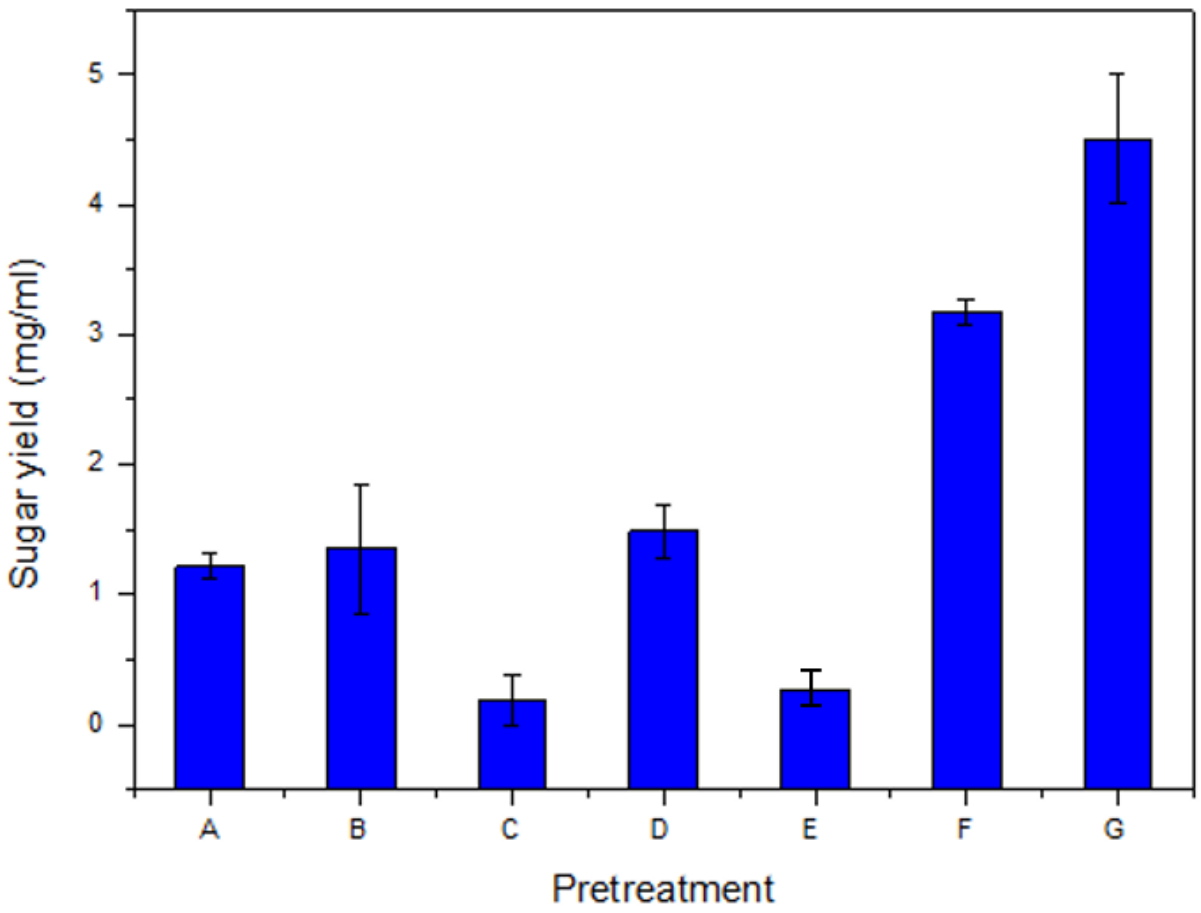


Figure 3.3 Yield of total sugars in liquid fraction after MHS pretreatment. Average values and standard deviations reported for duplicates. (A): $ZnCl_2.4H_2O$. (B): $LiClO_4.3H_2O$. (C): Urea. (D): $ZnCl_2.4H_2O$/Urea. (E): $LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$ (F):$LiClO_4.3H_2O$/Urea. (G): $LiClO_4.3H_2O$/$ZnCl_2.4H_2O$/Urea.

The standard curve used to estimate sugar yield in Figure 3.3 can be found in Appendix A. The highest total sugar quantities were found in pretreatment G ($LiClO_4.3H_2O$/$ZnCl_2.4H_2O$/Urea) at levels of 4.5 mg/mL. For all the other pretreatment except pretreatment F ($LiClO_4.3H_2O$/Urea), the total sugar concentrations were significantly lower in comparison. The lower sugar formation in pretreatment A ($ZnCl_2.4H_2O$), B ($LiClO_4.3H_2O$), C (Urea) and E ($LiClO_4.3H_2O$/$ZnCl_2.4H_2O$) hydrolysates correlate with the results found in Figure 3.2 which shows these pretreatment solvents to yield the highest hemicellulose recovery in the solid fraction. The higher sugar formation observed in Pretreatment D ($ZnCl_2.4H_2O$/Urea), F ($LiClO_4.3H_2O$/Urea) and G ($LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$/Urea), is mainly due to the fact that lower amounts of oligomeric hemicellulose were retained in the solid fraction which indicates that solubilisation of hemicellulose to

monomers in the liquid fraction was more severe in these pretreatment solvents. These results also indicate that MHS pretreatment solvents can dissolve hemicellulose into xylose sugars, rather than degrade it into fermentation inhibitors such as furfurals, unlike most acid and hydrothermal treatments.

3.3.2 Crystallinity and surface chemistry determination

3.3.2.1 XRD spectra

XRD analysis allows for measuring the crystallinity of the material and considers disordered cellulose, hemicellulose and lignin. Cellulose accounts for the highest proportion of the crystalline structure therefore, The XRD spectra is usually an indication of the cellulose crystallinity. The XRD spectra and Crystallinity index (CrI) values for the effect of MHS pretreatment on corn cob are shown in Figure 3.4 and Table 3.1, respectively. In this study, all samples presented the same diffraction patterns. Two typical diffraction peaks which correspond to (101) and (002) peaks were observed at $2\theta = 15^{\circ}$ and 22.5°, respectively. The highest peak observed at 002 was observed for spectra E ($ZnCl_2.4H_2O$/ Urea) which also happens to have one of the highest cellulose recoveries in Figure 3.2 while Urea which has one of the lowest cellulose recoveries has a low peak intensity shown as spectra D in Figure 3.4.

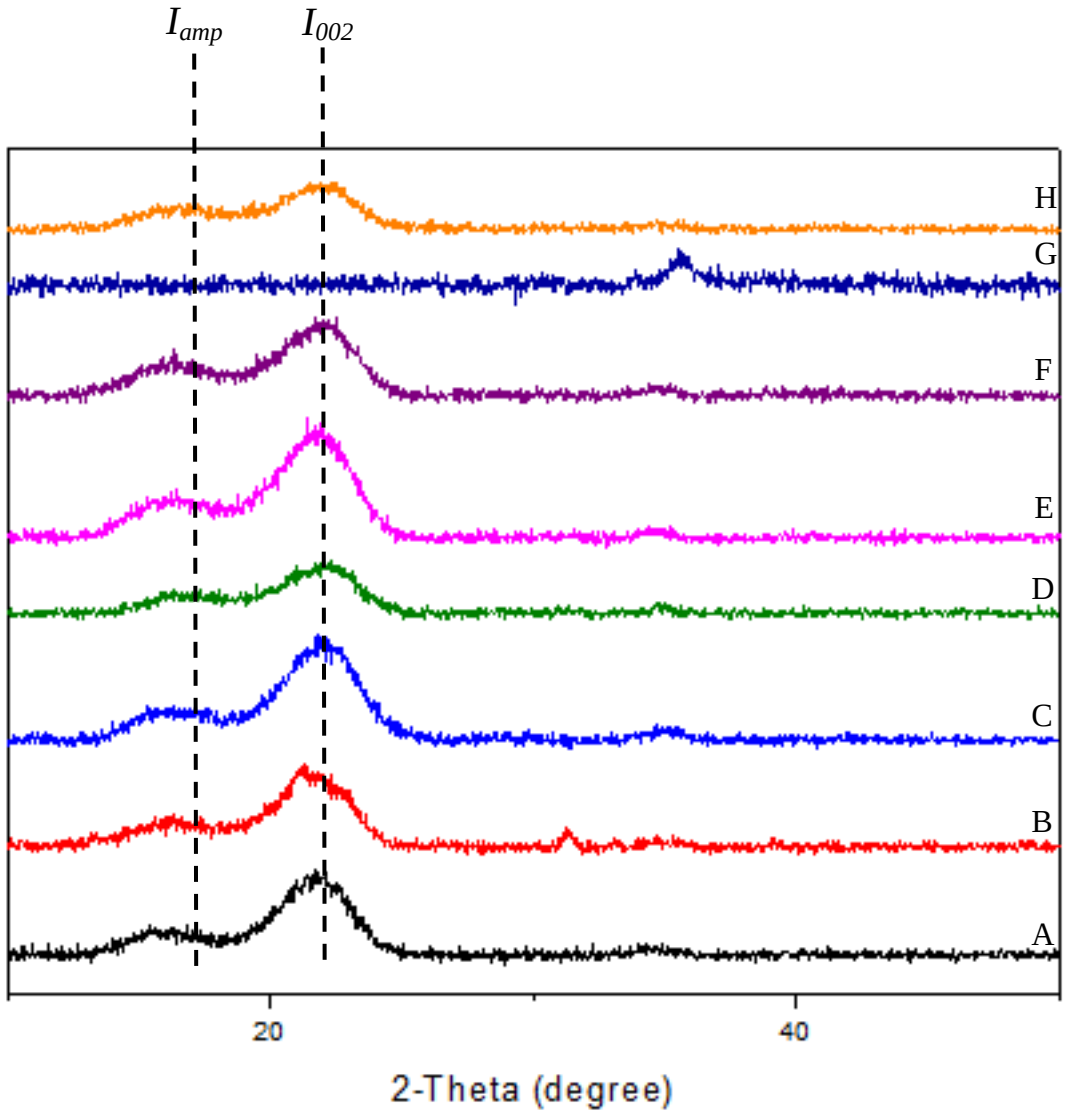


Figure 3.4 XRD spectra of (A): Raw (B): $ZnCl_2.4H_2O$. (C): $LiClO_4.3H_2O$. (D): Urea. (E): $ZnCl_2.4H_2O$/Urea. (F): $LiClO_4.3H_2O$/$ZnCl_2.4H_2O$ (G):$LiClO_4.3H_2O$/Urea. (H): $LiClO_4.3H_2O$/$ZnCl_2.4H_2O$/Urea.

3.3.2.2 FTIR spectra

The FTIR technique was used to check the chemical nature of the fractions and residue after the pretreatment. The main chemical bond vibrations of lignocellulosic materials are present between 800 – 1800 cm^{-1}. The characteristic absorption bands characteristic of holocellulose components of both hemicellulose and cellulose are 1112–1120, 1161 and 897 cm^{-1}. Other cellulose characteristic bands are found at 1376, 1061 and 1025 cm^{-1} while bands observed at 1251, 1046 and 990–996 cm^{-1} are hemicellulose characteristic bands. The lignin absorption

bands are at 1718, 1702, 1654, 1508, 1597, 1458, 1420, 1261, 1242, 1224 and 840 cm^{-1}. The bands mentioned have been characterised in detail in Table 3.1.

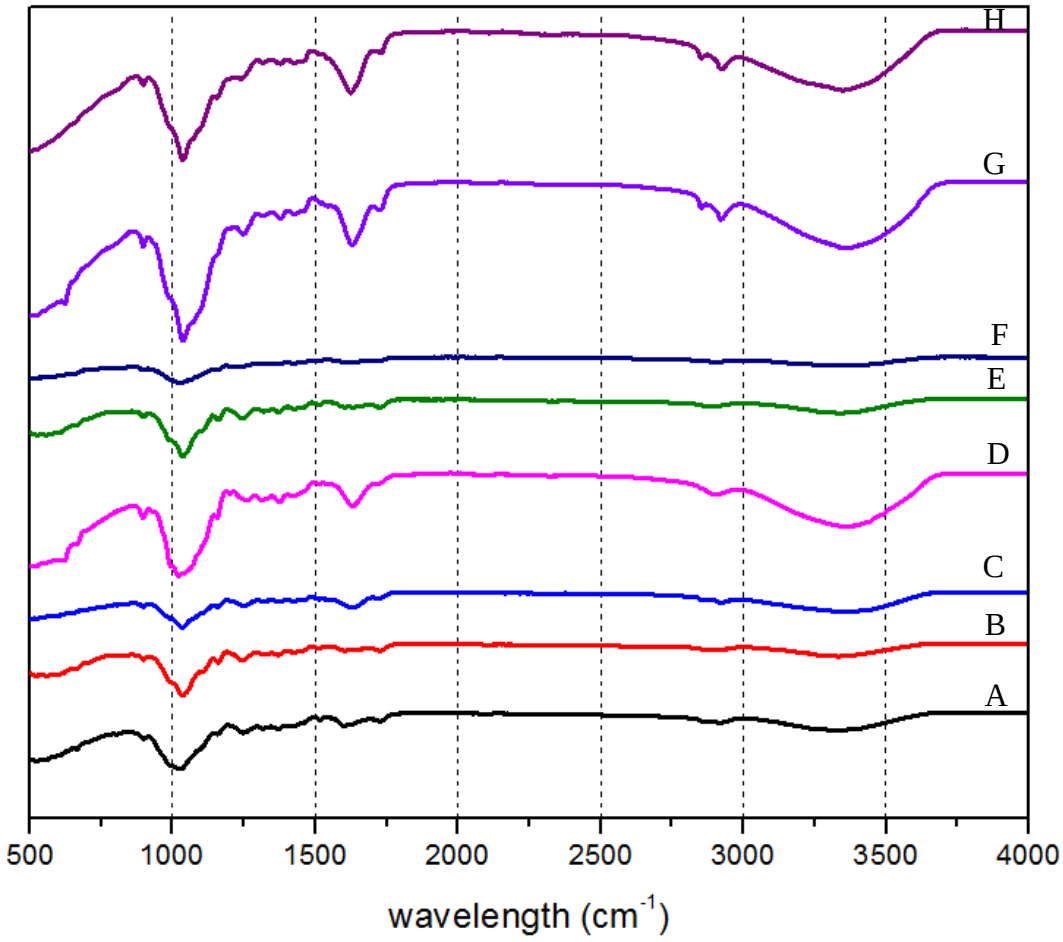


Figure 3.5 FTIR Spectra of (A) Raw, (B): $ZnCl_2.4H_2O$. (C): $LiClO_4.3H_2O$. (D): Urea (E): $ZnCl_2.4H_2O$/Urea (F): $LiClO_4.3H_2O/ZnCl_2.4H_2O$ (G):$LiClO_4.3H_2O$/Urea. (H): $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea.

The band at a wavelength of around 1730 cm^{-1} in Figure 3.5 was assigned to an unconjugated C=O stretch of ester, acetyl or carboxylic acid groups in hemicellulose components. In this peak, linkages between lignin and carbohydrates may exist. FTIR intensities at this wavenumber showed increased peaks after pretreatment G ($LiClO_4.3H_2O$/Urea) and H ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea). These peaks are diminished in the raw samples (A). The peaks at wavelength 1730 cm^{-1} showed slight changes in the hemicellulose content after $ZnCl_2.4H_2O$/ Urea (E) pretreatment and a high hemicellulose recovery after pretreatment using G ($LiClO_4.3H_2O$/Urea) and H ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea). These spectra correspond to the results in Figure 3.3 where the highest percentages of hemicellulose lost

were found in samples treated with $LiClO_4.3H_2O$/Urea and $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea. This also indicates breakage of the carbohydrate and lignin linkages.

The major functional group of lignin is an aromatic compound (phenolic hydroxyl group) with a benzene ring. Structural changes in lignin and loss of aromatic compounds were shown by the changes in intensities at the 1600 cm^{-1} band. Pretreated samples E ($ZnCl_2.4H_2O$/ Urea), G ($LiClO_4.3H_2O$/Urea) and H ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea) increased the intensity of the 1,600 cm^{-1} band. These spectra correlate with the results shown in Figure 3.2, whereby corn cob pretreatment, $ZnCl_2.4H_2O$/Urea, $LiClO_4.3H_2O$/Urea and $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea all showed similar lignin recovery when compared to one another.

The peaks at around 2900 cm^{-1} as shown in Figure 3.5 indicate C-H stretching vibration which shows an alkane functional group and indicates the rupture of methyl/methylene group of cellulose. After pretreatment D, G and H, the peaks of the FTIR spectrum increase. This result shows that the methyl (CH_3) groups of the samples were disrupted. This characteristic reduction of band intensities at 2900 cm^{-1} wavelength was also found by Ciolacu et al. (2011) and Kumar et al. (2009) who concluded that the samples became more amorphous.

There were noticeable changes in the peaks at the 3100-3600 cm^{-1} wavelength range for most of the pretreated samples. This corresponds to alcohols (O-H) stretching vibration which gives significant information regarding the hydrogen bonds. These changes occur as the molten hydrate salt pretreatment solvents become bound to the lignocellulose structure causing hydrogen bond breakage between O-H bonds. This also indicates possible crystalline cellulose disruption. This implies that highly ordered hydrogen bonds in the corn cob samples were disrupted through cellulose dissolution in all seven MHS pretreatment.

3.3.2.3 Crystallinity Index

Using data from FTIR, LOI and TCI peak ratios have been found and are tabulated in Table 3.3. XRD data gave information on CrI calculated using Equation (3.4) and the results were also tabulated in Table 3.3.

Table 3.3 FTIR crystallinity ratio and XRD Crystallinity index of raw and pretreated biomass

	FTIR crystallinity ratio		XRD Crystallinity index
	$\alpha_{1430}\ cm^{-1}$/ $A_{898}\ cm^{-1}$	$\alpha_{1372}\ cm^{-1}$ / $A_{2900}\ cm^{-1}$	$(I_{200} - I_{am})$/ I_{200}
Pretreatment	LOI	TCI	CrI
Raw	1.224	0.935	48.044
$LiClO_4.3H_2O$	1.038	0.975	71.260
$LiClO_4.3H_2O$/Urea	1.027	0.994	77.389
$LiClO_4.3H_2O/ZnCl_2.4H_2O$	1.130	0.958	54.134
Urea	1.043	0.973	67.747
$ZnCl_2.4H_2O$	1.033	0.988	62.081
$LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea	1.075	0.954	63.626
$ZnCl_2.4H_2O$/ Urea	1.140	0.979	72.764

LOI: Lateral order index or crystallinity index based on FTIR ; TCI: Total crystallinity index based on FTIR; CrI: Crystallinity index based on XRD

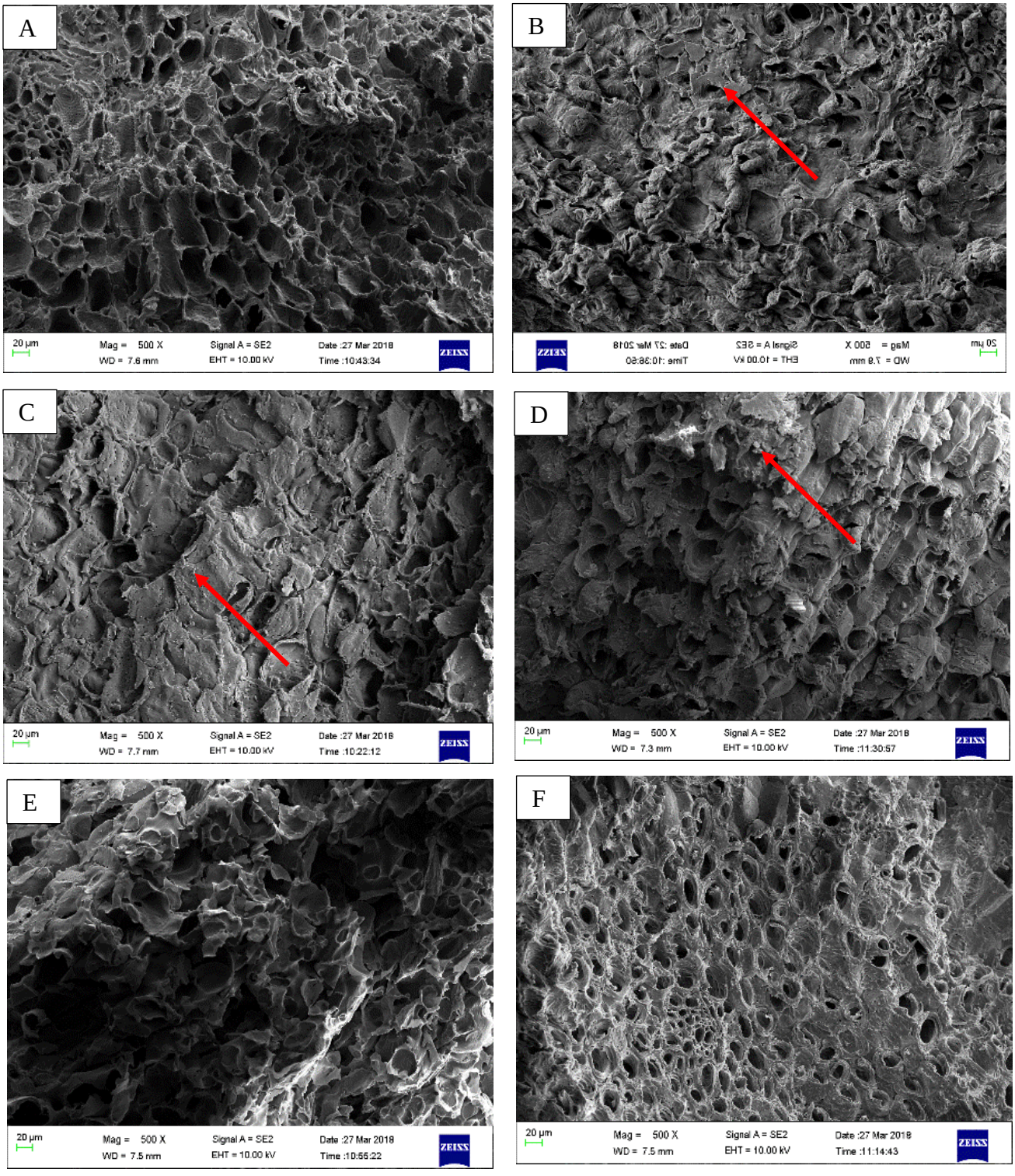
Higher values of TCI and LOI are characteristic of biomass samples with more ordered structures and higher crystallinity. LOI decreased after MHS pretreatment as shown in Table 3.3. TCI was relatively constant. Thus in this case, LOI was more sensitive to predict the crystallinity of the samples. In addition, it has been shown in previous literature that the LOI is a better indicator of crystallinity changes than the TCI for lignocellulosic samples (Nelson & O'Connor, 1964; Zhao et al., 2004; Ayeni & Daramola, 2017). The results of LOI showed the transformation of the cellulose from a highly crystalline structure to a more amorphous structure in the corn cob after pretreatment with MHS. LOI decreased from 1.224 in the raw sample to the lowest value (1.027) found in the samples treated with $LiClO_4.3H_2O$/Urea. Decrease in crystallinity of switchgrass (Li et al., 2010) and straw (Fu and Mazza, 2011a), have also been reported after pretreatment with other cellulose solvents such as ionic liquids.

Awosusi et al. (2017) reported a significant difference in the crystallinity indices as well as surface morphologies between the raw and $ZnCl_2.4H_2O$ treated biomass.

CrI values of corncob were shown in Table 3.3. It was notable that the CrI values of corncob residues changed approximately proportionally to the amount of cellulose recovered. Crystallinity is strongly influenced by the biomass composition. It was initially hypothesized that the crystallinity of the biomass would decrease due to the pretreatment, however since XRD measures all components and not just cellulose, there were interferences from the other amorphous components (hemicellulose, lignin). Observation of this data shows a good correlation between the CrI and cellulose content recovery (Figure 3.1) in the analysed material. The raw sample presented the lowest crystallinity (CrI), because it has a higher content of hemicellulose and lignin, which are amorphous (Xu et al., 2007). The results thus show an increase in crystallinity with pretreatment and also show the pretreatment which retained the highest amount of cellulose (%) with one of the highest crystallinity index of 72,769 ($ZnCl_2.4H_2O$/ Urea) as a result of the removal of the lignin and amorphous hemicellulose fractions. The samples with lower content of lignin and hemicellulose in the compositional analysis were the samples that presented higher crystallinity, as can be noted for the pretreatment (C, D and E). Due to the lack of regular crystal structure in hemicellulose and lignin the interpretation of the crystallinity of cellulose through XRD analysis is possibly hindered by the hemicellulose and lignin fractions.

FTIR and XRD results suggest that pretreatment using MHS can reduce the crystallinity of cellulose in corn cob. A mechanism was proposed which bases the reduction of cellulose crystallinity on the ability of cellulose molecules to compete for the water molecules of the coordination sphere of a specific ion and once dissolution takes place, the hydroxyl groups of cellulose will substitute part of the coordinated water. It was suggested by Li et al. (2009) that the decrease of CrI, was due to the water rapidly precipitating, which prevented the dissolved lignocellulose from rearranging into the original crystalline structure; this resulted in biomass with more amorphous structure.

3.3.3 Morpological analysis for evaluation of biomass



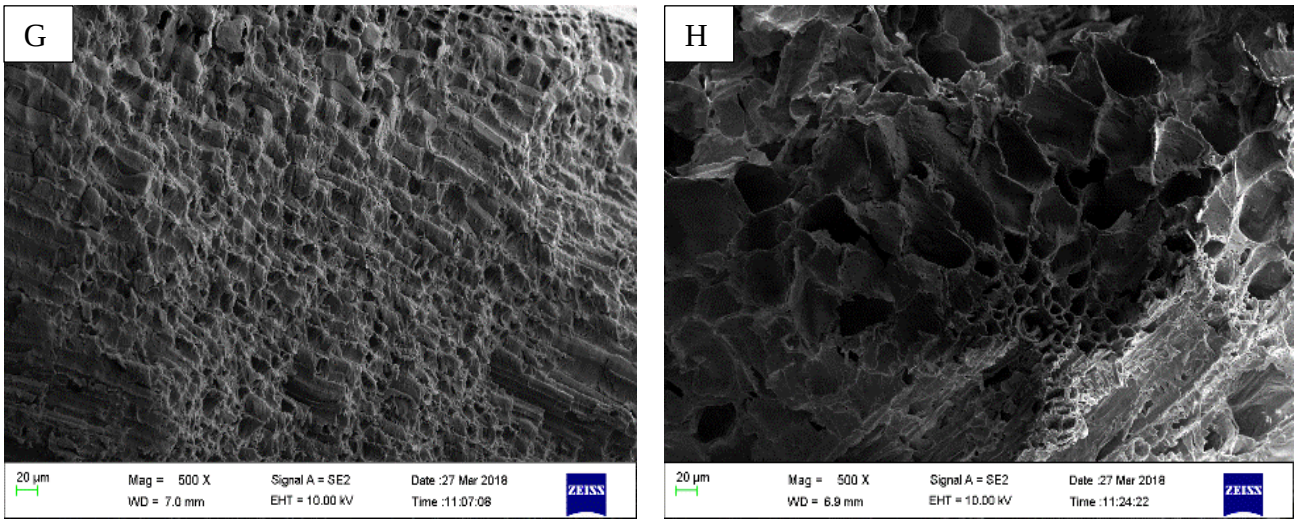


Figure 3.6 Scanning Electron micrograph of untreated and pretreated corn cob. (A) untreated (B) $ZnCl_2.4H_2O$ treated (C) $LiClO_4.3H_2O$ treated (D) Urea treated (E) $ZnCl_2.4H_2O$/Urea treated (F) $LiClO_4.3H_2O$/Urea treated (G) $LiClO_4.3H_2O/ZnCl_2.4H_2O$ treated (H) $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea treated at 500X Magnification. Red arrows indicate globular structures.

SEM results for the raw samples show intact lamellar structure where hemicellulose and lignin wraps around the cellulose in a hetero-matrix. In addition, the structure of the raw sample is visibly ordered. However, the molten hydrated salt treated (Figures 3.6B-H) corncob samples showed disrupted lamellar structures due to the removal of hemicellulose and lignin. The samples also show distinct inner cavities of the biomass, thus increasing the surface area. The particle size of the corn cob was also reduced in Figures 3.6B-H. In addition, there was damage in the intact cell structure as well as a more exposed internal cell structure. The pretreatment certainly increased the external surface area and the porosity of the material for each of the pretreatment. The most obvious change from the original fibre can be noted in the Figure 3.6B. These samples were not homogenous therefore; quantification based on surface deconstruction cannot be done. The change in surface morphology is in agreement with the finding of Selig et al. (2009) which showed that there was similar change on the biomass surface structure occurs when corn stover is pretreated and delignified using alkaline peroxidase.

Further proof of modifications which occurred after pretreatment and another interesting feature of some of these samples is the presence of globular structures (indicated by the red

arrows). These globular structures are associated with lignin condensation that were observed on the surface of the corn cob after pretreatment (Donohoe et al., 2008). The formation of these lignin agglomerates on the surface have been observed in many other types of lignocellulosic biomass when they undergo organosolv steam-explosion and diluted acid pretreatment (Donohoe et al., 2008). This droplet phenomenon has in previous studies been related to the severity of pretreatment conditions. The droplets were observed in the corn cob samples pretreated with (B) $ZnCl_2.4H_2O$ (C) $LiClO_4.3H_2O$ (D) Urea. The more severe the pretreatment and its conditions, the more droplets formed on the surface. The lignin molecules liquefy and then coalesce. Once the lignin phase transition is reached, droplets are formed within the cell wall matrix. Due to the hydrostatic pressures within the layers of the cell wall, some of the lignin fractions are forced to the outer surface of the biomass samples. The small droplets bind to the pretreatment mass and are then re-deposited on the biomass surface when the samples are cooled (Donohoe et al., 2008).

Biomass morphology is a major factor that contributes to effective hydrolysis. The larger the surface area, the more MHS molecules are able to access the plant cell wall polymers, as well as being able to separate the main fibres into fibrils and strands. SEM analysis results supported the FTIR and XRD results which showed that MHS pretreatment was effective in reducing the crystallinity of the biomass overall.

CHAPTER FOUR

4. Optimisation of processing conditions for MHS pretreatment of corn cob by response surface methodology

4.1 Introduction

To reduce our dependence on petroleum based sources and mitigate global climate change, Alternative and renewable fuels derived from lignocellulosic biomass need to be investigated (Waldron, 2010). To effectively utilise lignocellulosic resources, new technologies are needed to overcome the barriers associated with the conversion of biomass to chemicals and fuels (Ragauskas et al., 2006). These much needed breakthroughs include improvement of pretreatment processes that are able to fractionate biomass to the main components of lignin, cellulose and hemicellulose in high volumes in order to improve overall biomass utilisation (Wildschut et al., 2013).

The conventional method for the multi-factorial system optimisation uses one-factor at a time (OFAT). However, this type of method is time-consuming and fails to consider any possible interaction between the factors (Myers and Montgomery, 2002). Response surface methodology (RSM) is the most popular statistically-based optimisation strategy which uses a quadratic polynomial model to establish optimal conditions (Myers and Montgomery, 2002). RSM is used in investigating the effects of individual as well as interactive effects of factors in a process. An efficient alternative to factorial design is central composite design (CCD), this design is popularly used for second order response surfaces (Hongqiang and Xu, 2012). Determining the optimal operating conditions for effective pretreatment techniques can contribute to overcoming the cost barriers associated with the use of biomass by reducing the number of experimental trials needed to evaluate multiple parameters and their interactions (Khuri and Cornell, 2018). RSM has been successfully applied in the study and optimisation of chemical pretreatment by ionic liquids (Trinh et al., 2018), alkaline peroxide (Ayeni et al., 2018), alkaline and dilute acids (Ávila-Lara et al., 2015). In addition, the central composite design (CCD) of RSM has previously been successfully applied in the optimisation of several pretreatment processes (Ávila-Lara et al., 2015; Hongqiang and Xu, 2012).

In Chapter 3, a pretreatment solvent ($ZnCl_2.4H_2O$/Urea) was identified that effectively fractionated the corn cob into cellulose, hemicellulose and lignin with high recovery of

cellulose in the solid fraction and high recovery of hemicellulose in the liquid fraction. It is however important to optimise both the chosen solvent and conditions used, not just from a process point-of-view, but also from an economic perspective. If the chosen pretreatment is not efficient enough, the yield from the downstream enzymatic hydrolysis and fermentation steps will be low and of low quality.

In this chapter, the effect of pretreatment conditions (time, temperature and solvent concentration) on the use of $ZnCl_2.4H_2O$/ Urea molten hydrate was investigated and optimised for the fractionation of corn cob. RSM was adopted to investigate the effect and optimise the pretreatment conditions for fractionating cellulose, hemicellulose and lignin of corn cob. The main and interaction effects of the parameters were studied. CCD, a class of three level complete factorial design was used as a design experiment which considered pretreatment time, temperature and solvent concentration as the independent variables and the responses were % recovery of cellulose, % recovery of hemicellulose and % recovery of lignin.

4.2. Materials and method

4.2.1 $ZnCl_2.4H_2O$/ Urea pretreatment

The pretreatment was carried out at varying temperatures (60-90 °C) in a shaking incubator for varying times (60-120 min) using $ZnCl_2.4H_2O$/ Urea at varying concentration ratios (50-90 % w/w) (Table 4.1) at a constant biomass to solvent ratio of 1:10 (5 g dry biomass will be added to 50 g of each solvent) and at a constant agitation speed of 270 rpm. The slurries obtained from each pretreatment method were recovered and separated into solid and liquid fractions by vacuum filtration.

4.2.2 Compositional analysis of solids

Refer to Section 3.2.3 for the in depth compositional analysis method.

4.2.3 Experimental Design

The experimental design is shown in Table 4.1, the experimental data were analysed using RSM to fit the second order polynomial equation created by Design-Expert 7 software (Stat-Ease Inc., USA) The parameters were selected for the studies as follows: reaction temperature (60-90 °C), $ZnCl_2.4H_2O$/ Urea molar ratio (50- 90% w/w) and time (60-120 min). The variable ranges are shown in Table 4.1. The ranges were selected to keep the intervals within mild reaction conditions. Table 4.1 describes the five coded levels ($-\alpha$, -1,

0, 1, α) and un-coded independent factors, the test ranges for the parameters and the overall experimental design. The CCD design made up of 20 experiments (8 factorial points, 6 centre points and 6 axial point) were carried out with three variables and each variable varied at three levels to allow for the evaluation of the curvature (Jeong et al., 2009). The value of α is the distance from the axial points to the central point $\alpha = (2^n)^{1/4}$, in which n is the number of independent factors, for three factors α=1.68 (Barker et al., 1985). The factorial design experiments were run in duplicates to ensure reliability; the average error was calculated and used.

Table 4.1 Independent variables and their levels in the experimental design

Independent variable	Symbols	Unit	Code Levels				
			-1.68 (-α)	-1	0	1	1.68 (+α)
Time	A	min	39.55	60	90	120	140.45
Temperature	B	°C	49.77	60	75	90	100.23
Solvent concentration	C	%	36.36	50	70	90	103.64

4.2.4 Model formulation and statistical analysis

The experimental data was used to develop a quadratic polynomial model having % cellulose recovery (Y_1), % hemicellulose recovery in the liquid fraction (Y_2) and % lignin recovery (Y_3). The general regression model (Equation (4.1)) was adopted.

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{12} AB + \alpha_{13} AC + \alpha_{23} BC + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{33} C^2 + \varepsilon \quad (4.1)$$

Where *A*, *B*, *C* represent the independent variables: temperature, time and solvent concentration, respectively. The intercept term is α_0; α_1, α_2 and α_3 represent linear terms; α_{11}, α_{22} and α_{33} represent quadric terms; α_{12}, α_{13} and α_{23} are the interacting terms. All experiments were done in duplicate and data average values were calculated. The statistical analysis, plotting of response surfaces and optimization of the data were performed using "Design Expert" software (Trial version 7.0.3, Stat-Ease, Inc., Minneapolis, USA). The analysis of variance (ANOVA) was calculated. The fit quality of the polynomial equation was assessed

by the coefficient of determination (R^2), the significance of the regression and statistical coefficient was checked using t-test and F-test, respectively. The predictability of the developed model was then cross validated using laboratory experiments.

4.2.5 Desirability function

Numerical optimisation by desirability function was carried out to optimise the operating conditions of the pretreatment. The desirability function technique can be used to determine the optimum conditions of a process which involves one or more responses. The desirability procedure first determines the independent variable levels that simultaneously produce the most desirable prediction for the responses, thereafter the overall desirability of the outcome is maximised based on the factors which can be controlled.

In numerical optimisation, the desired goal for each factor and response were chosen. The goals defined were as follows: maximise, minimise, target, exact value (for factors only), none (for responses only) and within range (Khuri and Mukhopadhyay, 2010). Maximum and minimum levels for each factor were chosen. The factors can also be weighted according to priority. The goals were combined to get a desirability function. The desirability function is an objective value ranging from zero (outside the goal) and one representing the goal, the program thus aims at maximising this function. The goal seeking step starts randomly from one point and makes its way to a maximal point through the steepest slope.

4.3 Results and discussion

4.3.1 Central composite design studies

CCD was employed using the Design-Expert (Stat-Ease, Inc., Minneapolis, USA) software to study the simultaneous effects of reaction time, temperature and solvent concentration of pretreatment on cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery. The experimental conditions were obtained by a complete three-factor experimental design, which included six replications of the centre point and six axial points and eight factorial points. Table 4.2 shows the series of conditions of the experiments conducted for each run.

Table 4.2 Experimental response of the dependent variable after pretreatment using central composite design

Run	Variable			Response		
	A: Time (min)	B: Temperature (°C)	C: Concentration (%)	Y_1: Cellulose recovery (%)	Y_2: Hemicellulose recovery (%)	Y_3: Lignin recovery (%)
1	60	60	50	57.487	15.220	45.903
2	120	60	50	74.779	16.727	48.612
3	60	75	50	88.874	17.418	63.182
4	90	100.23	70	95.097	21.928	63.532
5	60	60	90	80.921	10.150	44.640
6	120	60	90	83.765	19.137	50.701
7	60	75	90	100.220	15.265	62.986
8	120	90	90	88.359	29.473	63.112
9	39.55	75	70	73.056	12.741	49.193
10	140.45	75	70	99.030	19.682	56.694
11	90	49.77	70	64.434	9.5438	43.350
12	90	100.23	70	96.729	19.459	56.409
13	90	75	36.36	71.993	14.009	49.943
14	90	75	103.64	94.561	20.753	55.441
15	90	75	70	80.192	14.728	49.319
16	90	75	70	78.353	16.903	50.018
17	90	75	70	81.149	20.438	52.334
18	90	75	70	84.803	16.269	48.437
19	90	75	70	86.734	13.168	51.740
20	90	75	70	87.210	15.220	51.597

4.3.2 Composition of solid fraction

The detailed recovery of the components of $ZnCl_2.4H_2O$/ Urea treated solid fraction for each run are shown in Figure 4.1. The highest solid recoveries of cellulose for the pretreatment were obtained in run 10 (39.55 min, 75 ^oC, 70/30 % (w/w) $ZnCl_2.4H_2O$ /urea) and 12 (90 min, 49.77 ^oC, 70/30 % (w/w) $ZnCl_2.4H_2O$/Urea) with 87.6% and 86.1%, respectively. This shows that cellulose is less affected by the severity of the pretreatment as opposed to hemicellulose. Hemicellulose recoveries were lowest at run 8 (120 min, 90 °C, 90/10 % (w/w) $ZnCl_2.4H_2O$/Urea) and for run 4 (90 min, 49.11 °C, 70/30 % (w/w) $ZnCl_2.4H_2O$/Urea). These severities are considered high due to longer times and higher temperatures. This is in agreement with various studies which state that hemicellulose is more susceptible to pretreatment conditions (FitzPatrick et al., 2010) . On the other hand, the lowest lignin recovery for the trials were obtained during run 3 (60 min, 75, 50%), 4 (60 min, 100.23 °C, 70/30% (w/w) $ZnCl_2.4H_2O$ /Urea) and 8 (120 min, 90 °C, 90/10 % (w/w) $ZnCl_2.4H_2O$ /Urea).

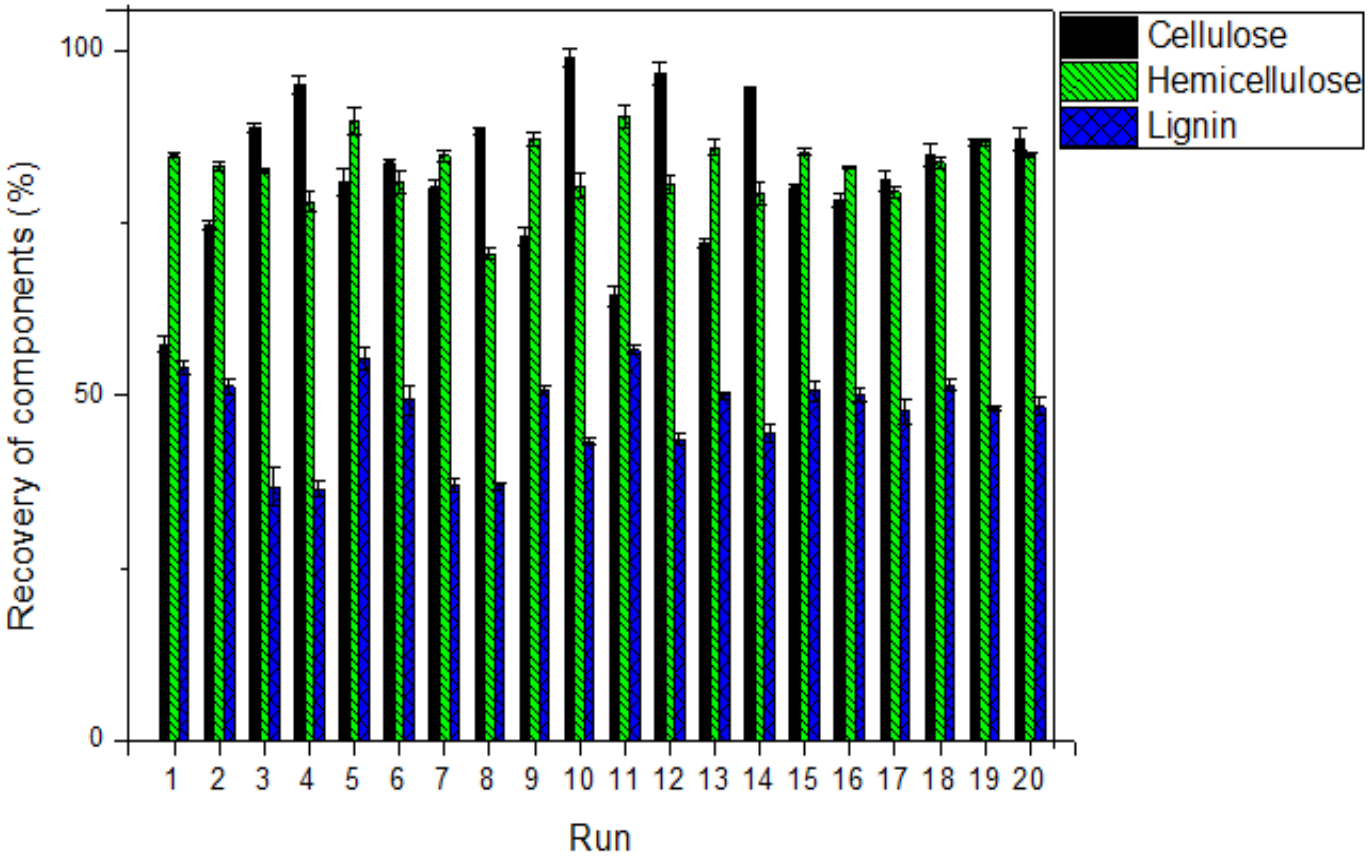


Figure 4.1 Recovery of polysaccharides (cellulose and hemicellulose) and lignin after pretreatment at different process conditions (error bars represent standard deviations of duplicates).

4.3.4 Models and statistical analysis

The experimental data was analysed using the Design-Expert software and a second-order polynomial equation was obtained. The equation included linear, quadratic and interactive terms for the variables tested. The polynomial equations, describing the cellulose recovery (Y_1), hemicellulose recovery (Y_2) and lignin recovery (Y_3) as a function of reaction time (A), temperature (B) and solvent concentration (C) of $ZnCl_2.4H_2O$/ Urea MHS pretreatment are shown in Equation (4.2), Equation (4.3) and Equation (4.4)

$$Y_1(\%) = +88.31 + 2.87A + 6.55B - 6.40C + 0.52AB - 1.81AC - 15.24BC + 0.22A^2 - 2.88B^2 - 0.15C^2 \quad (4.2)$$

$$Y_2(\%) = +18.67 + 1.79A + 4.62B + 3.15C + 0.35AB + 1.72AC + 3.37BC + 0.075A^2 + 0.14B^2 + 1.12C^2 \quad (4.3)$$

$$Y_3(\%) = +61.00 - 1.49A + 14.08B + 3.24C - 1.90AB + 1.02AC + 2.23BC + 0.31A^2 + 1.36B^2 + 1.77C^2 \quad (4.4)$$

Equations (4.2 - 4.4) reveal the effect of the individual variables (linear and quadratic) or double interactions on the responses. The negative coefficients of the values show the negative effect of the factors on the responses (decrease the response), for example, solvent concentration (C) decreases cellulose recovery (Y_1), whereas the positive coefficients of the values mean that the factors have an additive effect on the responses (increase the response).

4.3.4.1 Analysis of variance

The significance of the developed model, the pretreatment variable (linear and quadratic) as well as the interaction effects were analysed ANOVA. ANOVA is a tool used to test the statistical significance of each variable by comparing the estimated experimental error to the mean square. The *p*-value showed that some terms where significant while others were not. However, all the coefficients were considered in the design and polynomial equation to minimise the error. The results of ANOVA for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery after $ZnCl_2.4H_2O$/Urea pretreatment are presented in Table 4.3, Table 4.4 and Table 4.5 respectively.

The model analysis gave F-value of 10.23, 6.45 and 9.18 for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery respectively. This implies that the models are significant for each of the responses. The F-value of the model is calculated as the ratio of mean square values for individual terms to the mean square values for the residuals. The Prob> F represents the probability of the F-statistics value. This is used as a test for the null hypothesis. For a model to be considered significant, the *p*-value needs to be lower than 0.05, this value indicates that there is only a 5% chance that the models the

values represent could occur due to noise. The models of cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery are significant and effectively describe the responses. The cellulose recovery response model has a lower *p*-value (0.0006) than the hemicellulose recovery in the liquid fraction model (0.0037) and lignin recovery model (0.0009) indicating that the model for cellulose recovery is most significant.

Table 4.3 ANOVA Analysis for Responses Y_1 [Cellulose recovery (%)]

Source	Sum of squares	Degree of freedom	Mean squares	*F-values*	*p*-value *Prob > F*
Model	1934.95	9	214.99	10.23	0.0006
A	71.26	1	71.26	3.39	0.0953
B	34.03	1	34.03	1.62	0.2319
C	62.64	1	62.64	2.98	0.1149
AB	2.26	1	2.26	0.11	0.7495
AC	49.57	1	49.57	2.36	0.1555
BC	316.45	1	316.45	15.06	0.0031
A^2	10.84	1	10.84	0.52	0.4889
B^2	18.75	1	18.75	0.89	0.3671
C^2	0.14	1	0.14	6.547E-003	0.9371
Residual	210.10	10	21.01		
Lack of Fit	140.97	4	35.24	3.06	0.1073
Pure error	69.13	6	11.52		
Total	2145.05	19			

In Table 4.3, A (Time) and BC (Temperature * solvent concentration) are significant model terms in the model Equation (4.2) and they significantly affect cellulose recovery, while the other coefficients do not influence cellulose recovery greatly in the range of this study. The results are in accordance with the observation that cellulose dissolution only takes place under harsh conditions such as high temperatures (Tan and Lee, 2012). A (Time) and AC (Time * solvent concentration) as seen in Table 4.4 also have significant effects on model Equation (4.3), they therefore significantly affect hemicellulose recovery in the liquid

fraction while the other factors do not. The solvent concentration influence is second order however there has been evidence showing the cellulose solvent (NaOH) concentrations having an impact on Hemicellulose dissolution (Costa Correia et al., 2013; Lehto and Alén, 2013). For lignin dissolution, the coefficients which are significant in the model are A (Time) and B (Temperature). This is in agreement with Vancov and McIntosh (2011) who noted that in the case of delignification, even in mild alkaline solvent conditions, an increase in the temperature improves the delignification rate.

The "Lack of Fit F-value" of 3.06, 1.00 and 1.78 for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery respectively, implies the lack of fit is not significant relative to the pure error. The "Lack of Fit F value" is not significant and there is only a 10.73% (Table 4.3), 47% (Table 4.4) and 25% (Table 4.5) chance that the large values of F could have occurred as a result of noise.

Table 4.4 ANOVA Analysis for Response Y_2 [Hemicellulose recovery in the liquid fraction (%)]

Source	Sum of squares	Degree of freedom	Mean squares	*F-values*	*p-value Prob > F*
Model	326.75	9	36.31	6.45	0.0037
A	27.84	1	27.84	4.95	0.0503
B	16.96	1	16.96	3.01	0.1132
C	15.20	1	15.20	2.70	0.1313
AB	1.04	1	1.04	0.18	0.6768
AC	44.91	1	44.91	7.98	0.0180
BC	15.42	1	15.42	2.74	0.1288
A^2	1.28	1	1.28	0.23	0.6438
B^2	0.045	1	0.045	7.919E-003	0.9308
C^2	7.18	1	7.18	1.28	0.2851
Residual	56.27	10	5.63		
Lack of Fit	22.48	4	5.62	1.00	0.4761
Pure error	33.79	6	5.63		
Total	383.02	19			

Table 4.5 ANOVA Analysis for Response Y_3 [Lignin recovery (%)]

Source	Sum of squares	Degree of freedom	Mean squares	F-values	p-value *Prob > F*
Model	674.73	9	74.97	9.18	0.0009
A	19.38	1	19.38	2.37	0.0504
B	157.40	1	157.40	19.27	0.0014
C	16.11	1	16.11	1.97	0.1905
AB	30.88	1	30.88	3.78	0.0805
AC	15.90	1	15.90	1.95	0.1932
BC	6.75	1	6.75	0.83	0.3847
A2	20.94	1	20.94	2.56	0.1404
B2	4.17	1	4.17	0.51	0.4912
C2	18.00	1	18.00	2.20	0.1685
Residual	81.67	10	8.17		
Lack of Fit	44.35	4	11.09	1.78	0.2508
Pure error	37.32	6	6.22		
Total	756.41	19			

4.3.4.2 Model summary

Table 4.6 shows the model summary for each response. The coefficient of variability (C.V) measures residual variations of the data in relation to the size of the mean. Higher C.V values usually indicate lower reliability while relatively low values of C.V indicate better reliability and precision of the conducted experiments. The C.V% was a low value of 5.56%, 14.03% and 5.41% for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery respectively which indicates that the experiments conducted were reliable and precise. The value of the signal-to-noise ratio (adequate precision ratio) was found to be 12.919, 11.593 and 9.672 indicating the models have an adequate signal. The adequate precision ratio and adjusted determination coefficient (Adj R^2) exceeded 4 and 0.70, respectively for all three models. This indicates that a good correlation between input and output variables could be drawn by the model developed and it was implied that the quadratic model could be used to explore the design space, predict the responses and find the optimal conditions of this process.

Table 4.6 Model summary for responses

Response	Standard deviation	Mean	C.V.%	PRESS	Adeq-precision
Cellulose recovery (%)	4.58	82.39	5.56	1460.56	12.91
Hemicellulose recovery (%)	2.37	16.91	14.03	300.85	11.59
Lignin recovery (%)	2.86	52.86	5.41	497.61	9.672

4.3.4.3 Pareto analysis

The percentage effect of each of the factors was done by the Pareto analysis (Khuri and Cornell, 2018). The analysis is a calculation of the percentage effect (*Pi*) of each factor on the response, according to the following equation:

$$Pi = \left(\frac{\beta_i^2}{\sum_{i=1}^{14}\beta_i^2}\right) \times 100 (i \neq 0) \quad (4.5)$$

Where βi is the regression coefficient of each variable.

Figure 4.2, Figure 4.3 and Figure 4.4 shows the Pareto plot. As seen in Figure 4.2 under the $ZnCl_2.4H_2O$/ Urea pretreatment, the most significant factor in cellulose recovery are B (Temperature) and C (Solvent concentration) in both linear and interactive forms. The interactive form (AB) however has the highest influence of 69.07%. Hemicellulose recovery in the liquid fraction was mainly affected by temperature (linear term), followed by interactive term (BC), solvent concentration (linear term), time (linear term) and interaction of time and temperature. Finally, lignin recovery was affected primarily and almost entirely by temperature (linear term) with 88.72% effect. Followed by solvent concentration (linear term) at 4.7%. The highest interactive term was temperature*solvent concentration (BC) with 2.2% effect on lignin recovery.

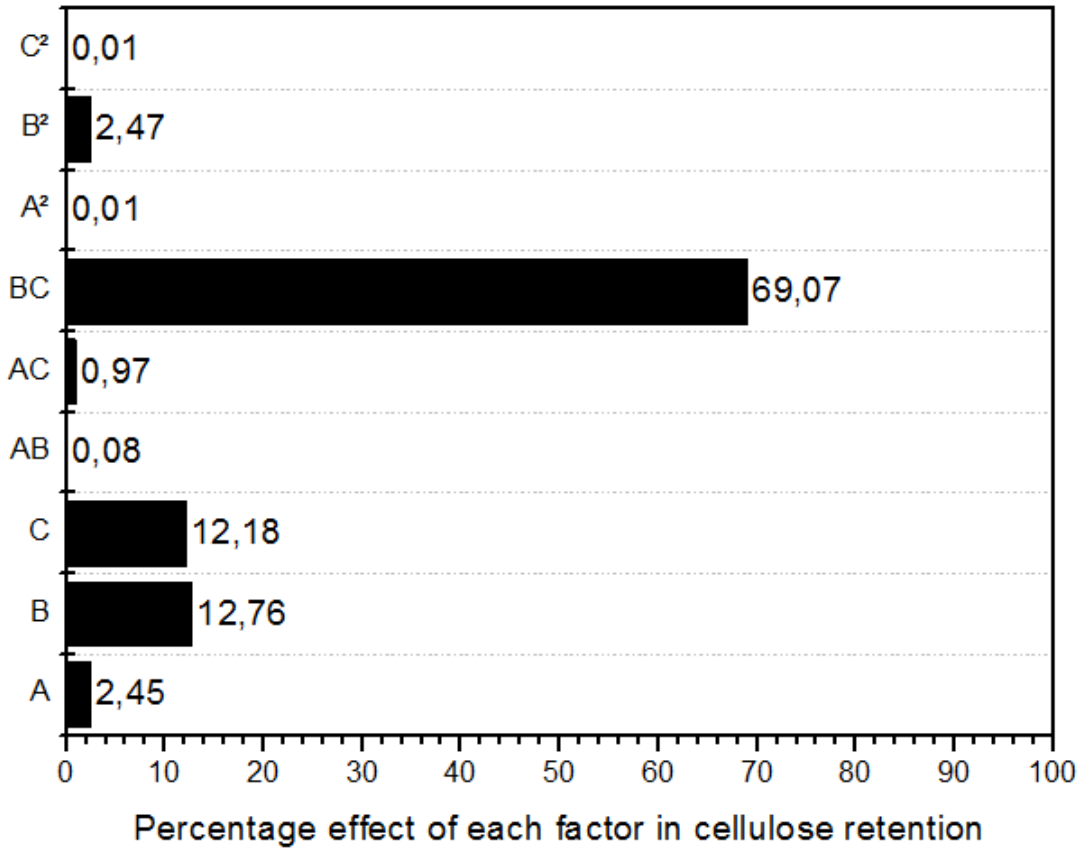


Figure 4.2 Pareto chart showing the percentage effect of each factor in cellulose recovery

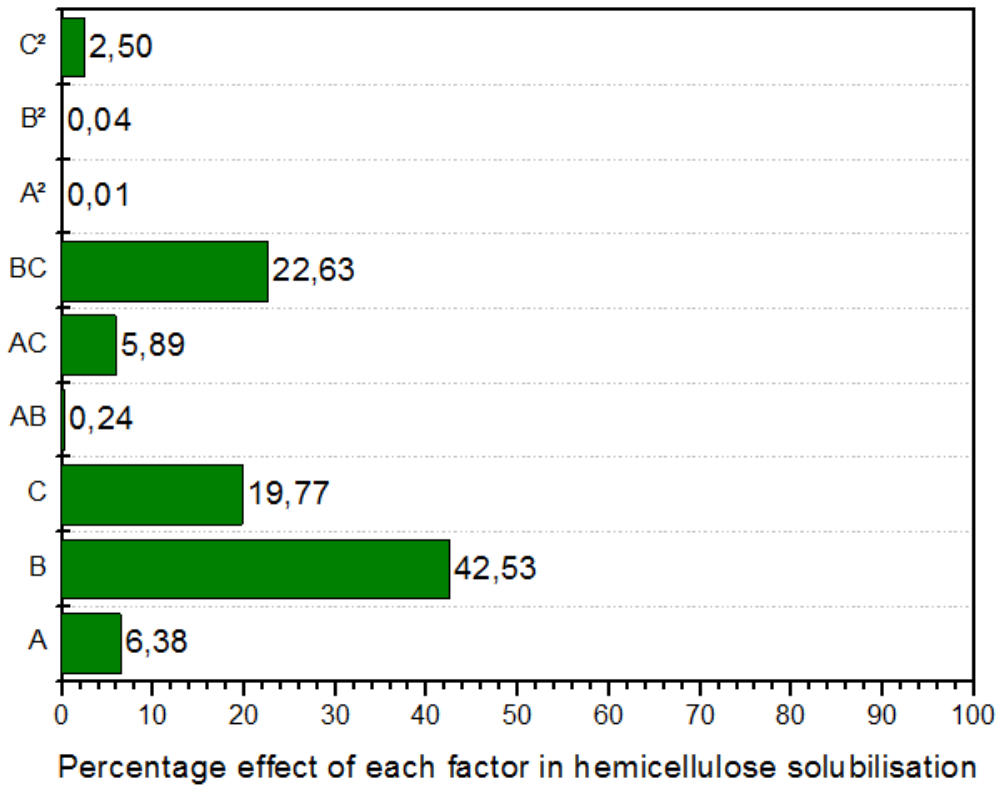


Figure 4.3 Pareto chart showing the percentage effect of each factor on hemicellulose recovery in the liquid fraction

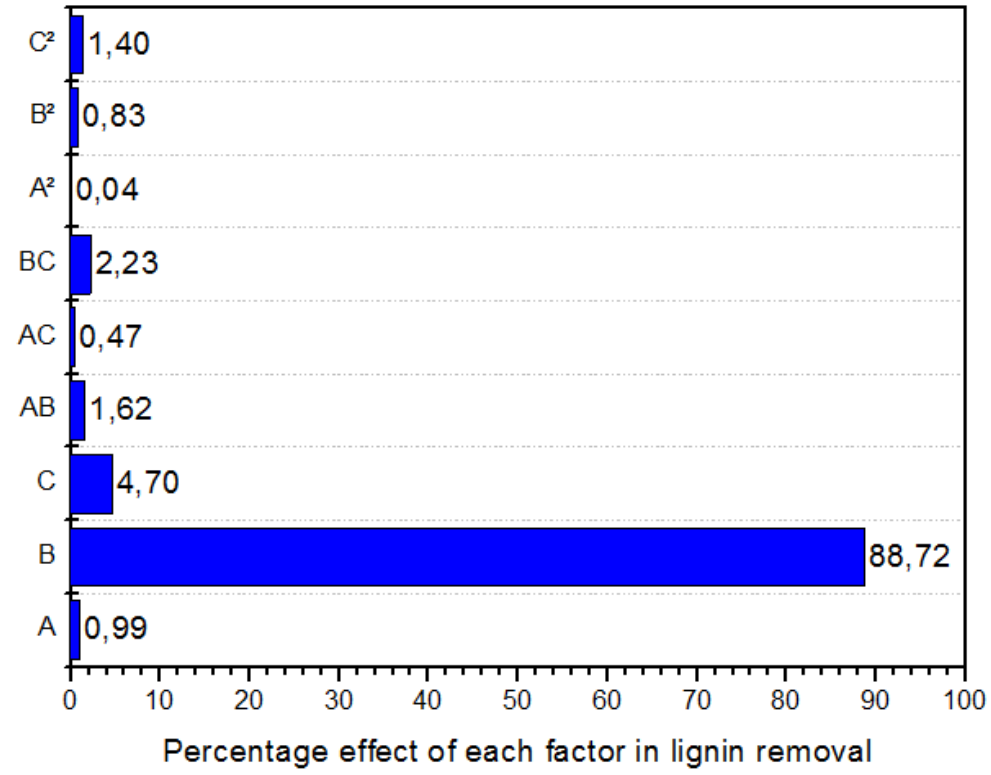


Figure 4.4 Pareto chart showing the percentage effect of each factor on lignin recovery

4.3.4.4 Model Fitting

The actual and predicted data for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery in addition to leverage, internally studentised residuals, externally studentised residuals, DFFITS and Cook's distance of the data can be obtained from diagnostic case statistics (Appendix B). The results show that the leverage value was within 0-1. The number of standard deviation separating actual and predicted values can be measured by internally studentised residuals. The limit of the internally studentised residuals is ±3 sigma. The analysis of diagnostic case statistics of data shows that the model fits well to optimise the independent variables for all three responses (Khuri and Cornell, 2018).

The reliability of the predicted models was additionally tested by comparing the predicted and experimental response results. The observed yield from the experimental and the predicted values are shown and represented as a parity plot in Figure 4.5 with the R^2 values for each response. In each case, there was satisfactory correlation between the predicted values and observed values from the experiment which was R^2=0.9021, 0.8531, 0.8920 for Figure 4.5A, Figure 4.5B and Figure 4.5C, respectively. This shows a close agreement between experimental and the theoretical values predicted. In Figure 4.5A, Figure 4.5B, Figure 4.5C, 9.8%, 14.69% and 22.3% of the total variation, respectively, were not explained by the model.

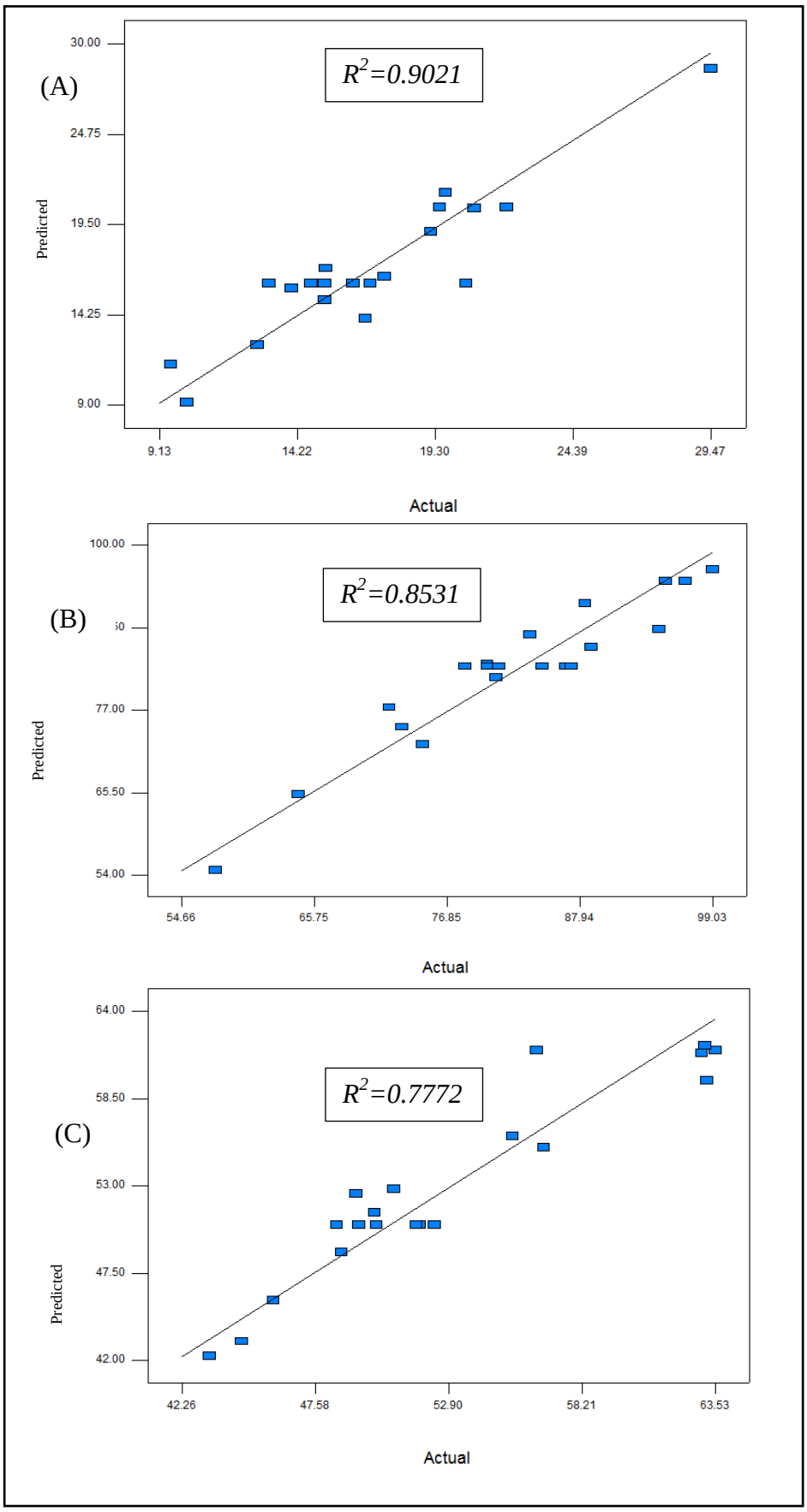


Figure 4.5 Parity plot showing distribution of predicted vs. actual values of responses.

(A) Cellulose recovery. (B) Hemicellulose recovery in the liquid fraction. (C) Lignin recovery.

4.3.5 Influence of pretreatment parameters combinations

The three-dimensional response surface plot described by the above-mentioned first order polynomial Equation (4.2), Equation (4.3) and Equation (4.4) were fitted to the experimental data as represented in Figure 4.6, Figure 4.7 and Figure 4.8. One of the variables was kept constant (at midpoint) while the other two variables were varied according to the experimental ranges determined previously. The shapes of the response surfaces indicate the type and amount of the interaction between the different independent variables (Ma et al., 2009). The surface plots for the cellulose recovery are shown in Figure 4.6. Figure 4.6A and Figure 4.6C. These plots show that the pretreatment temperature had the major increasing effect on cellulose recovery. As seen in Figure 4.6A, longer times result in higher cellulose recovery only for the pretreatments at higher temperatures while the effect of longer pretreatment time was negligible at lower pretreatment temperatures. At higher solvent concentrations, cellulose recovery increased, However as the concentration increased, the effects of increasing temperature became negligible after 77 ^{o}C (Figure 4.6C). Overall, the cellulose recovery was maximal at 100.23% with only a small increase when time is increased to approximately 90 min.

The response surface plots for the hemicellulose recovery in the liquid fraction are shown in Figure 4.6A, Figure 4.6B and Figure 4.6C. The results showed that temperature also has a large influence on hemicellulose recovery in the liquid fraction. Figure 4.6B shows a decrease in the recovery of hemicellulose in the liquid fraction with increasing concentration until 75% where an increase is observed thereafter. The largest recovery in the liquid fraction is observed at higher concentration and longer time. In Figure 4.6C, it was observed that at lower concentrations, the effect of temperature is negligible. While at lower temperatures, the effect of concentration is negligible. However, at higher temperatures and concentrations, we notice an increase in the hemicellulose recovery in the liquid fraction. As expected, the recovery of hemicellulose is highly dependent on the severity of the experiment. This is in agreement with various other studies which have shown a dependence of hemicellulose on severity compared to the other components (Li et al., 2010). The graph of the interaction between concentration and temperature (Figure 4.7C) gives the maximum recovery of 24.6% at 100% concentration, 100.23 ^{o}C and time at the midpoint of 75 minutes; these are the best conditions for achieving high yields of recovered hemicellulose in the liquid fraction. This validates the results of the Pareto chart in Figure 4.3 where Temperature* Solvent concentration (BC) had the highest interactive influence on the response.

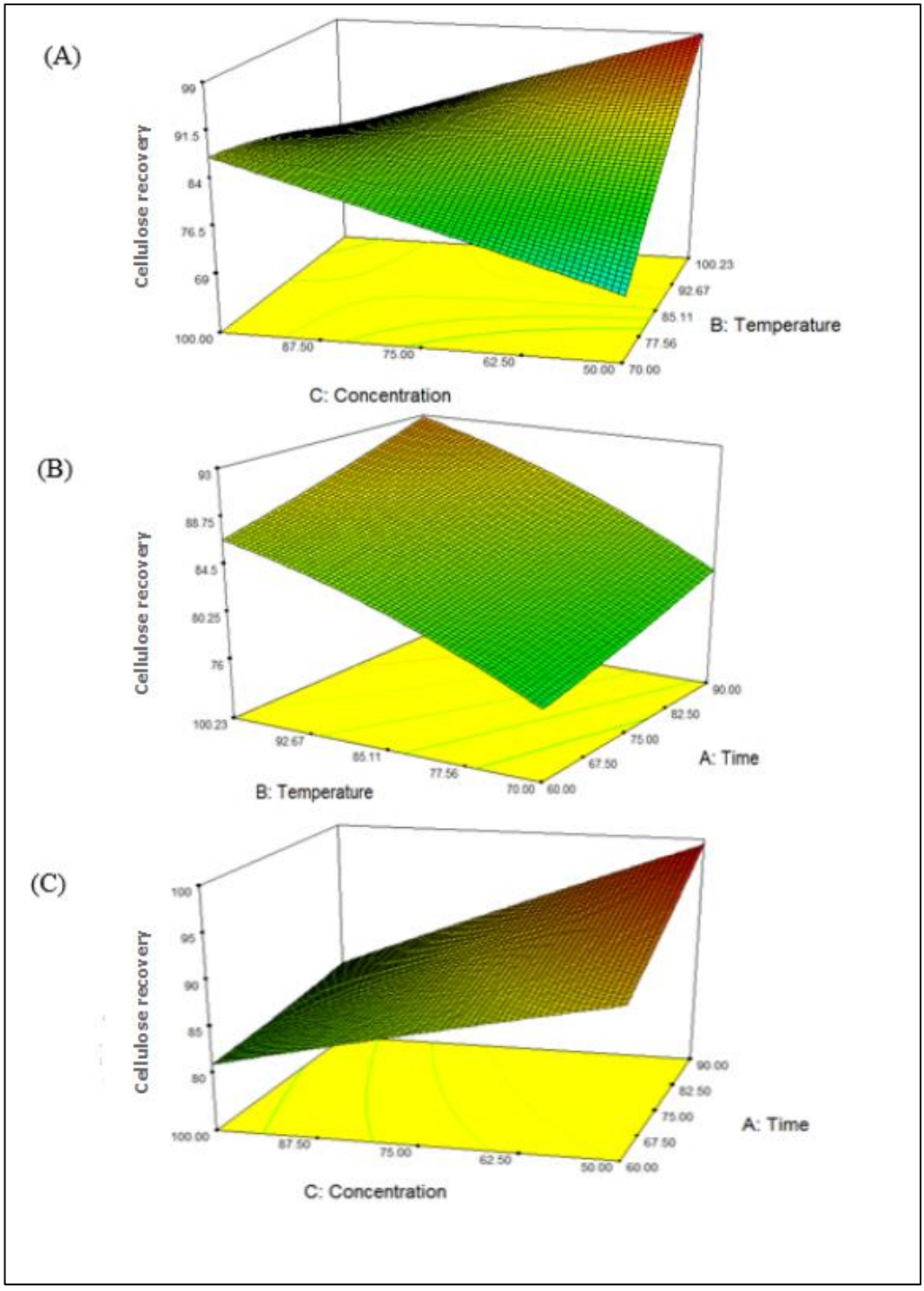


Figure 4.6 Response surface plot showing effect of pretreatment conditions on cellulose recovery. (A) Effect of solvent concentration and pretreatment temperature at retention time of 75 min. (B) Effect of pretreatment time and pretreatment temperature at solvent concentration of 75%/25% (w/w) $ZnCl_2.4H_2O$/ Urea. (C) Effect of solvent concentration and pretreatment time at retention temperature of 75 °C.

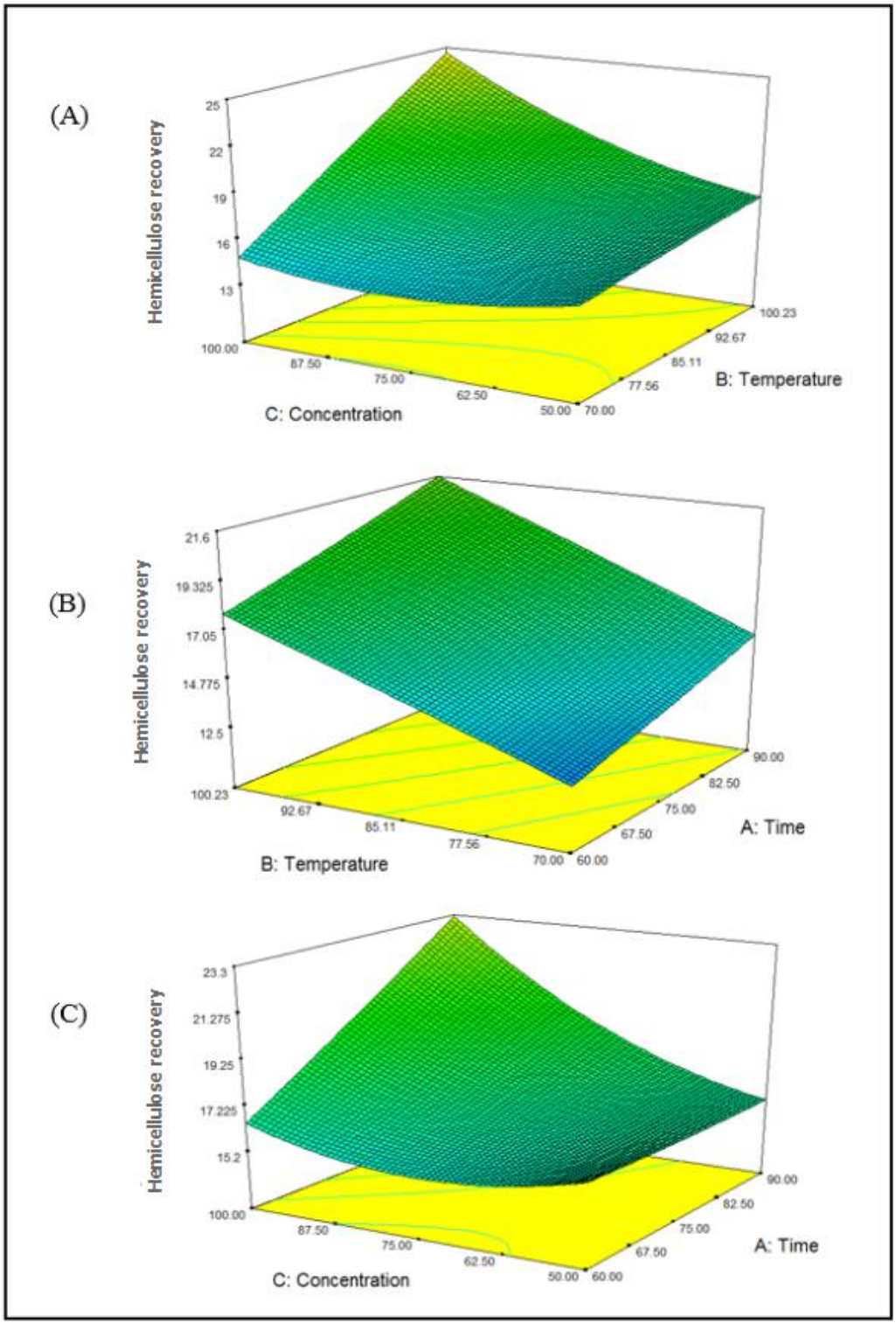


Figure 4.7 Response surface plot showing effect of pretreatment conditions on hemicellulose recovery. (A) Effect of solvent concentration and pretreatment temperature at retention time of 75 min. (B) Effect of pretreatment time and pretreatment temperature at solvent concentration of 75%/25% (w/w) $ZnCl_2.4H_2O$/ Urea. (C) Effect of solvent concentration and pretreatment time at pretreatment temperature of 75 °C.

Figure 4.8A represents the effects of temperature and time on lignin recovery at a constant solvent concentration of 75%. An increase in temperature resulted in a linear increase in lignin recovery. The length of time had no influence on the lignin recovery at lower temperatures; however, the lignin recovery was maximal at shorter times. At maximum temperatures, there was an increase from 62% to 66% lignin recovery as a result of the decrease in time from 90 min to 60 min at a temperature of 100.23 ^{o}C. Results in Figure 4.8 B show the effects of solvent concentration and time on lignin recovery at a constant temperature of 85.11 ^{o}C. An increase in time caused a linear decrease in lignin recovery at low solvent concentrations, however at higher solvent concentrations; there is a slight decrease in lignin recovery with increasing time. Solvent concentration at shorter times resulted in a decrease in lignin recovery until approximately 62.50% thereafter, an increase in lignin recovery was observed. Lignin recovery was maximal (60.2%) when concentration is maximal at 100% and time is 90 minutes. Figure 4.8C shows the effect of temperature and concentration on lignin recovery at a constant time of 75 min. Solvent concentration has a negligible effect on the recovery of lignin at lower temperatures. There is however a slight increase in lignin recovery with increasing concentration. Temperature has a linear effect on lignin recovery as the increase in temperature results in an increase in the response at both high and low solvent concentrations. Figure 4.8C shows the highest amount of recovery (69%) and this is in agreement with the Pareto chart (Figure 4.4) where BC is shown to have the highest interactive effect on lignin recovery.

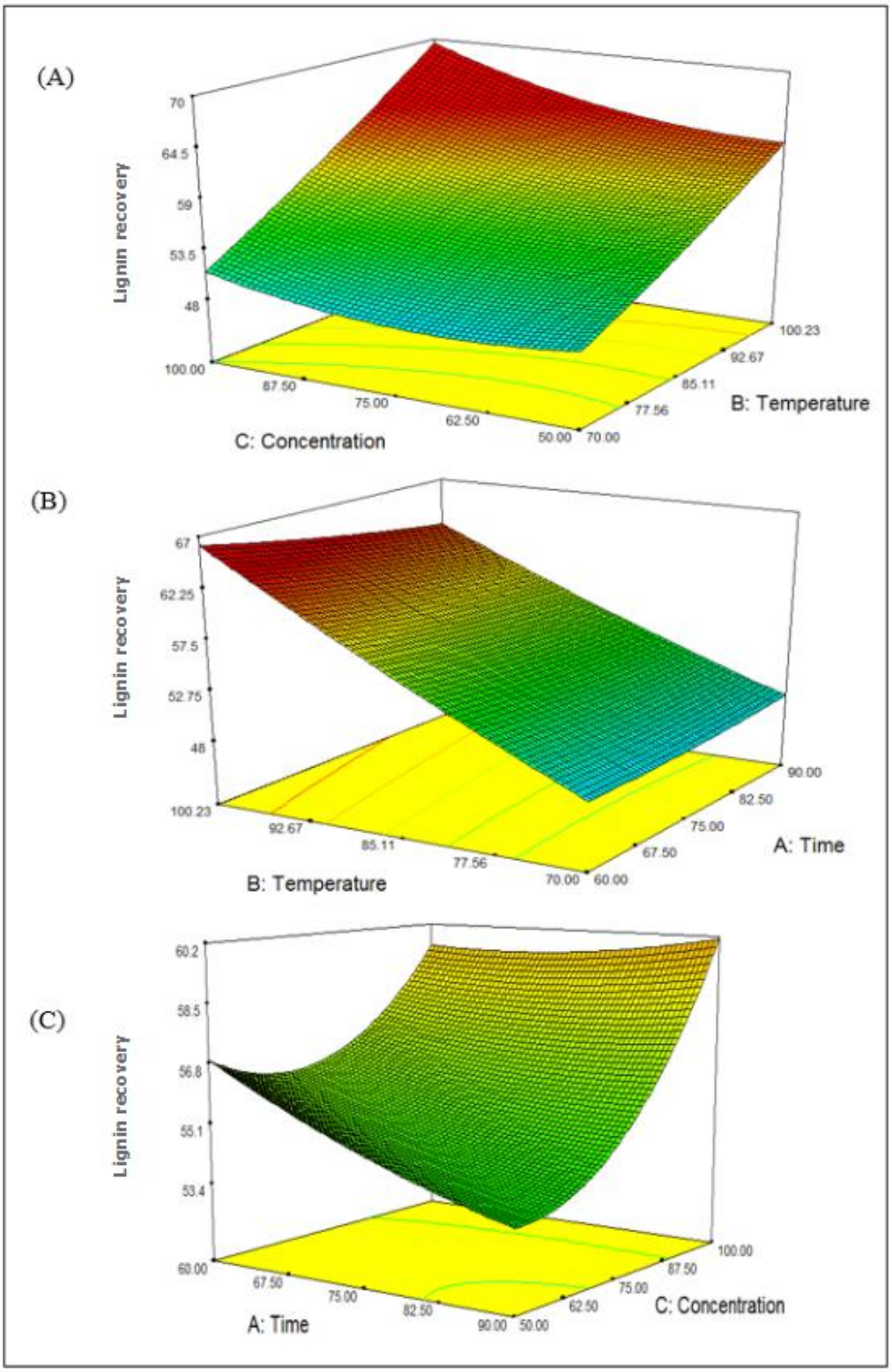


Figure 4.8 Response surface plot showing effect of pretreatment conditions on lignin recovery. (A) Effect of solvent concentration and pretreatment temperature at retention time of 75 min. (B) Effect of pretreatment time and pretreatment temperature at solvent concentration of 75%/25% (w/w) $ZnCl_2.4H_2O$/ Urea. (C) Effect of solvent concentration and pretreatment time at pretreatment temperature of 75 °C.

4.3.6 Optimisation by desirability function.

The mathematical model which was developed from the experimental data can be used for the effective prediction of the operating conditions which should be used for the optimisation of the model responses. The strategy that was used can determine the pretreatment temperature, pretreatment time and solvent concentration, resulting thereby in maximum responses. Desirability function (DF) assigns a scale-free value to all responses in the problem by the so-called individual desirability functions and then combine these values by taking usually their geometric mean to obtain an overall desirability function as a single objective. This multiple response numerical method was applied for optimisation of any combination of three factors (time, temperature, concentration). Figure 4.9 was obtained from design expert analysis and demonstrates the desirability values of the numerical optimisation procedure in which the criteria were set as follows: "in range" for time (60 min), "in range" for temperature (70-120 ^{o}C), "in range" for concentration (50-90%) the objective was to optimise (maximize) the responses, cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery to analyse economically viable optimal conditions. A variety of solutions were found however the best local maximum was shown as a desirability plot (Figure 4.9). The desirability plot shown in Figure 4.9 provides the optimised treatment conditions of 90 min, 120 ^{o}C and concentration of 71.32% (w/w) $ZnCl_2.4H_2O$/ Urea. At this condition, cellulose recovery was 99.03%, hemicellulose recovery in the liquid fraction was at 27.18% and lignin recovery was at 72.43% and desirability at 0.902. A full table of the other optimized conditions based on desirability can be found in appendix B. These optimum values were cross validated experimentally which resulted in 91% cellulose recovery, 29% hemicellulose recovery in the liquid fraction and 68% lignin recovery at the same conditions. These values indicate the suitability and accuracy of the model. Apparently, the recovery of cellulose needs different conditions compared to hemicellulose recovery in liquid fraction and lignin recovery. These were the best conditions to favourably optimise all three responses.

The optimised value for the solvent concentration is 71.32% (w/w) $ZnCl_2.4H_2O$/ Urea. This low solvent concentration is advantageous as it reduces the viscosity of the solvent. This reduces some challenges which are usually encountered with higher concentrations of MHS. The optimised temperature of 120 ^{o}C is higher than the glass transition temperature of both lignin and hemicellulose which are 40 ^{o}C and from 50 ^{o}C – 100 ^{o}C, respectively. This temperature is thus expected to delignify the biomass as well as increase the dissolution of

hemicellulose to an extent (Li et al., 2011). The pretreatment time of 90 min is desirable as it is expected to reduce the degradation of the polysaccharide components (cellulose and hemicellulose) which results in an increase in polysaccharide yield and decrease in fermentation inhibitors. The obtained desirable conditions differ largely from those obtained in the pretreatment of wheat straw using ionic liquid pretreatment. The conditions found in the study performed by Fu and Mazza (2011) were 158 °C, 49.5% ionic liquid concentration and 216 min. The large difference in the optimal conditions is caused by the responses for the optimisation being based on the recovery of fermentable sugars in the pretreatment of wheat straw as opposed to whole fractions of the components as calculated in this study (Fu and Mazza, 2011).

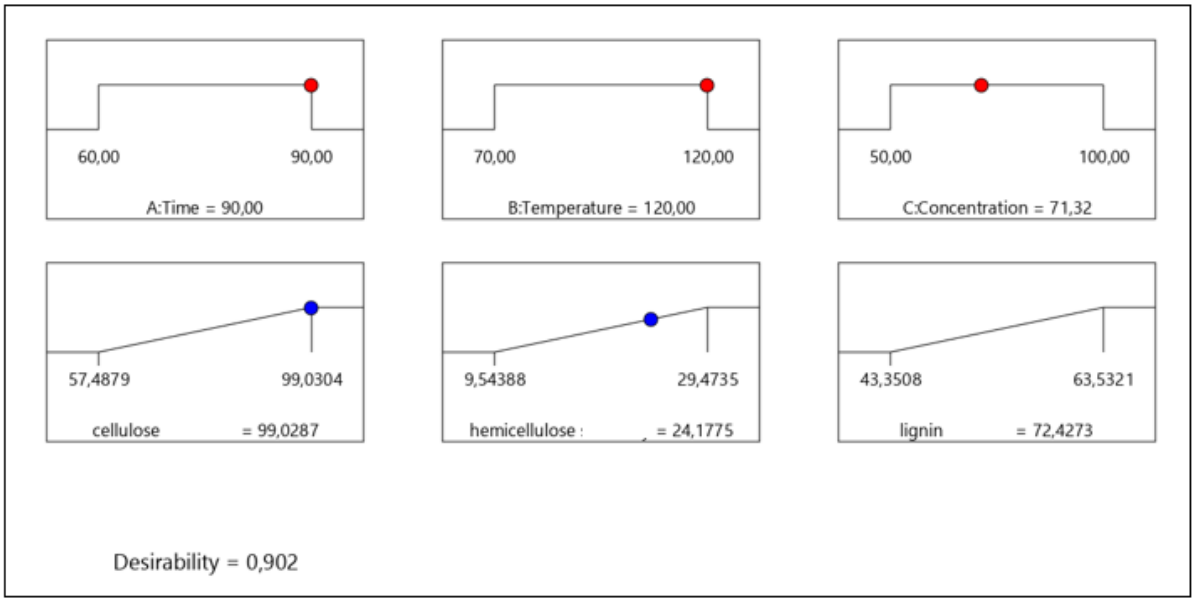


Figure 4.9 Desirability ramp of cellulose recovery, hemicellulose recovery in liquid fraction and lignin recovery

Mass balance calculations are necessary for the evaluation of the pretreatment efficiency of a method which is designed for lignocellulosic biomass fractionation (Hatzis et al., 1996). The mass balance of the whole process in Figure 4.10 was evaluated for the most effective operating conditions. It was also used to detect the changes in the main fractions of the biomass during MHS pretreatment as well as the separation and drying procedures which follow. Pretreatment conditions of 90 min, 120 ^{o}C and concentration of 71.32% (w/w) $ZnCl_2.4H_2O$/ Urea were used. For these pretreatment conditions, 206.4 g cellulose was recovered from 226.9 g cellulose input to the MHS pretreatment process; also, about 286.49 g

of hemicellulose was recovered from an input of 403.5 g. The lignin recovery was 101.15 g from an input of 316.1 g into the MHS pretreatment process. Approximately 240 g of total sugars were released in the liquid fraction during the MHS pretreatment process. This may include glucose, xylose and aromatic monomers of cellulose, hemicellulose and lignin respectively. The mass balance is not closed as there was loss in mass due to evaporation, human error and instrumentation errors.

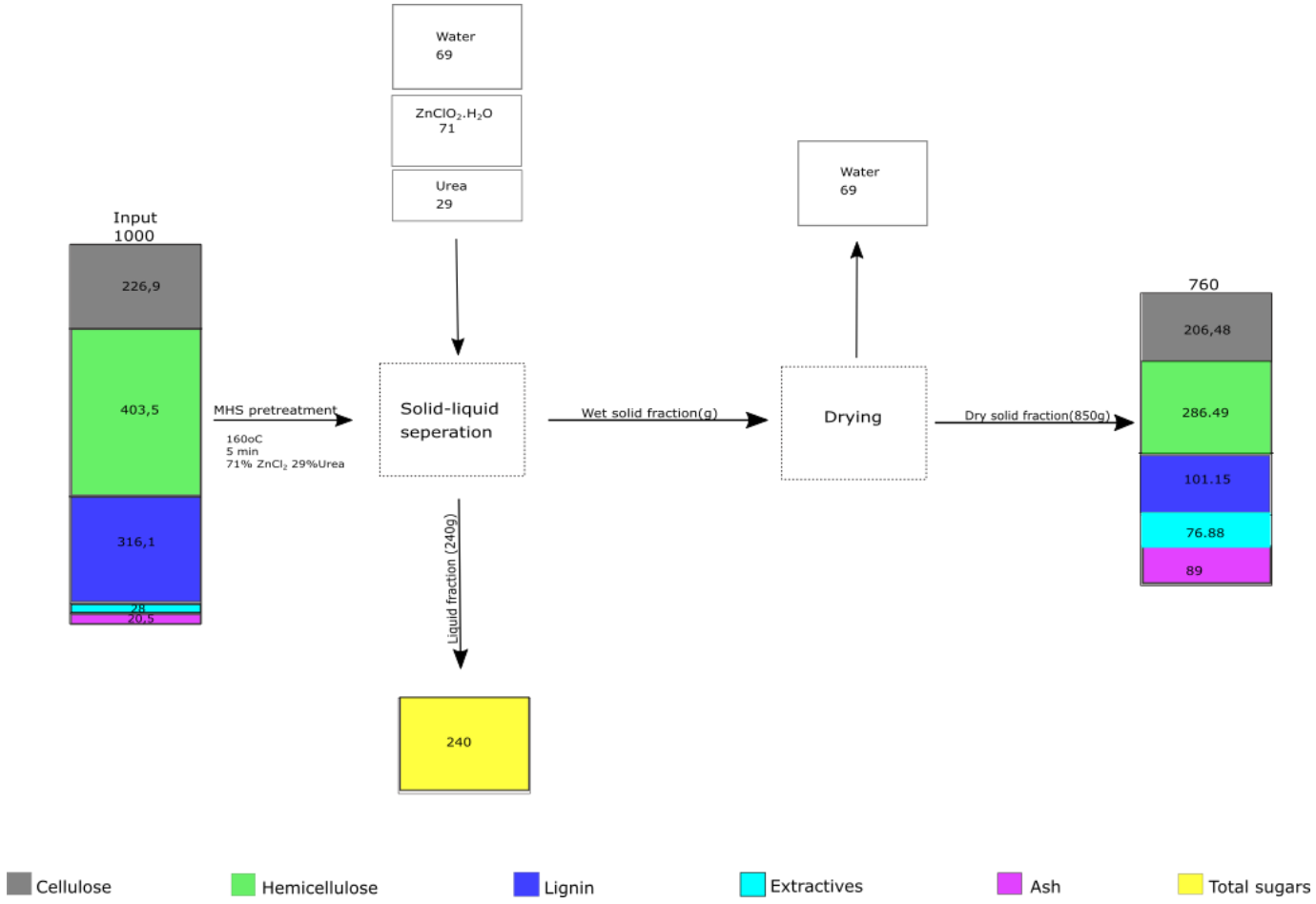


Figure 4.10 Mass balance of optimised molten hydrate salt pretreatment. The mass unit is grams (g)

CHAPTER FIVE

5. Kinetic study of corncob dissolution reactions in molten hydrate salts

5.1 Introduction

Pretreatment of lignocellulosic biomass with cellulose solvents such as ionic liquids and molten hydrate salts (MHS) under mild conditions has been found to effectively separate and break down hemicellulose, modify the lignin network and increase cellulose accessibility (Sathitsuksanoh et al., 2012). The use of these cellulose solvents are becoming widely investigated as they have the advantage of high boiling point, low vapour pressure and liquid status at moderate temperature. In addition, some molten salt hydrates are even able to swell and dissolve cellulose and biomass.

A key method for obtaining an in-depth understanding of the molten salt hydrate dissolution of biomass is the use of kinetic analysis. Kinetic modelling plays an important role in the design, development and operation of many chemical processes, kinetics of the reactions are also necessary to design and optimise efficient pretreatment processes (Dong et al., 2015). Corncob waste is mainly composed of cellulose, hemicellulose and lignin. During the course of the pretreatment, polysaccharides cellulose and hemicellulose are hydrolysed to monomers (glucose and xylose) and lignin is removed from the solid fractions. The recovery of cellulose, hemicellulose and lignin fractions during the pretreatment process are dependent on conditions of the process (Jacobsen and Wyman, 2000). In addition, precise process control is required to optimally fractionate the biomass as to increase the yield of products attained from the biorefinery.

In a recent study on the kinetic analysis of the recovery of lignin from corn stover was done by soaking with aqueous ammonia of different concentrations and temperatures between 30 °C and 70 °C (Gao et al., 2016). Also, there were several studies where pretreatment of corn stover was conducted at temperatures of 170 °C -230 °C and 150-210 °C using dilute acid and hot water for the recovery of lignin from the substrate. Several kinetic studies of corncob pretreatment with lime, hydrogen peroxide, sulphuric, formic and phosphorus acid have also been reported (Fuentes et al., 2011; Dong et al., 2015; Ayeni et al., 2016). In this study, a kinetic and thermodynamic analysis was reported on the molten hydrate salt dissolution of the major components (cellulose, hemicellulose and lignin) from corn cob biomass with

71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea pretreatment under wide temperature ranges of 50 °C－120 °C and pretreatment times from 0－120 min.

5.2 Materials and methods

5.2.1 Dissolution kinetics

Dissolution experiments were carried out in 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea. The reactions were carried out in a 250 mL Erlenmeyer flask at temperatures 50 ^oC, 70 ^oC, 90 ^oC, 120 ^oC at a biomass to solvent ratio of 1:10 (5g dry biomass will be added to 50g of each solvent) at agitation speed of 270 rpm. The reaction mixtures were run for 20, 40, 60, 80, 100, 120 min for each temperature. Once the reaction was stopped, the slurries were separated into solid and liquid fractions. The experimental procedures carried out in this chapter with regard to the preparation of materials, the reaction and compositional analysis were carried out the same as those mentioned in Chapter 3.

The recovery of each component in solid and liquid fraction is defined in Figure 5.1

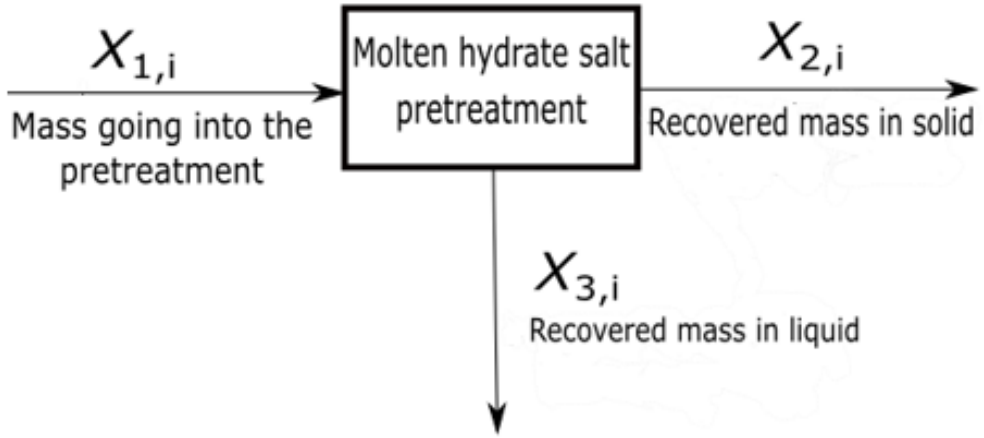


Figure 5.1 Mass flow diagram for MHS pretreatment

Where X is the measured weight; i is either the cellulose, hemicellulose or lignin. The recovery of each component can be calculated by the following equations:

$$\%\ Recovery\ of\ i\ in\ solid = \left(\frac{X_{3,i}}{X_{1,i}}\right) \times 100\% \quad (5.1)$$

$$\%\ Recovery\ of\ i\ in\ liquid = \left(\frac{X_{2,i}}{X_{1,i}}\right) \times 100\% \quad (5.2)$$

5.2.2 Dissolution kinetics study

The pretreatment process variables include temperature (°C) and time (min). These parameters influence the products through the kinetics of the reaction. Therefore, the information obtained from kinetics studies is essential in predicting product yields and optimising the process. Common models for pretreatment are kinetic models with a first order dependence of reaction rate on biomass components as well as an Arrhenius type relationship between temperature and rate constant.

To assess the rates and mechanism of the MHS dissolution of lignocellulose, a number of studies have been performed using a first order kinetic model (Fuentes et al., 2011). A single step reaction of lignocellulose to each component was assumed as unimolecular. It was also assumed that the sum of a series of elementary steps in the reaction system is represented by the overall reaction rate equation. Lastly, the steady state approximation was assumed, which implies that an intermediate in the dissolution reaction is consumed as quickly as it is formed and its concentration remains constant during the reaction.

5.2.2.1 Determination of kinetic parameters

The interaction between Lignin, Cellulose, Hemicellulose (A) and $ZnCl_2.4H_2O$/ Urea (B) to give solubilised product (C) was represented by the following stoichiometric relationship as given in Equation (5.3):

$$A + B \overset{k}{\rightarrow} C \tag{5.3}$$

The dissolution rate of component was assumed as a function of cellulose, hemicellulose and lignin recovery (A) from Equation (5.1) and time (t) as follows:

$$\frac{-dA}{dt} = k.A \tag{5.4}$$

Where $\frac{dA}{dt}$ = lignin, hemicellulose and cellulose recovery rate; k = chemical reaction rate constant; A = residual lignin, cellulose and hemicellulose after pretreatment,

In order to carry out the kinetic study, the reaction was done at different temperatures and the concentration of the products was determined at various time intervals. The rate law and the rate constant *(k*) was then determined at the temperature at which the reaction was performed. In order to determine the rate constant (*k*), a linear regression curve was created based on the equation below and *k* was determined from the slope of the line.

$$\ln[A] = -kt + \ln[A]_0 \tag{5.5}$$

Where A is total lignin, hemicellulose or cellulose content at time t, A_0 = total lignin, hemicellulose or cellulose content at time t = 0

After the rate law was determined, the reaction was then be studied at a wide temperature range. The rate constants at the different temperatures were then fitted to the Arrhenius equation (Fogler, 1999). Where the slope and the intercept of the plots depict the Ea/R and ln [A] in Equation (5.7), respectively. The Arrhenius equation is:

$$k = Ae^{-Ea/RT} \tag{5.6}$$

By taking the natural log for both sides of Equation (5.6), Equation (5.7) was obtained

$$\ln k = \frac{E_a}{RT} + lnA \quad (5.7)$$

Where k represents the rate constant of reaction (min^{-1}), A is the Pre-exponential factor (min^{-1}), Ea is the activation energy, ($J.mol^{-1}$), R is Gas Content, 8.314 $J.mol^{-1}.K^{-1}$ and T is Temperature, K.

5.2.2.2 Determination of thermodynamic parameters

To study the thermodynamics of the lignocellulose dissolution reaction, diverse parameters need to be determined as well.

The change in enthalpy of the process was calculated using the following equation:

$$\Delta H = E - RT \quad (5.8)$$

Where ΔH is the change in enthalpy of activation energy, E is the activation energy, R is Gas Content, 8.314 J/ mol.K and T is Temperature, K.

Eyring equation was used to calculate the entropies of activation (ΔS) from the values of enthalpy and the reaction rate constant. The equation is given as:

$$e^{\frac{\Delta S}{R}} = \frac{h}{k_b} T(ke^{\frac{\Delta H}{RT}}) \quad (5.9)$$

Where k_b is the Boltzmann's constant (1.381 x 10^{-23}J/K), T is temperature in Kelvin (K) and h is Planck's constant (6.626 x 10^{-34} Js).

The free energy change for the dissolution or Gibb's free energy (ΔG) was obtained using the following relation as shown:

$$\Delta G_{diss} = \Delta H_{diss} - T\Delta S_{diss} \quad (5.10)$$

5.3 Results and discussion

5.3.1 Dissolution kinetics of corn cob in $ZnCl_2.4H_2O$/ Urea MHS

The experimental data of cellulose, hemicellulose and lignin dissolution versus time using $ZnCl_2.4H_2O$/ Urea MHS at varying reaction temperatures to obtain the reaction rate constant (k) is shown in Figure 5.2. The results of the component dissolution show that hemicellulose dissolution has the greatest increase over time compared to the other components.

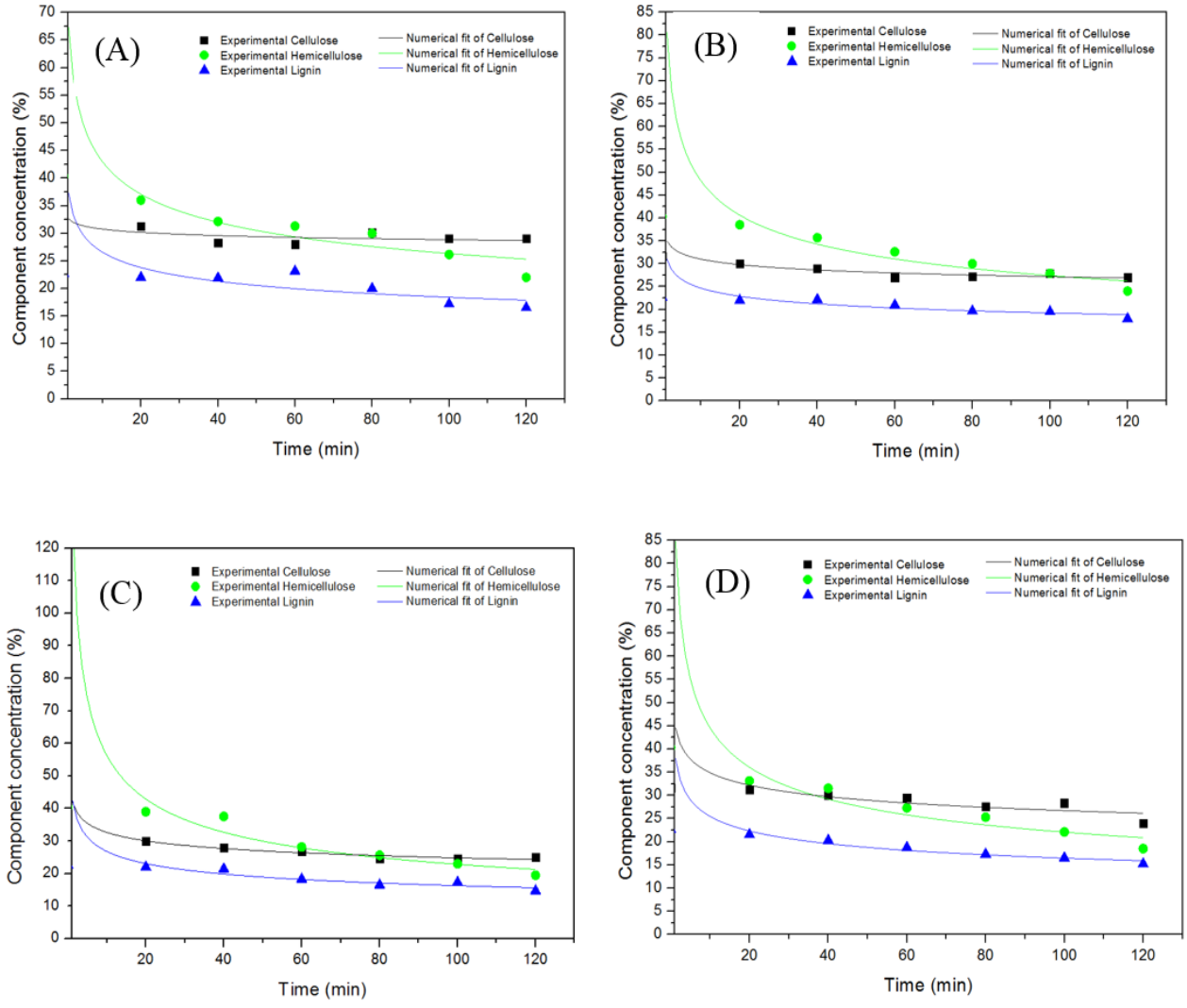


Figure 5.2: Reaction profiles of cellulose, hemicellulose and lignin dissolution using $ZnCl_2.4H_2O$/ Urea at (A) 50 ^oC (B) 70 ^oC (C) 90 ^oC (D) 120 ^oC

Figure 5.2 A-D shows the fit model prediction and experimental data for cellulose, hemicellulose and lignin dissolution at 50, 70, 90 and 120 °C using 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea. The diagrams indicate the effect of dissolution temperature and time on the concentration of cellulose, hemicellulose and lignin. The diagrams all show a decrease in concentration of each component with respect to time and to a smaller extent, temperature. The figures show the trend of steeper decreases in concentration for hemicellulose when compared to the other components (cellulose and lignin). It is evident from these results that higher temperature favours the dissolution of all the components. However, hemicellulose is more influenced by temperature as well as time than the other components this observation is drawn from the steep slope of the hemicellulose plots when compared to the other components. From Figure 5.2, it can be seen that at the different temperatures with regards to the reacting components, the modelled reaction follows first order kinetics for the range of the experiment studied. This was verified by curve fitting of the concentration-time data. The fit for all the plots have significantly low deviations from experimental data.

The component concentration of cellulose was lowest (24%) at 120 °C and highest (29%) at 70 °C which means at lower temperature severities cellulose is retained. This shows that temperature severity does not have a large effect on cellulose dissolution.

5.3.2 Reaction rates of component dissolution in $ZnCl_2.4H_2O$/ Urea MHS

The experimental result data obtained for $ZnCl_2.4H_2O$/ Urea MHS pretreatment of corn cob were fitted to an integrated pseudo first- order rate kinetics (Equation (2.3)). Figures 5.3 - 5.6 are plots of the natural log of concentration versus time for 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea pretreatment of corn cob at 50 °C , 70 °C , 90 °C and 120 °C respectively. From the plots, the rate of dissolution of cellulose, hemicellulose and lignin from corn cob is independent of concentration. The plots of the natural log versus time (t) resulted in straight lines for Figures 5.3-5.6, with each slope estimating the specific rate constant ‘k’. The straight line obtained in the figures for each temperature (50 - 120 °C) investigated suggests a first order rate of cellulose, hemicellulose and lignin dissolution in $ZnCl_2.4H_2O$/ Urea. The rate constant ‘k’ and the correlation coefficient ‘R’ for each plot is presented in Table 5.1.

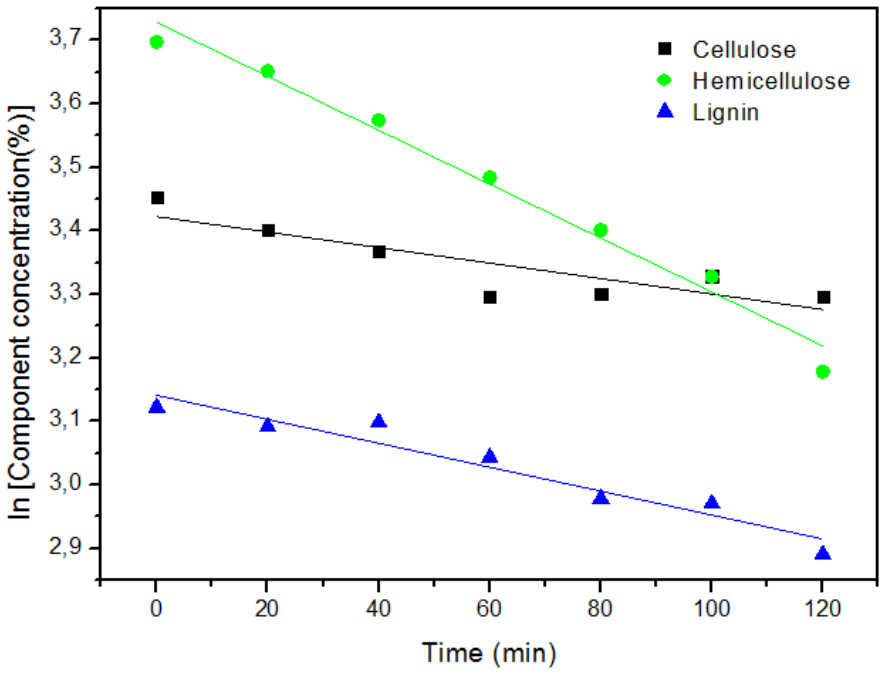


Figure 5.3 Kinetics of dissolution at 50 ^{o}C.

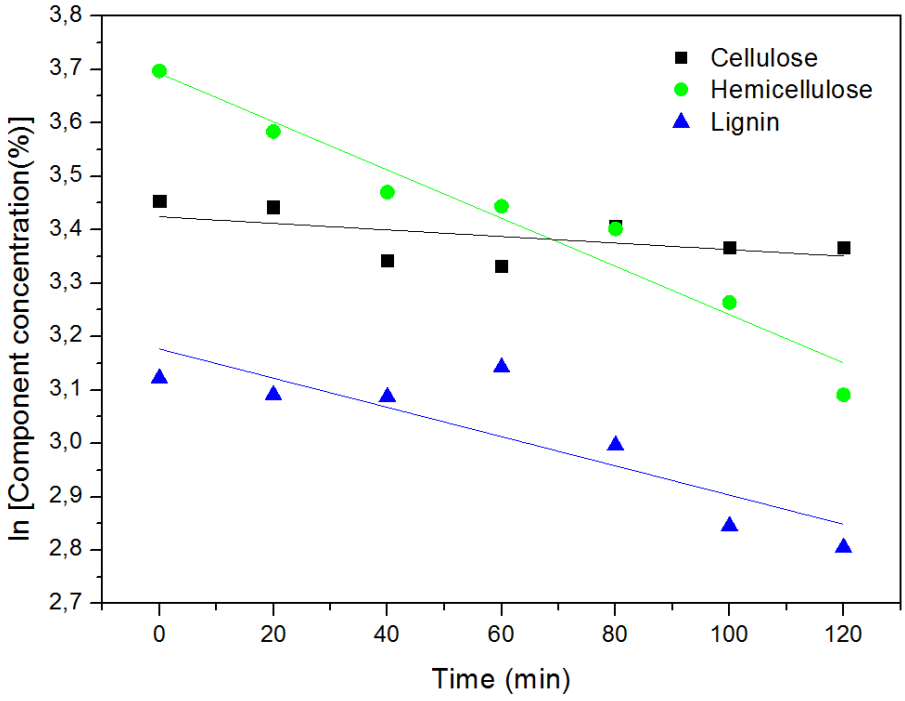


Figure 5.4 Kinetics of dissolution at 70 ^{o}C

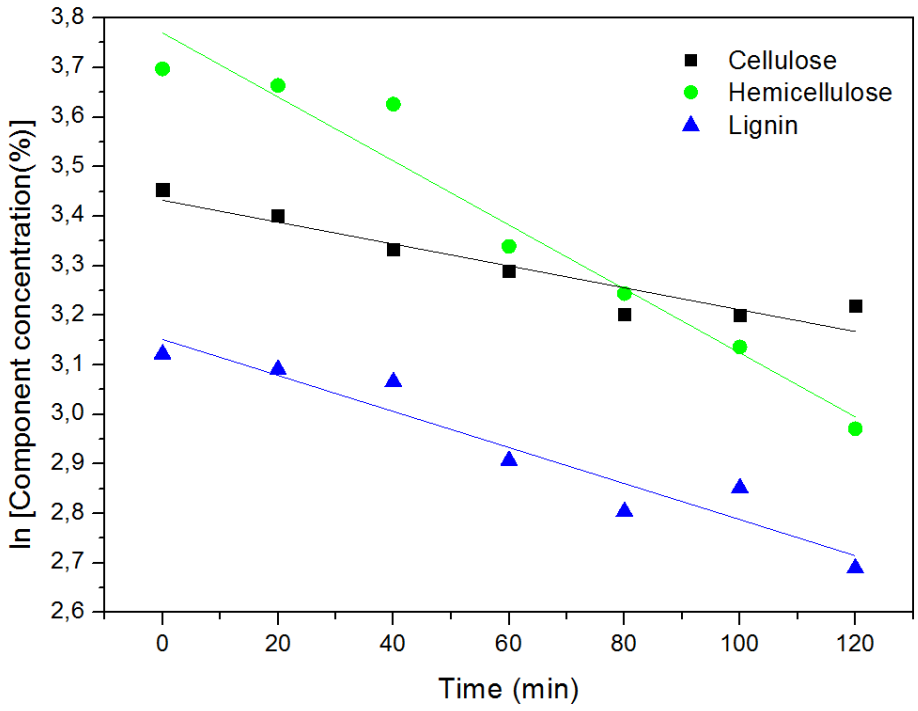


Figure 5.5 Kinetics of dissolution at 90 ^{o}C

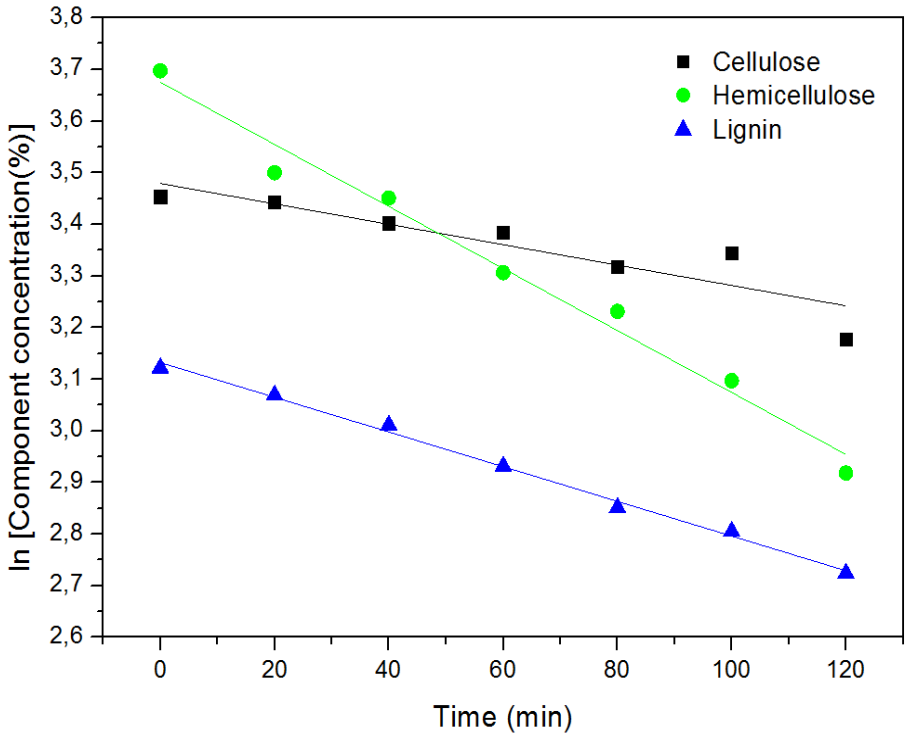


Figure 5.6 Kinetics of dissolution at 120 ^{o}C

Table 3.1 Estimated kinetics constants for $ZnCl_2.4H_2O$/ Urea pretreatment

T(K)	Cellulose		Hemicellulose (s^{-1})		Lignin (s^{-1})	
	k (s^{-1})	R^2	k (s^{-1})	R^2	k (s^{-1})	R^2
323	2.00×10^{-5}	0.8647	7.00×10^{-5}	0.9907	3.00×10^{-5}	0.9657
343	1.02×10^{-5}	0.5558	7.50×10^{-5}	0.9421	4.50×10^{-5}	0.8667
363	3.67×10^{-5}	0.9455	10.7×10^{-5}	0.9776	6.00×10^{-5}	0.9580
393	3.16×10^{-5}	0.9077	10.0×10^{-5}	0.9914	5.50×10^{-5}	0.9978

Notably, the temperature dependence of the rate constant is not observed, the rate constant increases as the reaction temperature does not increases proportionally from 50 ^oC to 120 ^oC for all the components. In the case of cellulose dissolution, the rate constant at 70 °C was lower than at 50 °C, at this temperature there is a low R^2 value of 0.5558 which indicates poor fit. This may be due to the method in which cellulose recovery is calculated from the difference of the other components, giving a margin of error. In addition, the errors may be due to the propagation errors of measuring devices. However, based on the results observed, 90 °C was considered the optimal temperature condition for cellulose, hemicellulose and lignin dissolution in $ZnCl_2.4H_2O$/ Urea MHS.

The rate constant values at the examined temperatures for cellulose were 2.00×10^{-5} (50 °C), 1.02×10^{-5} (70 °C), 3.67×10^{-5} (90 °C) and 3.16×10^{-5} (120 °C), respectively. The rate constants were generally higher at higher temperatures. However, a trend was not observed of proportional increase of rate with increasing temperature. There was a slight drop in the rate at the relatively lower temperatures (50 °C and 70 °C) which suggests that the crystalline regions of cellulose made up of the inter- and intra-molecular hydrogen bonds in cellulose, hinder cellulose dissolution at the lower temperatures as well as at higher temperatures when compared to hemicellulose and lignin.

A similar trend of increased reaction rates with increased temperatures was recorded in both the hemicellulose and lignin reaction rates. Where the rate constant increased from 7.00×10^{-5} to 7.50×10^{-5}, 10.7×10^{-5} and 10.0×10^{-5} as the reaction temperature was increased from 50 °C to 70 °C, 90 °C and 120 °C , respectively in hemicellulose dissolution. It is observed that hemicellulose dissolution occurs at a faster rate compared to the dissolution of the other two

components (lignin and cellulose). This is in agreement with the well-known fact that hemicellulose is more susceptible to pretreatment conditions and dissolution (Kumar et al., 2009). This is presumably due the ease of cleaving the hemicellulose backbone.

5.3.3 Activation energy and frequency factor determination

To determine the activation energy and frequency factor (A) for the dissolution reactions, the natural logarithm of k values at the different experimental temperatures are plotted versus the inverse of temperature (1/T) using Equation (5.6). The straight line in the plot indicates that the dissolution reactions follow Arrhenius law.

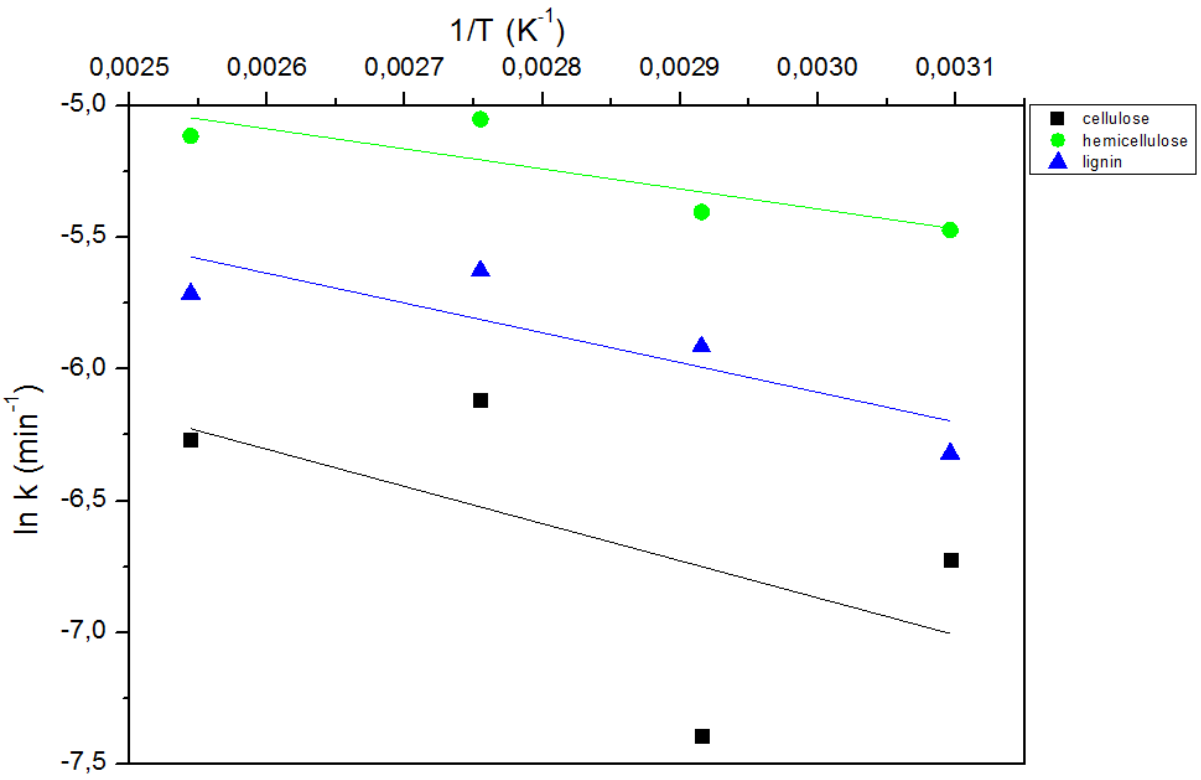


Figure 5.7 Arrhenius plot based on apparent component dissolution rate constants (k) at temperatures from 50 - 120 ^{o}C.

Figure 5.7 shows the plot of the rate constant fitted with the Arrhenius equation. Using Equation (5.7), the activation energy (Ea) of the component dissolution and frequency factors (A) were obtained from the slope and the intercept of plotting ln k versus 1/T. The activation energy and frequency factor are shown in Table 5.2.

Table 5.2 The activation parameters of lignocellulosic component dissolution

Component	*A* (min^{-1})	*E*a ($kJ.mol^{-1}$)	R^2
Cellulose	2.637	14.10	0.577
Hemicellulose	3.110	7.606	0.857
Lignin	2.699	11.29	0.859

The activation energy (Ea) and frequency factor (A) are 14.10 $kJ.mol^{-1}$ and 20×10^3 s^{-1} for cellulose respectively. This implies that the minimum energy necessary for the dissolution reaction of cellulose to occur using MHS is 14.10 $kJ.mol^{-1}$. This is the minimum amount of energy required to activate the molecules to initiate a chemical reaction during cellulose dissolution. When compared to cellulose dissolution by phosphoric acid (Zhang et al., 2009). The activation energy associated with dissolution of microcrystalline cellulose in phosphoric acid (83%) was 42.4 $kJ.mol^{-1}$. It was also reported that the activation energy associated with the dissolution of cotton linters pulp in NaOH/urea system was 101 $kJ.mol^{-1}$, which was much larger than *E*a shown in Table 5.2. This suggests that $ZnCl_2.4H_2O$/ Urea MHS solvents possess powerful dissolution towards cellulose. The R^2 for cellulose indicates a poor fit compared to the hemicellulose and lignin models. This poorer fit may be due to the loss in mass which is connected to the rapid degradation of cellulose under the prevailing conditions. Additional experimentation at other temperatures may be required to improve the fit.

The frequency factor value acquired from the intercept of the straight line indicates the collision rate of the reactant molecules at the applied operating conditions. The frequency factor is dependent on the reaction order and is thus affected by the reaction rate. The frequency factor is also dependent on the operating temperature. As the temperatures increase, the molecules tend to move faster, which leads to increase in the frequency of collisions. It was observed that hemicellulose dissolution has the highest frequency factor, followed by lignin and cellulose. The number of collisions in a minute occurring during the reaction is 2.637, 3.110 and 2.699 for cellulose, hemicellulose and lignin respectively. This may be due to the higher concentration of hemicellulose in the corn cob sample therefore resulting in more interactions.

Significantly larger activation energy of cellulose dissolution (compared to lignin and hemicellulose dissolution) shows the difficulty of cellulose dissolution in the range of temperature investigated in the experiments. This is attributed to the highly crystalline nature of cellulose. This is the reason for the higher reaction temperature necessary to initiate the chemical conversion upon the use of cellulose, as reported in Chapter 4. The higher activation energy also correlates with the temperature dependence of hemicellulose recovery found in Chapter 4 compared to the other components. It is evident that conversion of cellulose, lignin and hemicellulose in molten hydrate salts has different rate-limiting steps. In addition, the Ea value is an indication of the sensitivity of the reaction rate to temperature changes. At a relatively large activation (200 - 400 $kJ.mol^{-1}$), there is a sensitivity of the reaction rate to temperature. Such large Ea values are typical of combustion and gasification reactions which take place at high temperatures and are very slow at room temperature. For the biochemical reaction of $ZnCl_2.4H_2O$/Urea MHS in corn cob the Ea is relatively low (7-14 $kJ.mol^{-1}$) compared to gasification and combustion reactions due to the facile nature of the pretreatment. Thus, using MHS pretreatment may cut down the energy consumption in industrial processes (Bobleter, 1994; Garrote et al., 1999).

Finally, hemicellulose dissolution is much more significant than cellulose dissolution which is a major trait of the MHS pretreatment where the recovery of cellulose is optimised. In addition, Hemicellulose dissolution behaves more similar to lignin dissolution than cellulose dissolution. This is because of covalent bonds between hemicellulose and lignin present in the cell wall (Glasser et al., 2000).

5.3.4 Thermodynamic parameters determination

The calculated values of ΔG, ΔH and ΔS for the dissolution of each component are tabulated in Table 5.3. The enthalpies were all positive values which indicated that the nature of reactions were endothermic which means that energy is required in all dissolution reactions. The calculated values in Table 5.3 also show that a higher operating temperature (120 °C) required lower activation enthalpy than for lower temperature (50 °C) for dissolution of all the components. This shows that at higher temperatures, dissolution of the components can occur much quicker. The enthalpy gain from breaking H-bonds and van der Waals interactions provide enthalpy gains to the Gibbs free energy (ΔG) of dissolution which are important for cellulose dissolution in MHS solvents.

The values obtained tabulated in Table 5.3 indicated that by increasing the temperature, more stable end products could be achieved as a result of lower values of entropy. It is also important to note that all the entropy values are negative. The basic principles of thermodynamics, tell us that any product from a process with negative entropy is designated as "stable". This is because negative entropy represents increasing order in the system. Entropy can be thought of as a state of molecular "disorder". The values obtained tabulated in Table 5.3 indicated that by increasing the temperature, lower entropy values were obtained which indicate the production of stable end products. The trend was a general decrease in entropy for all the components; this is due to the fact that entropy changes favour the liquid state and thus the formation of a solution and thus the dissolution of the component. The hemicellulose dissolution has more negative entropy than the other components and on comparative basis, hemicellulose dissolution form relatively more stable products than lignin and cellulose dissolution. Cellulose dissolution forms less stable products however, cellulose dissolution is driven by the entropy gain upon the chain dissociation and hindered by the loss of solvent entropy (the solvent loses entropy by binding and causing an ordered structure). The entropy of dissolution favours dissociated cellulose chains over the crystalline form in $ZnCl_2.4H_2O$/ Urea. It is important to note that an increase in the molecular weight of the components especially cellulose due to its rigidity may cause a decrease in the entropy due to the formation of more ordered interaction between the components and solvents.

The Gibbs free energy change which occurs in any given process is an indication of the spontaneity of the process. Gibbs free energy changes of the components of lignocellulose in $ZnCl_2.4H_2O$/ Urea MHS are tabulated in Table 5.3. Positive values of Gibbs free energy for cellulose, hemicellulose and lignin dissolution indicates the non-spontaneous nature of the reaction. Non-spontaneous processes are those which occur with the addition of external energy, this explains and further shows the endothermic nature of the reactions. The ΔG values for dissolution of hemicellulose are higher than ΔG values for cellulose and lignin dissolution, which indicates that relative to hemicellulose, dissolution of cellulose and lignin are thermodynamically less favourable processes when compared to hemicellulose dissolution. This result is in correlation with the order of magnitude observed in the kinetic rate constants (Table 5.1) as well as the high recovery of cellulose, high recovery of hemicellulose and low lignin recovery observed in Chapter 3 for pretreatment done in $ZnCl_2.4H_2O$/Urea MHS pretreatment.

Table 5.3 Thermodynamic parameters of cellulose, hemicellulose and lignin dissolution in $ZnCl_2.4H_2O$/ Urea

Component	ΔH (kJ.mol^{-1})				ΔS (kJ.mol^{-1})				ΔG (kJ.mol^{-1})			
	ΔH_{323}	ΔH_{343}	ΔH_{363}	ΔH_{393}	ΔS_{232}	ΔS_{343}	ΔS_{363}	ΔS_{393}	ΔG_{323}	ΔG_{343}	ΔG_{363}	ΔG_{393}
Cellulose	11.414	11.248	11.082	10.832	-0.205	-0.210	-0.199	-0.199	54.930	60.940	61.300	67.750
Hemicellulose	4.920	4.754	4.588	4.338	-0.195	-0.193	-0.190	-0.190	58.060	61.760	64.570	70.490
Lignin	8.604	8.438	8.272	8.022	-0.202	-0.198	-0.195	-0.193	56.650	59.530	62.670	68.760

CHAPTER SIX

6. Conclusions and Recommendations

6.1 Conclusions

Lignocellulosic biomass is a prospective renewable energy resource that can be used to generate biochemical, bioproducts and biofuels. The major concern in lignocellulose conversion is the fractionation and pretreatment of biomass while still maintaining an energy efficient, green and cost-effective process. Corn cob is a promising energy crop with high polysaccharide content and good energy density. In this study, several pretreatment using molten hydrate salts (MHSs) ($ZnCl_2.4H_2O$, $LiClO_4.3H_2O$, Urea), binary ($LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$, $ZnCl_2.4H_2O$/ Urea, $LiClO_4.3H_2O$/Urea) and ternary ($LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$ /Urea) were developed and tested as solvents for the pretreatment of corn cob to improve subsequent enzymatic hydrolysis, separate cellulose, hemicellulose and lignin with high purity for value-added co-products and reduce the energy and chemical requirements.

The specific objectives of this dissertation were outlined in Chapter 1. The contributions in addressing these objectives are described below.

Objective 1

Investigate the effect of molten hydrate salt solvent systems on the pretreatment of corn cobs on the physical and chemical characteristics of corn cobs

The structural and chemical changes in the plant biomass before and after MHS pretreatment were analysed in terms of crystallinity (XRD, FTIR), morphology (SEM) and surface chemical composition (FTIR) of the biomass material.

All the seven MHSs investigated in this study are effective in altering the biomass crystallinity because of the significant decrease in Lateral Order Index (LOI) from 1.22 to 1.02 and the Crystallinity Index (CrI) from 54.13 to 77.38. SEM images revealed disordered and loose structures of biomass after pretreatment. FTIR analysis showed that MHS treated biomass exhibited a significant loss of native cellulose crystalline structure. It can be concluded that the seven MHS solvents proposed in this study are promising green solvents to reduce biomass and cellulose crystallinity and alter surface chemistry and surface

morphology during pretreatment step. The decrease of crystallinity and increase in surface area after the pretreatment will enhance the digestibility during enzymatic hydrolysis which will increase sugar yield for biochemical and biofuel production.

Objective 2

Identify the most efficient solvent system with respect to the recovery of cellulose, hemicellulose and from the biomass.

The fractionation of corn cob using the seven MHS pretreatment processes described were effective in separating the three components of biomass (cellulose, hemicellulose and lignin). Among the pretreatment solvents studied, $ZnCl_2.4H_2O$/ Urea pretreated samples was the most efficient in terms of highest cellulose recovery, highest hemicellulose recovery in the liquid fraction and highest lignin recovery. It resulted in significant cellulose recovery (100%), significant hemicellulose recovery in liquid fraction (42%) as well as lignin recovery (44%). The yield of these components depended on pretreatment efficiency. It is expected that this pretreatment can improve the utilization of biomass significantly due to the significant recovery yield of cellulose and lignin in the solid fraction and hemicellulose in the liquid fraction which shows that effective fractionation has occurred at mild conditions using a mild pretreatment solvent ($ZnCl_2.4H_2O$/ Urea).

Objective 3

Investigate the effect of dissolution parameters such as solvent concentration, reaction time and temperature on the pretreatment efficiency of the solvent system.

It is evident that optimising the conditions for the pretreatment process of $ZnCl_2.4H_2O$/ Urea in corn cob is required. To obtain optimum conditions, a statistical design of RSM known as CCD was employed which was able to reduce the number of experiments, while still being sufficient enough to describe the responses. Experimental results were analysed and appropriate predictive empirical linear models were developed for each response (cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery). The quadratic models representing all the responses were expressed as a function of time, temperature and solvent concentration. The model validation was accomplished using analysis of variance (ANOVA) as well as R^2 tests. The effects of the individual variables and their interactions

were found to be statistically significant. The results, however, showed that the quadratic terms among the investigated variables were statistically insignificant. For cellulose recovery, A (Time) and BC (Temperature * solvent concentration) were significant terms. Hemicellulose recovery in the liquid fraction showed significance of A (Time) and AC (Time * solvent concentration). For lignin recovery, the coefficients which are significant in the model are A (Time) and B (Temperature).

Objective 4

Optimise the pretreatment conditions of the pretreatment process to maximize cellulose recovery, hemicellulose recovery in the liquid fraction and delignification of the biomass.

Within the range of parameters studied, the optimum conditions established from the statistically based optimisation model and numerical optimisation were found at 90 minutes, 120 °C and 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea which resulted in 99.07% cellulose recovery, 24.17% hemicellulose recovery in the liquid fraction and 72.42% lignin recovery. To validate the optimised results, repeated confirmatory runs were carried out under optimised conditions. The results found experimentally were 91% cellulose recovery, 29% hemicellulose recovery in the liquid fraction and 68% lignin recovery at the same conditions. This study demonstrates optimisation of a lignocellulosic biomass pretreatment method that allows the production of value-added products from cellulose, hemicelluloses and lignin.

Objective 5

Develop a kinetic model to explain the dissolution behaviour of corncob the recovery of cellulose, recovery of hemicellulose in the liquid fraction and delignification of corn cobs.

The process of dissolution of cellulose, hemicellulose and lignin followed first-order reaction kinetics, thus the Arrhenius equation was chosen to describe the dissolution of lignocellulose step. The values of kinetic and thermodynamic parameters which were determined extensively demonstrated the importance of the process parameters (time and temperature) in the dissolution process of each component. The rate constant (k), activation energy (Ea) and pre-exponential factor (A) obtained for component dissolution reactions have the following

order of magnitude: Cellulose > Lignin> Hemicellulose. The rates were also found to increase with increasing temperatures. Activation energy obtained for the dissolution of cellulose is higher than the activation energy obtained during the dissolution of hemicellulose in 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea and follow an order of magnitude of Cellulose (14.10 $kJ.mol^{-1}$) > Lignin (11.29 $kJ.mol^{-1}$) > Hemicellulose (7.606 kJ. mol^{-1}). Thermodynamically, ΔH shows that all the reactions were endothermic. The hemicellulose dissolution reaction has a higher value of entropy change corresponding to more disorder in their structure which shows that the reaction occurs more rapidly and is more favourable in $ZnCl_2.4H_2O$/ Urea pretreatment.

6.2 Recommendations

The following recommendations were made for future work based on the findings of this study:

- To maximize the advantage of the $ZnCl_2.4H_2O$/ Urea fractionation processes, further studies for conversion of various other lignocellulosic biomass materials to various co-products are required.
- The results from this study are from lab scale experimentation. When scaled up, pilot scale processes can encounter several unanticipated problems which were not encountered during lab experimentation. Scale up studies as well as techno-feasibility studies are required to ensure technically feasible processes in industries.
- To understand the viability of the $ZnCl_2.4H_2O$/ Urea pretreatment process, a techno-economic analysis on this process is required.
- Using $ZnCl_2.4H_2O$/ Urea solvent as a medium for the efficient and direct production of (bio) chemicals in a one-pot process.

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Appendices

Appendix A: Compositional analysis

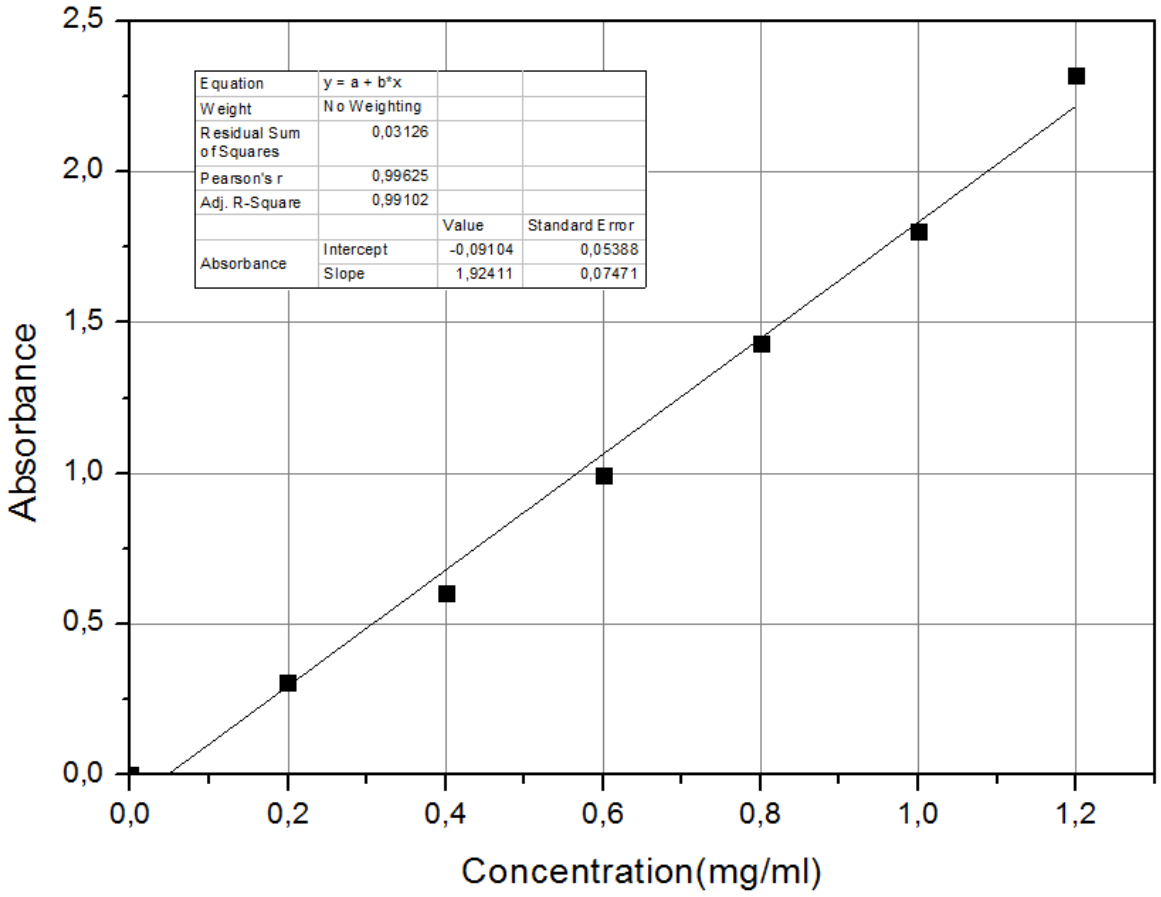

Figure A.1 Standard glucose concentration vs. absorbance

Table A.1 Concentration of sugars in pretreatment solvent

Pretreatment	Absorbance	Concentration(mg/mL)
$ZnCl_2.4H_2O$	0,217	0,267
$LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea	0,821	1,484
$LiClO_4.3H_2O$/Urea	0,756	1,353
$LiClO_4.3H_2O$	1,659	3,173
Urea	2,318	4,501
$LiClO_4.3H_2O/ZnCl_2.4H_2O$	0,178	0,188
$ZnCl_2.4H_2O$/ Urea	0,687	1,214

Appendix B: Optimisation

The value of actual and predicted removal percentage, leverage, internally studentized residuals, externally studentized residuals, DFFITS and Cook's distance of the data can be obtained from diagnostic case statistics .

Table B.1 Diagnostics case statistics for cellulose recovery.

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	81,15	83,10	-1,95	0,166	-0,466	-0,447	0,004	-0,200	17
2	57,49	54,66	2,83	0,707	1,139	1,158	0,312	1,797	1
3	74,78	72,24	2,54	0,671	0,967	0,964	0,190	1,375	2
4	94,56	88,27	6,29	0,606	2,186	2,870	0,734	3,558(1)	14
5	80,19	83,10	-2,91	0,166	-0,695	-0,676	0,010	-0,302	15
6	86,73	83,10	3,63	0,166	0,868	0,857	0,015	0,383	19
7	88,87	85,73	3,15	0,646	1,154	1,176	0,244	1,590	3
8	96,73	95,02	1,71	0,419	0,488	0,469	0,017	0,398	12
9	83,77	87,56	-3,79	0,707	-1,528	-1,655	0,562	-2,569(1)	6
10	78,35	83,10	-4,75	0,166	-1,134	-1,153	0,026	-0,515	16
11	71,99	77,37	-5,38	0,721	-2,220	-2,957	1,273(1)	-4,753(1)	13
12	87,21	83,10	4,11	0,166	0,982	0,980	0,019	0,438	20
13	80,92	81,57	-0,6470	0,827	-0,339	-0,323	0,055	-0,706	5
14	73,06	74,64	-1,58	0,606	-0,549	-0,529	0,046	-0,656	9
15	88,36	91,83	-3,47	0,646	-1,275	-1,321	0,297	-1,787	8
16	64,43	65,30	-0,8657	0,600	-0,299	-0,285	0,013	-0,349	11
17	84,80	83,10	1,70	0,166	0,407	0,390	0,003	0,174	18
18	80,18	83,37	-3,19	0,707	-1,286	-1,336	0,399	-2,076	7
19	99,03	96,53	2,50	0,721	1,031	1,034	0,274	1,662	10
20	95,10	95,02	0,0742	0,419	0,021	0,020	0,000	0,017	4

(1) Exceeds limits.

Table B.2 Diagnostics case statistics for hemicellulose recovery in the liquid fraction.

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	20,44	16,08	4,36	0,166	2,012	2,475	0,081	1,106	17
2	15,22	15,10	0,1213	0,707	0,094	0,090	0,002	0,139	1
3	16,73	14,02	2,70	0,671	1,986	2,422	0,803	3,455(1)	2
4	20,75	20,44	0,3176	0,606	0,213	0,203	0,007	0,251	14
5	14,73	16,08	-1,35	0,166	-0,624	-0,604	0,008	-0,270	15
6	13,17	16,08	-2,91	0,166	-1,345	-1,409	0,036	-0,630	19
7	17,42	16,46	0,9618	0,646	0,682	0,662	0,085	0,896	3
8	19,46	20,50	-1,04	0,419	-0,576	-0,555	0,024	-0,472	12
9	19,14	19,08	0,0584	0,707	0,045	0,043	0,000	0,067	6
10	16,90	16,08	0,8234	0,166	0,380	0,363	0,003	0,162	16
11	14,01	15,77	-1,76	0,721	-1,404	-1,487	0,509	-2,390(1)	13
12	15,22	16,08	-0,8596	0,166	-0,397	-0,380	0,003	-0,170	20
13	10,15	9,13	1,02	0,827	1,033	1,037	0,508	2,263(1)	5
14	12,74	12,50	0,2428	0,606	0,163	0,155	0,004	0,192	9
15	29,47	28,57	0,8990	0,646	0,637	0,617	0,074	0,835	8
16	9,54	11,37	-1,83	0,600	-1,220	-1,255	0,224	-1,538	11
17	16,27	16,08	0,1890	0,166	0,087	0,083	0,000	0,037	18
18	15,27	16,95	-1,68	0,707	-1,312	-1,368	0,416	-2,126(1)	7
19	19,68	21,37	-1,69	0,721	-1,345	-1,410	0,467	-2,265(1)	10
20	21,93	20,50	1,43	0,419	0,790	0,774	0,045	0,657	4

(1) Exceeds limits.

Table B.3 Diagnostics case statistics for lignin recovery.

Run Order	Actual Value	Predicted Value	Residual	Leverage	Internally Studentized Residuals	Externally Studentized Residuals	Cook's Distance	Influence on Fitted Value DFFITS	Standard Order
1	52,33	50,52	1,82	0,166	0,697	0,678	0,010	0,303	17
2	45,90	45,76	0,1453	0,707	0,094	0,089	0,002	0,138	1
3	48,61	48,79	-0,1752	0,671	-0,107	-0,101	0,002	-0,145	2
4	55,44	56,11	-0,6724	0,606	-0,375	-0,358	0,022	-0,444	14
5	49,32	50,52	-1,20	0,166	-0,458	-0,440	0,004	-0,196	15
6	51,74	50,52	1,22	0,166	0,469	0,450	0,004	0,201	19
7	63,18	59,65	3,53	0,646	2,076	2,611	0,788	3,531(1)	3
8	56,41	61,54	-5,13	0,419	-2,356	-3,350	0,400	-2,845(1)	12
9	50,70	52,78	-2,08	0,707	-1,341	-1,405	0,433	-2,181(1)	6
10	50,02	50,52	-0,4976	0,166	-0,191	-0,181	0,001	-0,081	16
11	49,94	51,32	-1,38	0,721	-0,914	-0,906	0,216	-1,456	13
12	51,60	50,52	1,08	0,166	0,415	0,397	0,003	0,177	20
13	44,64	43,19	1,45	0,827	1,220	1,254	0,709	2,738(1)	5
14	49,19	52,51	-3,32	0,606	-1,847	-2,159	0,524	-2,676(1)	9
15	63,11	61,81	1,31	0,646	0,769	0,752	0,108	1,017	8
16	43,35	42,26	1,09	0,600	0,603	0,583	0,055	0,714	11
17	48,44	50,52	-2,08	0,166	-0,797	-0,781	0,013	-0,349	18
18	62,99	61,36	1,63	0,707	1,052	1,058	0,267	1,644	7
19	56,69	55,43	1,26	0,721	0,836	0,822	0,180	1,321	10
20	63,53	61,54	1,99	0,419	0,914	0,905	0,060	0,769	4

(1) Exceeds limits.

Table B.4 Desirability table

100 Solutions found

Number	Time	Temperature	Concentration	cellulose recovery	hemicellulose solubility	lignin recovery
1	75,590	118,889	82,000	103,157	23,972	74,155
2	83,834	116,100	76,205	101,711	23,215	69,935
3	61,564	118,549	81,612	107,055	21,396	76,500
4	62,679	114,712	97,716	105,337	24,478	76,692
5	61,714	116,340	81,881	106,288	21,072	75,225
6	86,332	111,458	93,907	97,465	28,051	71,577
7	89,052	119,581	84,675	99,210	27,488	72,681
8	83,004	117,013	92,924	98,880	28,434	74,554
9	85,586	119,833	73,289	103,020	23,274	70,418
10	68,218	117,534	82,999	104,722	22,657	74,978
11	66,173	116,212	76,352	105,377	20,658	73,382
12	73,162	119,621	78,306	104,564	22,723	74,175
13	75,124	113,702	80,069	102,223	22,396	71,208
14	71,226	119,870	70,746	106,302	20,717	73,072
15	74,033	109,660	99,152	100,671	26,224	72,764
16	81,275	115,826	78,436	101,720	23,375	70,815
17	66,344	117,856	71,319	106,418	19,966	73,278
18	86,733	113,849	89,303	98,249	27,236	71,608
19	68,878	119,302	79,500	105,409	22,269	75,120
20	83,388	117,580	76,227	102,166	23,413	70,673
21	77,043	113,539	92,650	100,467	26,094	73,420
22	89,058	113,338	95,095	96,580	29,630	72,498
23	87,661	119,851	77,251	101,570	24,804	71,040
24	67,254	116,163	76,923	105,066	20,920	73,256
25	89,499	111,954	87,629	97,686	26,798	69,963
26	85,030	118,841	75,398	102,347	23,644	70,672
27	74,633	115,845	70,740	104,135	20,584	70,420
28	60,820	115,025	78,493	106,138	20,076	74,067
29	63,830	107,301	97,106	103,656	22,608	71,718
30	69,573	109,245	97,816	102,109	24,657	72,623
31	82,434	119,962	73,950	103,476	23,030	71,339
32	71,084	119,256	70,814	106,086	20,632	72,822
33	74,541	114,158	78,195	102,673	21,948	71,156
34	69,083	114,031	83,212	103,515	22,167	72,942
35	60,159	119,408	75,404	108,153	20,124	76,186
36	72,411	118,640	67,448	106,120	20,012	71,509

37	80,652	116,598	72,806	103,064	21,944	69,974
38	82,080	119,866	78,417	102,480	24,247	72,502
39	78,271	116,393	88,305	100,993	25,761	73,839
40	65,752	117,088	75,765	105,845	20,622	73,827
41	84,506	116,891	94,091	98,189	29,160	74,574
42	79,621	109,587	97,969	98,913	27,228	72,070
43	88,938	108,301	96,597	96,291	28,683	70,388
44	**65,820**	**113,302**	**77,013**	**104,319**	**20,260**	**71,994**
45	88,149	112,069	91,699	97,346	27,888	71,160
46	62,557	107,745	96,882	104,151	22,387	72,063
47	72,787	113,013	92,005	101,742	24,842	73,505
48	77,609	115,256	78,148	102,365	22,612	71,143
49	78,864	116,959	71,806	103,667	21,516	70,274
50	76,788	118,366	75,954	103,771	22,473	72,295
51	89,123	111,133	97,195	96,211	29,767	71,916
52	85,822	109,896	92,968	97,604	27,203	70,638
53	65,349	118,915	73,834	106,808	20,463	74,522
54	83,957	107,775	99,170	97,440	28,150	71,018
55	88,195	118,135	98,810	95,959	32,205	75,944
56	82,959	119,113	72,884	103,399	22,674	70,590
57	64,781	118,911	75,234	106,803	20,660	74,906
58	88,125	108,179	99,541	96,206	29,456	71,041
59	74,660	112,351	98,529	100,688	26,970	74,178
60	87,231	119,959	76,515	101,877	24,522	70,975
61	89,623	113,573	96,200	96,218	30,238	72,827
62	77,818	119,969	67,077	105,851	20,678	70,723
63	71,097	114,877	83,372	103,210	22,728	73,118
64	62,554	113,906	92,477	105,224	22,974	75,349
65	81,503	114,268	78,096	101,330	23,032	69,984
66	87,489	109,557	97,902	96,575	29,143	71,424
67	65,531	119,328	74,732	106,826	20,727	74,875
68	78,549	114,261	75,743	102,220	21,989	69,985
69	86,420	118,576	74,531	102,242	23,553	70,045
70	88,126	107,626	99,133	96,240	29,146	70,668
71	79,347	113,275	89,221	100,128	25,515	72,271
72	66,945	116,763	88,323	104,494	23,551	75,761
73	74,002	106,048	97,983	100,281	24,854	70,437
74	73,447	110,697	89,307	101,261	23,711	71,629
75	70,748	115,685	79,019	103,909	21,816	72,762
76	64,842	117,316	93,763	104,993	24,626	77,400
77	62,052	119,766	74,807	107,850	20,327	75,875

78	70,307	109,493	82,879	101,869	21,395	70,235
79	84,976	106,523	99,992	97,052	28,295	70,458
80	89,983	112,031	95,613	96,219	29,670	71,892
81	85,582	118,697	79,974	101,104	25,094	71,682
82	85,063	119,384	96,823	97,426	30,988	76,474
83	89,777	119,733	79,204	100,571	25,775	71,095
84	71,271	108,323	94,165	101,366	23,871	71,338
85	80,109	109,738	90,258	99,292	25,104	70,565
86	88,663	109,308	98,721	96,141	29,650	71,394
87	62,623	118,988	74,882	107,398	20,305	75,337
88	78,485	117,264	93,490	100,248	27,644	75,442
89	70,736	112,047	81,378	102,614	21,656	71,287
90	70,212	111,050	81,842	102,402	21,474	70,912
91	84,060	114,846	97,278	97,756	29,612	74,342
92	88,694	114,636	82,213	99,253	25,519	69,928
93	74,885	113,742	74,484	102,856	21,100	70,146
94	62,385	113,823	82,281	105,253	20,793	73,708
95	64,238	108,663	84,218	103,046	20,450	70,734
96	72,893	108,668	98,354	100,952	25,436	72,119
97	79,565	119,769	73,236	104,144	22,396	71,711
98	61,704	113,607	97,186	105,541	23,820	75,992
99	67,982	119,137	75,841	106,038	21,276	74,474
100	61,544	106,750	98,086	104,410	22,140	71,635

International Journal of Industrial Chemistry

Kinetic and thermodynamic study of dissolution of corn cob in ZnCl2.4H2O/ Urea solvent system

--Manuscript Draft--

Manuscript Number:	
Full Title:	Kinetic and thermodynamic study of dissolution of corn cob in ZnCl2.4H2O/ Urea solvent system
Article Type:	Research
Funding Information:	National Research Foundation (CSRU180415320913) — Prof Michael Daramola
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Kinetic and thermodynamic study of dissolution of corn cob in $ZnCl_2.4H_2O$/ Urea solvent system.

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Abstract

The main concern associated with the conversion of lignocellulose is overcoming biomass recalcitrance using pretreatment while still maintaining a green, cost-effective and energy efficient process. A pretreatment solvent ($ZnCl_2.4H_2O$/ Urea) was previously optimised for the effective fractionation of biomass. Further kinetic and thermodynamic parameters of dissolution were determined in this study. Dissolution experiments were carried out in 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea. The reactions were carried out in a 250 mL Erlenmeyer flask at temperatures 50 ^oC, 70 ^oC, 90 ^oC, 120 ^oC at a biomass to solvent ratio of 1:10. The results reveal the dissolution to be a 1st order kinetics and the obtained activation energy for cellulose, hemicellulose and lignin dissolution were 14.10 $kJ.mol^{-1}$, 11.29 $kJ.mol^{-1}$ and 7.606 $kJ.mol^{-1}$, respectively. The positive values for ΔH and ΔG indicate that the dissolution process for all three components are endothermic and endergonic. The less negative ΔS for hemicellulose dissolution (-0.190; -0.195 $kJ.mol^{-1}$) showed that the process of dissolution of hemicellulose occurred more rapidly and produced more stable products.

Keywords*:* Lignocellulose, dissolution, Kinetics, Thermodynamics

1 Introduction

Due to the world's increased energy and petrochemical demand, insecurity in energy supply, the increase fossil fuel price as well as increased environmental pollution, alternative sources of energy which can replace conventional fossil fuels need to be investigated [1]. These alternative sources include proposed biorefinery solutions which seek to convert waste lignocellulosic biomass to fuels, materials and platform chemicals currently produced from oil refinery [2]. Lignocellulosic biomass are agricultural residues, such as corn cobs and corn stover as well as quickly grown grasses or plants, such as switchgrass. These kinds of biomass are abundant, do not interfere with food crops and require less energy input [1]. Lignocellulosic biomass is composed primarily of cellulose, hemicellulose and lignin. The lignocellulosic biomass cell wall is a combination of crystalline cellulose fibrils joined together by amorphous hemicellulose which is cross-linked with lignin.

Bioconversion of biomass involves three main steps involved in the bioconversion of lignocellulosic biomass to fuels and chemicals [3]. These main steps being pretreatment, hydrolysis and fermentation. The bioconversion of biomass is hindered by its recalcitrance which is caused by the inherent association of cellulose, hemicelluloses and lignin in the lignocellulosic biomass [4]. To overcome this recalcitrance, a pretreatment process is required. The main purpose of the pretreatment step is to break down and fractionate the matrix of hemicellulose and lignin in order to expose the cellulose and hemicellulose to enzymatic degradation thereby

increasing substrate accessibility [5]. Developing effective pretreatment techniques and discovering the optimal operating conditions of these techniques can contribute to overcoming the cost barriers associated with the use of biomass. Pretreatment is able to influence downstream costs as it determines fermentation toxicity, enzymatic hydrolysis rate, as well as enzyme loading [4]

Pretreatment of lignocellulosic biomass with cellulose solvents such as ionic liquids and molten hydrate salts (MHS) under mild conditions has been effective in separating and breaking down hemicellulose, increasing cellulose accessibility and modifying the lignin network [6]. Ionic liquids are known for their ability to dissolve cellulose, hemicellulose, lignin and even raw lignocellulose directly at low temperature. For these reasons, they have been used as solvents for biomass green conversion. However, ionic liquids still have the disadvantage of being expensive and their recovery and reuse is still challenging. Inorganic molten salt hydrates, are a kind of green solvent ionic liquid-like system with similar physicochemical properties to ionic liquids [7]. Molten hydrate salts are known to have excellent ability to dissolve polysaccharides, such as cellulose and hemicellulose, in an inexpensive and recyclable manner [8]. However, their use in the pretreatment and fractionation of lignocellulose is still currently being researched at infancy stage, there are limited accounts in the scientific literature of dissolution of whole lignocellulosic biomass using molten hydrate salts in relation to biomass fractionation.

A key method for obtaining an in-depth understanding of the molten salt hydrate dissolution of biomass is the use of kinetic analysis. Kinetic modelling plays an important role in the design, development and operation of many chemical processes. Kinetics of the reactions are also necessary to design and optimise efficient pretreatment processes [9]. Corncob waste is mainly composed of cellulose, hemicellulose and lignin. During the course of the pretreatment, polysaccharides cellulose and hemicellulose are hydrolysed to monomers (glucose and xylose) and lignin is removed from the solid fractions [10]. The recovery of cellulose, hemicellulose and lignin fractions during the pretreatment process are dependent on conditions of the process. In addition, precise process control is required to optimally fractionate the biomass as to increase the yield of products attained from the biorefinery [4].

In a recent study on the kinetic analysis of the removal of lignin from corn stover was done by soaking with aqueous ammonia of different concentrations and temperatures between 30 °C and 70 °C [11]. Also, there were several studies where pretreatment of corn stover was conducted at temperatures of 170 °C -230 °C and 150-210 °C using dilute acid and hot water for the removal of lignin from the substrate. Several kinetic studies of corncob pretreatment with lime, hydrogen peroxide, sulphuric, formic and phosphorus acid have also been reported [9], [12], [13]. In this study, a kinetic and thermodynamic analysis was reported for the molten hydrate salt dissolution of the major components (cellulose, hemicellulose and lignin) from corn cob biomass with 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea pretreatment under wide temperature ranges of 50 °C—120 °C and pretreatment times from 0—120 min. A single step reaction of lignocellulose to each component was assumed as uni-molecular. It was also assumed that the sum of a series of elementary steps in the reaction system is represented by the overall reaction rate equation. Lastly, the steady state approximation was assumed, which implies that an intermediate in the dissolution reaction is consumed as quickly as it is formed and its concentration remains constant during the reaction [14].

2 Materials and methods

2.1 Materials

Raw corn cob of (*Zea mays*) were collected from domestic farming homes in Mukula Village, Limpopo South Africa. The dried samples were stored in capped plastic bottles tightly and were used shortly after collection. The cobs were cut to size using a band-saw (Mössner August, GmbH Co., Germany) and then milled using a rotor mill (Retsch® SE-200, Germany) with a 640 μm - 4 mm sieve. Deionized water produced from Milli-Q water (Millipore) with resistivity of 18 MΩ·cm. $ZnCl_2.4H_2O$ (98%) was bought from Rochelle Chemical Co. Ltd. In addition, H_2SO_4 (98%) analytical-grade, $LiClO_4.3H_2O$ (99%), Urea (99%), Acetone (99%) and glucose (99%) were purchased from Sigma Aldrich Chemical Company.

2.2 Dissolution reaction experiments

Dissolution experiments were carried out in 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea. The reactions were carried out in a 250 mL Erlenmeyer flask at temperatures 50 ºC, 70 ºC, 90 ºC, 120 ºC at a biomass to solvent ratio of 1:10 (5g dry biomass will be added to 50g of each solvent) at agitation speed of 270 rpm. The reaction mixtures were run for 20, 40, 60, 80, 100, 120 minutes for each temperature. Once the reaction was stopped, the slurries were separated into solid and liquid fractions.

2.3 Compositional analysis and recovery

The compositional analysis of biomass for hemicellulose, lignin, extractive and ash content were done as documented by the National Renewable Energy Laboratory (NREL) following Laboratory Analytical Procedures (LAP TP-510-42618, 42619, 42622) [15], [16].

The recovery (%) was determined according to the Equation (1) and Equation (2) and biomass yield after pretreatment was calculated as follows [17]:

$$\text{Biomass yield}\left(\%\left[\frac{\text{w}}{\text{w}}\right]\right) = \frac{Dry\ biomass\ weight\ after\ pretreatment \times 100\%}{Dry\ weight\ of\ untreated\ biomass} \quad (1)$$

The recovery of each component was calculated as follows [17]:

$$\text{Recovery of component}\left(\%\left[\frac{\text{w}}{\text{w}}\right]\right) = \frac{Component\ content\ in\ pretreated\ biomass \times biomass\ yield \times 100\%}{Total\ component\ in\ untreated\ biomass} \quad (2)$$

2.4 Determination of kinetic

The interaction between Lignin, Cellulose, Hemicellulose (A) and $ZnCl_2.4H_2O$/ Urea (B) to give solubilised product (C) is represented by the stoichiometric relationship in Equation (3):

$$A + B \xrightarrow{k} C \quad (3)$$

The dissolution rate of component was assumed as a function of cellulose, hemicellulose and lignin recovery (A) from Equation (2) and time (t) as follows:

$$\frac{-dA}{dt} = k.A \quad (4)$$

Where $\frac{dA}{dt}$ = lignin, hemicellulose and cellulose removal rate; k = chemical reaction rate constant; A = residual lignin, cellulose and hemicellulose after pretreatment.

To determine the rate constant (*k*), a linear regression curve was created based on the equation below and *k* was determined from the slope of the line.

$$\ln[A] = -kt + \ln[A]_0 \quad (5)$$

Where A is total lignin, hemicellulose or cellulose content at time t; A_0 = total lignin, hemicellulose or cellulose content at time t = 0.

The rate constants at the different temperatures was then fitted to the Arrhenius equation [18]. Where the slope and the intercept of the plots represent the (Ea/R) and ln A in Equation (5), respectively. The Arrhenius equation is:

$$k = Ae^{-Ea/RT} \quad (6)$$

By taking the natural log for both sides of Equation (6), Equation (7) was obtained.

$$\ln k = \frac{Ea}{RT} + lnA \quad (7)$$

Where k represents the rate constant of reaction (min^{-1}); A represents the Pre-exponential factor (min^{-1}); Ea is activation energy, ($J.mol^{-1}$); R is Gas Content, 8.314 $J.mol^{-1}.K^{-1}$; T is Temperature, K.

2.5 Determination of thermodynamic parameters

The change in enthalpy of the process was calculated using the following equation:

$$\Delta H = E - RT \quad (8)$$

Where ΔH is the change in enthalpy of activation energy; E is the activation energy; R is Gas Content, 8.314 J/ mol.K; T is Temperature, K. Eyring equation was used to calculate the entropies of activation (ΔS) from reaction rate constants and enthalpy values. The equation is given as:

$$e^{\frac{\Delta S}{R}} = \frac{h}{k_b} T(k e^{\frac{\Delta H}{RT}}) \quad (9)$$

Where k_b is the Boltzmann's constant (1.381×10^{-23}J/K); T is temperature in Kelvin (K); h represents Planck's constant (6.626×10^{-34} Js).

The free energy change for dissolution or Gibb's free energy (ΔG) was obtained using the following equation shown as:

$$\Delta G_{diss} = \Delta H_{diss} - T\Delta S_{diss} \quad (10)$$

3 Results and discussion

3.1 Dissolution kinetics of corn cob in $ZnCl_2.4H_2O$/ Urea MHS

The experimental data of cellulose, hemicellulose and lignin dissolution versus time in $ZnCl_2.4H_2O$/ Urea MHS at varying reaction temperatures to obtain the reaction rate constant (k) is shown in Figure 1. The results of the component dissolution show that hemicellulose dissolution has the greatest increase over time compared to the other components.

Fig.1 Reaction profiles of cellulose, hemicellulose and lignin dissolution using $ZnCl_2.4H_2O$/ Urea at (A) 50 ºC (B) 70 ºC (C) 90 ºC (D) 120 ºC

Figure 1A-D shows the fit model prediction and experimental data for cellulose, hemicellulose and lignin dissolution at 50, 70, 90 and 120 °C using 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea. The diagrams show the effect of dissolution temperature and time on the concentration of cellulose, hemicellulose and lignin. Figure 1 indicates a

decrease in concentration of each component with respect to time and to a smaller extent, temperature. The figures show the trend of steeper decreases in concentration for hemicellulose when compared to the other components (cellulose and lignin). The results show evidence that higher temperature favour the dissolution of all the components [19]. However, hemicellulose is more influenced by temperature as well as time than the other components this observation is drawn from the steep slope of the hemicellulose plots when compared to the other components [20]. This indicates the susceptibility of hemicellulose to dissolution by solvents. From Figure 1, it can be seen that at the different temperatures with regards to the reacting components, the modelled reaction follows first order kinetics for the range of the experiment studied. This was verified by curve fitting of the concentration-time data. The fit for all the plots have significantly low deviations from experimental data.

The component concentration of cellulose was lowest (24%) at 120 °C and highest (29%) at 70 °C which means at lower temperature severities cellulose is retained. This shows that temperature severity does not have a large effect on cellulose dissolution.

3.2 Reaction rates of component dissolution in $ZnCl_2.4H_2O$/ Urea MHS

The experimental result data obtained for $ZnCl_2.4H_2O$/ Urea MHS pretreatment of corn cob were fitted to an integrated pseudo first- order rate kinetics (Equation (5)). Figures 2A - D show plots of the natural log of concentration versus time for 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea pretreatment of corn cob at 50 °C, 70 °C, 90 °C and 120 °C respectively. From the plots, the rate of dissolution of cellulose, hemicellulose and lignin from corn cob is independent of concentration. The plots of the natural log versus time (t) resulted in straight lines for Figures 2A - D, with each slope estimating the specific rate constant 'k'. The straight line obtained in the figures for each temperature (50 - 120 °C) investigated suggests a first order rate of cellulose, hemicellulose and lignin dissolution in $ZnCl_2.4H_2O$/ Urea. The rate constant 'k' and the correlation coefficient 'R' for each plot is presented in Table 1.

Fig.2 Kinetics of dissolution at (A) 50 ºC (B) 70 ºC (C) 90 ºC (D) 120 ºC, 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea

Table 1: Estimated kinetics constants for $ZnCl_2.4H_2O$/ Urea pretreatment

T(K)	Cellulose		Hemicellulose (s^{-1})		Lignin (s^{-1})	
	k (s^{-1})	R^2	k (s^{-1})	R^2	k (s^{-1})	R^2
323	2.00×10^{-5}	0.8647	7.00×10^{-5}	0.9907	3.00×10^{-5}	0.9657
343	1.02×10^{-5}	0.5558	7.50×10^{-5}	0.9421	4.50×10^{-5}	0.8667
363	3.67×10^{-5}	0.9455	10.7×10^{-5}	0.9776	6.00×10^{-5}	0.9580
393	3.16×10^{-5}	0.9077	10.0×10^{-5}	0.9914	5.50×10^{-5}	0.9978

Notably, the temperature dependence of the rate constant is not observed, the rate constant increases as the reaction temperature does not increases proportionally from 50 °C to 120 °C for all the components. In the case of cellulose dissolution, the rate constant at 70 °C was lower than at 50 °C, at this temperature there is a low R^2 value of 0.5558 which indicates poor fit. This may be due to the method in which cellulose recovery is calculated from the difference of the other components, giving a margin of error. In addition, the errors may be due to the propagation errors of measuring devices. However, based on the results observed, 90 °C was considered the optimal temperature condition for cellulose, hemicellulose and lignin dissolution in $ZnCl_2.4H_2O$/ Urea MHS.

The rate constant values at the examined temperatures for cellulose were 2.00×10^{-5} (50 °C), 1.02×10^{-5} (70 °C), 3.67×10^{-5} (90 °C) and 3.16×10^{-5} (120 °C), respectively. The rate constants were generally higher at higher temperatures. However, a trend was not observed of proportional increase of rate with increasing temperature. There was a slight drop in the rate at the relatively lower temperatures (50 °C and 70 °C) which suggests that the crystalline regions of cellulose made up of the inter- and intra-molecular hydrogen bonds in cellulose, hinder cellulose dissolution at the lower temperatures as well as at higher temperatures when compared to hemicellulose and lignin.

A similar trend of increased reaction rates with increased temperatures was recorded in both the hemicellulose and lignin reaction rates. Where the rate constant increased from 7.00×10^{-5} to 7.50×10^{-5}, 10.7×10^{-5} and 10.0×10^{-5} as the reaction temperature was increased from 50 °C to 70 °C, 90 °C and 120 °C ,respectively, in hemicellulose dissolution. It is observed that hemicellulose dissolution occurs at a faster rate compared to the dissolution of the other two components (lignin and cellulose). This is in agreement with the well-known fact that hemicellulose is more susceptible to pretreatment conditions [21] and dissolution. This is presumably due the ease of cleaving the hemicellulose backbone.

3.3 Activation energy and frequency factor determination

To determine the activation energy and frequency factor (A) for the dissolution reactions, the natural logarithm of k values at the different experimental temperatures are plotted versus the inverse of temperature (1/T) using Equation (7) in Figure 3. The straight lines in the plots indicate that the dissolution reactions follow Arrhenius law.

Fig.3 Arrhenius plot based on apparent component dissolution rate constants (k) at temperatures from 50 - 120 °C.

Figure 3 shows the plot of the rate constant fitted with the Arrhenius equation. Using Equation (7), the activation energy (*E*a) of the component dissolution and frequency factors (A) were obtained from the slope and the intercept of plotting ln k versus 1/T, respectively. The activation energy and frequency factor are shown in Table 2.

Table 2: The activation parameters of lignocellulosic component dissolution

Component	*A* (min^{-1})	*E*a ($kJ.mol^{-1}$)	Arrhenius Fit, R^2
Cellulose	2.637	14.10	0.577
Hemicellulose	3.110	7.606	0.857
Lignin	2.699	11.29	0.859

The activation energy (Ea) and frequency factor (A) are 14.10 $kJ.mol^{-1}$ and 20×10^3 s^{-1} for cellulose respectively. This implies that the minimum energy necessary for the dissolution reaction of cellulose to occur using MHS is 14.10 $kJ.mol^{-1}$. This is the minimum amount of energy required to activate the molecules to initiate a chemical reaction during cellulose dissolution. When compared to cellulose dissolution by phosphoric acid, the activation energy associated with dissolution of microcrystalline cellulose in phosphoric acid (83%) was 42.4 $kJ.mol^{-1}$ [22]. It was also reported that the activation energy associated with the dissolution of cotton linters pulp in NaOH/urea system was 101 $kJ.mol^{-1}$ [23]. In addition, the Ea of ionic liquid pretreated rubber wood was reported as 118 $kJ.mol^{-1}$ [24]. These results were much larger than *E*a shown in Table 2. This suggests that $ZnCl_2.4H_2O$/ Urea MHS solvents possess powerful dissolution towards cellulose. The R^2 for cellulose indicates a poor fit compared to the hemicellulose and lignin models. This poorer fit may be due to the loss in mass which is as a result of the cellulose being calculated from the mass balance as opposed to being analysed experimentally. Additional experimentation at other temperatures may be required to improve the fit.

The frequency factor value acquired from the intercept of the straight line indicates the collision rate of the reactant molecules at the applied operating conditions. The frequency factor is dependent on the reaction order and is thus affected by the reaction rate. The frequency factor is also dependent on the operating temperature [14]. As the temperatures increase, the molecules tend to move faster, which leads to increase in the frequency of collisions. It was observed that hemicellulose dissolution has the highest frequency factor, followed by lignin and cellulose. The number of collisions in a minute occurring during the reaction is 2.637, 3.110 and 2.699 for cellulose, hemicellulose and lignin, respectively. This may be due to the higher concentration of hemicellulose in the corn cob sample therefore resulting in more interactions.

Significantly larger activation energy of cellulose dissolution (compared to lignin and hemicellulose dissolution) shows the difficulty of cellulose dissolution in the range of temperature investigated in the experiments. This is attributed to the highly crystalline nature of cellulose. This is the reason for the higher reaction temperature necessary to initiate the chemical conversion upon the use of cellulose. The higher activation energy also correlates with the temperature dependence of hemicellulose dissolution compared to the other components. It is evident that conversion of cellulose, lignin and hemicellulose in molten hydrate salts all have different rate-limiting steps. In addition, the Ea value is an indication of the sensitivity of the reaction rate to temperature changes. At a relatively large activation (200 - 400 $kJ.mol^{-1}$), there is a sensitivity of the reaction rate to temperature. Such large Ea values are typical of combustion and gasification reactions which take place at high

temperatures and are very slow at room temperature. For the biochemical reaction of $ZnCl_2.4H_2O$/Urea MHS in corn cob the Ea is relatively low (7-14 $kJ.mol^{-1}$) compared to gasification and combustion reactions due to the facile nature of the pretreatment. Thus, using MHS pretreatment may cut down the energy consumption in industrial processes [25], [26].

Finally, hemicellulose dissolution is much more significant than cellulose dissolution which is a major trait of the MHS pretreatment where the recovery of cellulose is optimised. In addition, Hemicellulose dissolution behaves more similar to lignin dissolution than cellulose dissolution. This is because of covalent bonds between hemicellulose and lignin present in the cell wall [27].

3.4 Thermodynamic parameters determination

The calculated values of ΔG, ΔH and ΔS for the dissolution of each component are tabulated in Table 3.

Table 3 Thermodynamic parameters of cellulose, hemicellulose and lignin dissolution in $ZnCl_2.4H_2O$/ Urea

Component	ΔH (kJ.mol^{-1})				ΔS (kJ.mol^{-1})				ΔG (kJ.mol^{-1})			
	ΔH_{323}	ΔH_{343}	ΔH_{363}	ΔH_{393}	ΔS_{232}	ΔS_{343}	ΔS_{363}	ΔS_{393}	ΔG_{323}	ΔG_{343}	ΔG_{363}	ΔG_{393}
Cellulose	11.414	11.248	11.082	10.832	-0.205	-0.210	-0.199	-0.199	54.930	60.940	61.300	67.750
Hemicellulose	4.920	4.754	4.588	4.338	-0.195	-0.193	-0.190	-0.190	58.060	61.760	64.570	70.490
Lignin	8.604	8.438	8.272	8.022	-0.202	-0.198	-0.195	-0.193	56.650	59.530	62.670	68.760

The enthalpies were all positive values which indicated the endothermic nature of the reactions meaning that energy is required in all dissolution reactions. The calculated values in Table 3 also show that a higher operating temperature (120 °C) required lower activation enthalpy than lower temperature (50 °C) for dissolution of all the components. This shows that at higher temperatures, dissolution of the components can occur much quicker. The enthalpy gain from breaking H-bonds and van der Waals interactions provide enthalpy gains to the Gibbs free energy (ΔG) of dissolution which are important for cellulose dissolution in MHS solvents.

The values obtained tabulated in Table 3 indicated that by increasing the temperature, more stable end products could be achieved as a result of lower values of entropy. It is also important to note that all the entropy values are negative. The basic principles of thermodynamics, tell us that any product from a process with a negative entropy is designated as "stable". This is because negative entropy represents increasing order in the system. Entropy can be thought of as a state of molecular "disorder". The values obtained tabulated in Table 3 indicated that by increasing the temperature, lower entropy values were obtained which indicate the production of stable end products. The trend was a general decrease in entropy for all the components, this is due to the fact that entropy changes favour the liquid state and thus the formation of a solution and thus the dissolution of the component. The hemicellulose dissolution has a more negative entropy than the other components and on comparative basis, hemicellulose dissolution form relatively more stable products than lignin and cellulose dissolution. Cellulose dissolution forms less stable products however, cellulose dissolution is driven by the entropy gain upon the chain dissociation and hindered by the loss of solvent entropy (the solvent loses entropy by binding and causing an ordered structure). The entropy of dissolution favours dissociated cellulose chains over the crystalline form in $ZnCl_2.4H_2O$/ Urea. It is important to note that an increase in the molecular weight of the components especially cellulose due to its rigidity may cause a decrease in the entropy due to the formation of more ordered interaction between the components and solvents.

The Gibbs free energy change which occurs in any given process is an indication of the spontaneity of the process. Gibbs free energy changes of the components of lignocellulose in $ZnCl_2.4H_2O$/ Urea MHS are tabulated in Table 3. Positive values of Gibbs free energy for cellulose, hemicellulose and lignin dissolution indicates the non-spontaneous nature of the reaction. Non-spontaneous processes are those which occurs with the addition of external energy, this explains and further shows the endothermic nature of the reactions. The ΔG values for dissolution of hemicellulose are higher than ΔG values for cellulose and lignin dissolution, which indicates that relative to hemicellulose, dissolution of cellulose and lignin are thermodynamically less favourable processes when compared to hemicellulose dissolution. This result is in correlation with the order of magnitude observed in the kinetic rate constants (Table 1).

4 Conclusion

The process of dissolution of cellulose, hemicellulose and lignin followed first-order reaction kinetics, thus the Arrhenius equation was chosen to describe the dissolution of lignocellulose step. The values of kinetic and thermodynamic parameters which were determined extensively demonstrated the importance of the process parameters (time and temperature) in the dissolution process of each component. Comparatively, the rates of component dissolution reactions have a following order: Cellulose > Lignin> Hemicellulose. The rates were also found to increase with increasing temperatures. Activation energy obtained for the dissolution of cellulose

is higher than the activation energy obtained during the dissolution of hemicellulose in 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea and follow an order of magnitude of Cellulose (14.10 $kJ.mol^{-1}$) > Lignin (11.29 $kJ.mol^{-1}$) > Hemicellulose (7.606 kJ. mol^{-1}). Thermodynamically, ΔH shows that all the reactions were endothermic. The hemicellulose dissolution reaction has a higher value of entropy change corresponding to more disorder in their structure which shows that the reaction occurs more rapidly and is more favourable in $ZnCl_2.4H_2O$/ Urea pretreatment.

Conflict of interest

The authors declare that they have no conflict of interest.

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Development of Non-derivatizing Hydrate Salt Pretreatment solvent for pretreatment and Fractionation of Corn Cob

Olayile Ejekwu[1], Augustine Omoniyi Ayeni[2], Orevaoghene Eterigho-Ikelegbe[1], Michael Olawale Daramola[1*]

Lignocellulosic biomass is a renewable energy resource that can be used to produce biofuels and bio-products. The major concern in lignocellulose conversion is overcoming biomass recalcitrance through pretreatment while still maintaining a green, energy efficient and cost-effective process. The aim of the study was to compare seven molten hydrate salt pretreatment solvent systems including unary, binary and ternary mixtures of $ZnCl_2.4H_2O$, $LiClO_4.3H_2O$ and Urea for the effective pretreatment of corncob in terms of physicochemical properties and pretreatment efficiencies and to optimise these efficiencies. The pretreatment experiments were carried out at 70 °C for 60 minutes at a biomass: solvent ratio of 1:10. Physicochemical change after pretreatment was checked by Fourier Transform infrared, X-ray diffraction and scanning electron microscopy. Physicochemical analysis showed a decrease in crystallinity and an increase in surface area after the pretreatment in all the MHS solvents tested. This work has successfully shown the use of $ZnCl_2.4H_2O$/ Urea, to pre-treat and fractionate corn cob with high recovery of cellulose (100%), low recovery of hemicellulose (42%) and lignin (44%) when compared to the other proposed systems. The results presented in this study demonstrate the potential of molten hydrate salt solvents for the pretreatment of lignocellulosic biomass such as corn cob.

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Public Interest Statement

Pretreatment is a crucial but expensive processing step in the biochemical conversion of lignocellulosic biomass to basic sugars and finally to fuels and chemicals. It is required to alter the structure of lignocellulosic biomass and make the major components of biomass (cellulose, hemicellulose and lignin) more accessible to the enzymes for hydrolysis to sugars. The effectiveness of lignocellulosic pretreatment is in the ability of the treatment to improve the separation of the major components of the biomass. Molten hydrate salts are able dissolve and separate these components. This study describes the effect of different molten hydrate salts systems composing of $LiClO_4.3H_2O$, $ZnCl_2.4H_2O$ and Urea to pre-treat and fractionate waste biomass.

Classifications & Keywords

Classification: *Chemical Engineering, Biochemical Engineering, Bioconversion, Bioenergy*

Keywords: *Molten hydrate salt, Pretreatment, Fractionation, Corn cob*

1 Introduction

In view of environmental concerns, in global warming resulting from the over use of fossil fuels, there is a need for platform chemicals, materials and fuels from renewable lignocellulosic resources by biochemical conversion. Lignocellulosic biomass is the most abundant plant material on the planet [1]. It has been proven to give promising yields in processes designed to produce fuels, chemicals and materials. Lignocellulosic feedstock makes the process cheap, viable as well as eco-friendly. These feedstocks can be derived from woody biomass, municipal waste and agricultural waste. Corn cob waste, a type of agricultural waste is the residue left from removing the maize grains and is what remains of the ear on which the kernels grow. Cobs make up about 20 wt. % of the yield of the corn residue. Corn cob residues are sufficiently available in South Africa and are produced by the maize industry in large quantities of approximately 14.5 million tons annually (National Agricultural Marketing Council, 2012).

All sources of lignocellulosic biomass consist of three fractions (lignin, hemicellulose, and cellulose), which are tightly bound in a hetero-matrix. Optimal utilisation of all fractions would cause an improvement in the economics of the overall bioconversion process. Bioconversion of the lignocellulosic feedstock involves the hydrolysis of cellulose and hemicellulose fractions to fermentable reducing monomeric sugars such as hexose and pentose. Which then undergoes fermentation and further processing to produce biofuels and biochemical. The main limiting factors to efficient hydrolysis include porosity (accessible surface area), cellulose crystallinity, cellulose sheathing by hemicellulose and lignin content in the biomass. To overcome these, a pretreatment process needs to be employed to remove lignin fractions, increase the surface area to allow for

accessible binding of enzymes and decrease crystallinity of cellulose in the biomass. Currently, a variety of fractionation and pretreatment processes including chemicals, physical and biological pre-treatment methods are being explored in academia and industry. Examples of pretreatment methods include; oxidative delignification, acid and alkali hydrolysis [2] ionic liquid [3], hydrothermal [4], inorganic salt immersion, steam explosion [5], microbial [6]. However, these pretreatment processes are either economically unfeasible, form harsh fermentation inhibitory by-products or are inefficient.

For sustainability purposes, biomass utilisation will heavily depend on the successful deployment of innovative-green chemistry. Green chemistry is defined as the efficient use of raw materials (preferably renewable), the elimination of waste and avoidance of hazardous or toxic reagents and solvents in chemical processes (Fargo, 2013). Examples of some green solvents include cheap, eco-friendly and recyclable systems are molten hydrate salts (MHS) solvent systems. Molten hydrate salts are aqueous solutions of inorganic salts at extraordinarily high concentration, which varies for each salt, depending on the coordination number of the salt cation [7]. MHSs have cations that are highly hydrated (coordinated with water), while the anion is coordination-free in the system. This nature gives molten salt hydrates some unique properties such as high boiling point, low vapour pressure and liquid status at moderate temperature, MHS are even able to swell and dissolve cellulose and biomass in a non-derivatising manner. Molten salt hydrates have been used as solvent and reaction medium in various chemical reactions and processes including biomass conversion. Because of similar properties and performance with ionic liquids, molten hydrate salts are often known as inorganic ionic liquids. However, molten hydrate salts usually have lower cost, lower viscosity and lower toxicity than organic ionic liquids. Some examples of MHSs are $LiClO_4 \cdot 3H_2O$, $LiSCN \cdot 2H_2O$, $LiI_2 \cdot 2H_2O$, $ZnCl_2 \cdot 4H_2O$ and their mixtures which have been proven to enable the dissolution of cellulose at relatively mild conditions [7].

The establishment of sustainable production of biofuels, biochemical and biomaterials in the future depends on the integration of low environmentally friendly technologies and green chemistry into biorefinery. Green chemistry is a set of principles which govern the manufacture and application of products with the aim of eliminating the use and generation of environmentally hazardous chemicals [8]. The development of processes technologies which are both innovative and sustainable is mandatory in achieving the shift towards bio-based production of fuels, chemicals and materials.

In this study, seven molten salt hydrate pretreatment systems classified into unary ($ZnCl_2.4H_2O$, $LiClO_4.3H_2O$, Urea), binary ($LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$, $ZnCl_2.4H_2O$/ Urea, $LiClO_4.3H_2O$/Urea) and ternary ($LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$ /Urea) were investigated for their abilities to pre-treat and fractionate biomass. The evaluation of the compositional changes in the major components of corncob namely, hemicellulose, lignin and cellulose; and to characterise multi-level structures of solid residues by a combination of methods, such as X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) to evaluate the efficiency of the different pretreatment with respect to cellulose, hemicellulose and lignin recovery and identifying the most efficient system. The screening results will form the basis for the selection of a molten hydrate salt solvent system for pretreatment of biomass with the highest potential for further optimisation of process parameters.

2 Materials and methods

2.1 Materials

Raw corn cobs of (*Zea mays*) were collected from domestic farming homes in Mukula Village, Limpopo South Africa. The dried samples were stored in capped plastic bottles tightly and were used shortly after collection. The cobs were cut to size using a band-saw (Mössner August, GmbH Co., Germany) and then milled using a rotor mill (Retsch® SE-200, Germany) with a 640 μm - 4 mm sieve. Deionized water produced from Milli-Q water (Millipore) with resistivity of 18 MΩ·cm. $ZnCl_2.4H_2O$ (98%) was bought from Rochelle Chemical Co. Ltd, South Africa. In addition, H_2SO_4 (98%) analytical-grade, $LiClO_4.3H_2O$ (99%), Urea (99%), Acetone (99%) and glucose (99%) were purchased from Sigma Aldrich Chemical Company.

2.2 Pretreatment of corn cob

Seven molten salt hydrate pretreatment systems classified into unary ($ZnCl_2.4H_2O$, $LiClO_4.3H_2O$ and Urea), binary ($LiClO_4.3H_2O/ZnCl_2.4H_2O$, $ZnCl_2.4H_2O$/Urea and $LiClO_4.3H_2O$/Urea) and ternary ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea) at a ratio of 1:1 and 1:1:1 for the binary and ternary complexes respectively were investigated for the treatment of corncob. The pretreatment was carried out at 70 °C for 60 minutes at a constant biomass to solvent ratio of 1:10 (5 g dry biomass will be added to 50g of each solvent) at agitation speed of 270 rpm. The slurries that were obtained from pretreatment with each solvent were recovered and separated into solid and liquid fractions using vacuum filtration.

2.3 Compositional analysis of the solid and liquid fractions of the corn cob

2.3.1 Solid fraction compositional analysis

The compositional analysis of untreated and MHS-treated biomass was analysed for hemicellulose, lignin, extractive and ash content as documented by the National Renewable Energy Laboratory (NREL) following Laboratory Analytical Procedures (LAP TP-510-42618, 42619, 42622). The analysis is summarised in Figure 1.

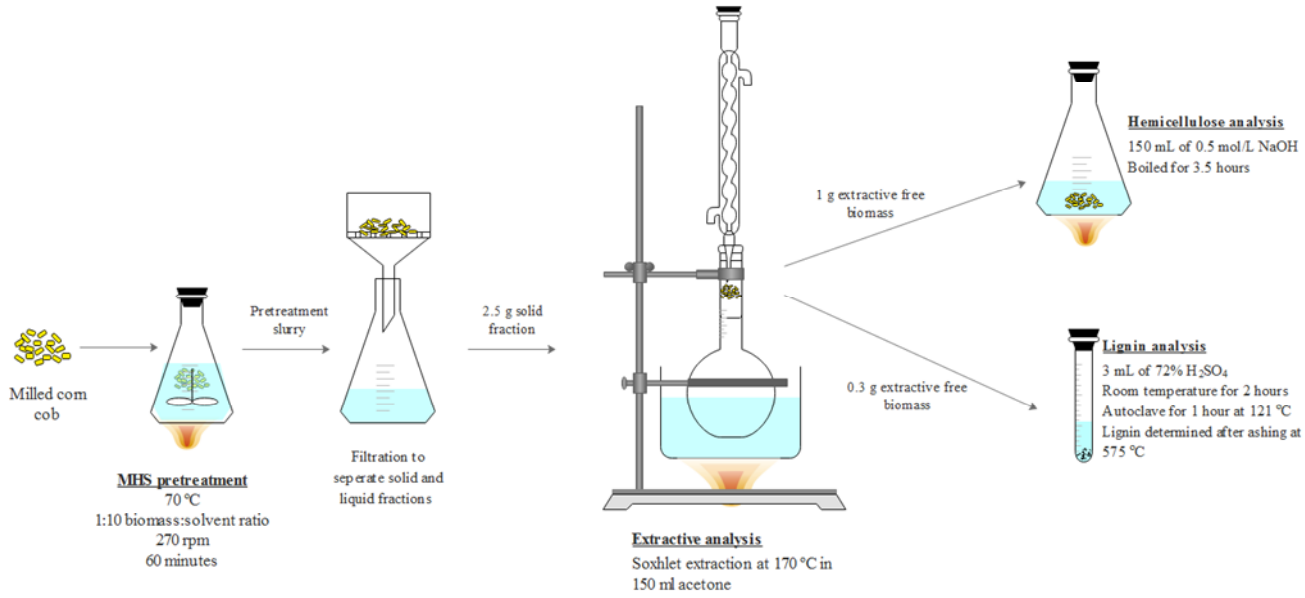

Figure 1 Method of compositional analysis for biomass

(Cellulose % = 100%- Extractive% - Hemicellulose% - Lignin% - Ash content)

2.3.2. Quantitative evaluation of pretreatment to determine the efficiency of the solvents systems

To quantitatively evaluate the effects of various pretreatment methods on each component (cellulose, hemicellulose and lignin), recovery ratio (or yield) of each component was estimated. The pretreatment efficiencies were evaluated based on the recovery/ yield of polysaccharides (cellulose and hemicellulose) and lignin. Adequate recovery of hemicellulose and cellulose sugars is important for an ideal pretreatment. Biomass yield after pretreatment was calculated as follows [9]:

$$\text{Biomass yield}\left(\%\left[\frac{w}{w}\right]\right) = \frac{Dry\ biomass\ weight\ after\ pretreatment \times 100\%}{Dry\ weight\ of\ untreated\ biomass} \quad (1)$$

The recovery of each component was calculated as follows [9]:

$$\text{Recovery of component}\left(\%\left[\frac{w}{w}\right]\right) = \frac{Component\ content\ in\ pretreated\ biomass \times biomass\ yield \times 100\%}{Total\ component\ in\ untreated\ biomass} \quad (2)$$

2.3.3 Total sugar determination of the liquid fraction

In this method, 3 mL of modified 3,5-Dinitrosalicylic acid (DNS) reagent was added to 1 mL of the liquid sample in a test tube with 1 mL of distilled water. The test tubes were then placed in a boiling water bath for 15 minutes and thereafter cooled for 15 minutes. The absorbance of the sample and DNS was then measured at 575 nm. Glucose standard curves were prepared and the values of total reducing sugar for the samples were then read from these glucose curve [10].

2.3.4 Cellulose crystallinity determination using X-ray diffraction (XRD)

The corn cob sample crystallinity was analysed using a D2 advance diffractometer (Bruker, Germany) with a scan rate of 10 min^{-1} from 10° to 90° and Co Kα radiation (1.789 A°) generated at 30 kV and 10 mA. In order to analyse the data, a method developed by Segal et al. (1959) will be used. The crystallinity indices (CrI) was calculated from the XRD patterns using the following equation:

$$CrI = \frac{I_{002} - I_{amp}}{I_{002}} \times 100\% \quad (3)$$

Where I_{002} is the intensity of 002 lattice diffraction ($2\theta = 22.6$) and I_{amp} represents the intensity of amorphous section ($2\theta = 18$).

2.3.5 Surface chemistry analysis using Fourier Transform infrared (FTIR)

Lignocellulosic materials contain alcohols, aromatics, alkanes, ketones, esters and other oxygen based functional groups which can be analysed using FTIR. Surface chemistry of the raw and pre-treated biomass samples were evaluated using a PerkinElmer Frontier Fourier transform infrared spectrometer (PerkinElmer, USA) using the attenuated total reflectance (ATR) method. A diamond ATR plate with angle of incidence of 45° was used as the internal reflection element. Spectra was obtained from a range of 400 to 4000 cm^{-1} and obtained at a resolution of 4 cm^{-1}. The spectra were used to investigate the surface chemistry information of the samples while the crystallinity index (CrI) of biomass can also be measured using FTIR. There are two well-established indices which exist which are a measure of biomass crystallinity. The Lateral Order Index (LOI), also defined as the empirical CrI is a ratio of absorption band at 1430 cm^{-1} to absorption at 898 cm^{-1}. Total Crystallinity Index (TCI), was also determined based on the ratio of the absorption band at 1372 cm^{-1} and 2900 cm^{-1}.

2.3.6 Morphological analysis for evaluation of biomass using Scanning Electron Microscope (SEM)

SEM was used to check the surface morphology of the pre-treated and untreated biomass. Using conductive tape, the dried samples were fixed onto aluminium stubs. The samples where then analysed by Carl Zeiss Sigma scanning electron microscope. This was followed by sputter coating by carbon and gold-palladium at five nanometre scale making the fibres conductive, avoiding degradation and charge build-up on the specimen surface. The SEM was operated under a vacuum and the images were captured.

3 Results and discussion

188 **3.1 Biomass compositional analysis**

189 **3.1.1 Solid fraction**

190 The raw material was characterised in terms of the composition of extractives, ash, cellulose, hemicelluloses and
191 lignin. The characterisation is necessary to determine the extent of the chemical compositional changes resulting
192 from the pretreatment. The composition of the raw and pre-treated biomass samples is shown in Table 1. The
193 total carbohydrate (cellulose and hemicelluloses i.e. the holocellulose) composition was 63.03% (w/w). The
194 high saccharide content implies that analysed corn cob waste materials are promising feedstock with potentials
195 for very high yields of valuable bio-based chemicals and products. Table 1 also shows the composition of the
196 pre-treated samples. However, the biomass recoveries were not shown, biomass recoveries are needed to analyse
197 the effect of each pretreatment on the corn cob. The results in Table 1 for the pre-treated samples do not include
198 the fractions which are unrecovered in the solid fraction, as some mass was lost in the solid fraction during
199 pretreatment. The recovery percentages are shown in Figure 2.

200

201

202

Table 1: Composition of raw and pre-treated solid fraction (70 °C , 60 min, 100 mL total liquid volume, 5 g biomass loading) corn cob solid (%w/w)

		Raw	A $ZnCl_2.4H_2O$	B $LiClO_4.3H_2O$	C Urea	D $LiClO_4/ZnCl_2$	E $ZnCl_2$/Urea	F $LiClO_4$/Urea	G $LiClO_4/ZnCl_2$/Urea
Biomass yield		100.00	73.40	75.20	87.90	78.35	80.30	88.50	90.80
Extractives		2.80	16.3	6.50	4.80	17.80	14.40	6.40	16.08
Hemicellulose		40.34	34.46	40.00	38.00	29.00	40.84	28.00	30.00
Cellulose		22.69	27.89	24.61	19.60	31.21	25.9	25.93	18.63
Lignin	AIL	29.55	17.00	27.00	33.33	20.10	16.68	37.00	31.99
	ASL	1.88	3.75	1.35	2.74	1.85	1.64	1.11	1.65
Ash		2.56	0.60	0.54	1.53	0.35	0.56	1.56	1.74

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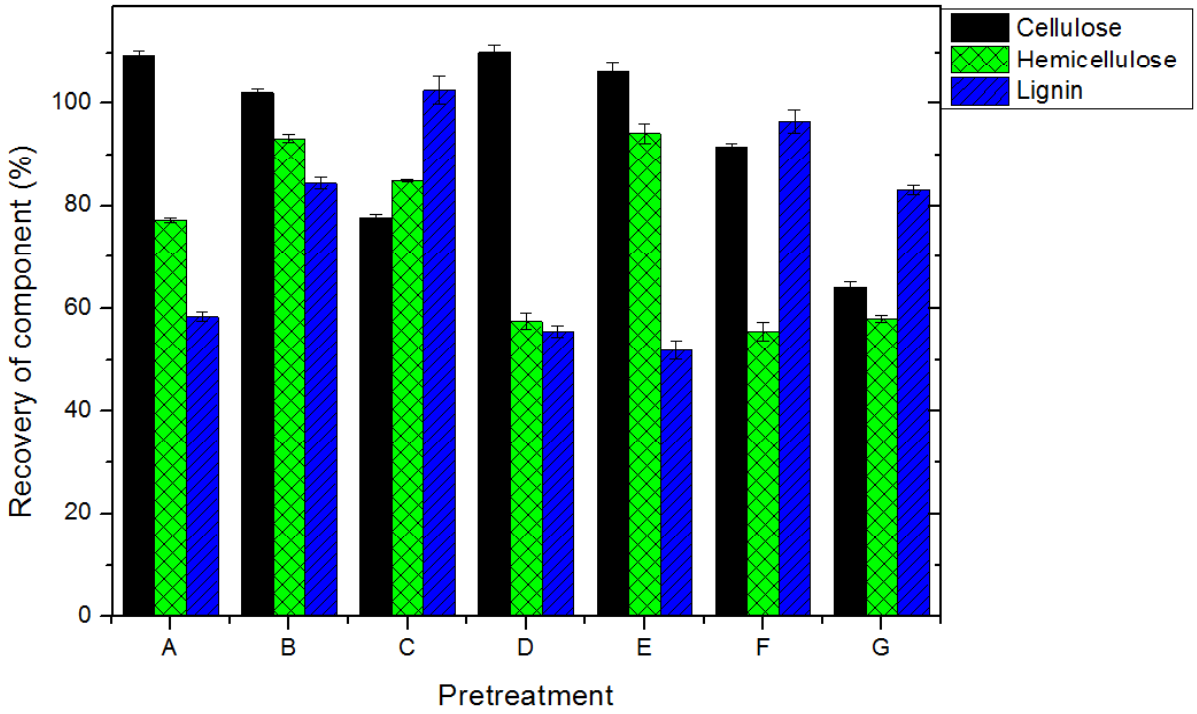

204

205 **Figure 2** Recovery of polysaccharides cellulose, hemicellulose and lignin after
206 pretreatment (error bars represent standard deviations of duplicates) (A): $ZnCl_2.4H_2O$. (B): $LiClO_4.3H_2O$. (C):
207 Urea. (D): $ZnCl_2.4H_2O$/Urea. (E): $LiClO_4.3H_2O/ZnCl_2.4H_2O$ (F):$LiClO_4.3H_2O$/Urea. (G):
208 $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea

209

210 The recovery was calculated using Equation (1) and Equation (2). The MHS solvent systems pretreatment was
211 predicted to separate the lignocellulosic biomass into a liquid fraction which contains mostly solubilised
212 hemicellulose as monomers and a solid fraction which is enriched with mostly oligomeric cellulose components.
213 Therefore, a high recovery of cellulose and a low recovery of hemicellulose and lignin are required. Cellulose
214 recovery in the solid fraction was over 75% for all the tested biomass samples except pretreatment G
215 ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea). The highest recovery of cellulose was approximately 109% and was
216 recovered by pretreatment D ($ZnCl_2.4H_2O$/ Urea). Martin et al. (2007) in comparison treated sugarcane bagasse
217 biomass using wet oxidation at alkaline pH, 195 ^oC and 15 min and was able to achieve 70% cellulose recovery
218 [12]. Hemicellulose recovery was within the range of 57-93% with the lowest being D ($ZnCl_2.4H_2O$/ Urea), F
219 ($LiClO_4.3H_2O$/Urea) and G ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea) with 99%, 55%, 66% recovery respectively.
220 Georgieva et al. (2008) recovered 93% of cellulose and 72% of hemicellulose from wet explosion pretreatment
221 of wheat straw at 35% (v/v) hydrogen peroxide and 185 ^oC for 15 min [13].

222 Lignin recovery was lowest for Pretreatment A, D and E with 66%, 77%, 88% recovery respectively. The high
223 recovery of cellulose (109%) and low recovery of hemicellulose (58%) and lignin (56%) in the biomass samples
224 pretreatment in sample D ($ZnCl_2.4H_2O$/ Urea) gave an indication that this is the most efficient solvent of the
225 seven tested for the fractionation of corn cob. These results are in good agreement with formic acid pretreatment

by Zhang et al. (2010) who under mild reaction conditions removed a relatively high ratio of hemicellulose (85%) and lignin (70%), while obtaining almost all the cellulose in the solid residue [14]. This type of pretreatment produces toxic by products such as acetic acid and furfural which are usually unavoidable in pretreatment using acid solvents [15].

The pretreatment in this study using MHSs occurred at a constant temperature of 70 ^{o}C and the results from the solid and liquid recovery reveal an efficient separation of the hemicellulose from cellulose as well as a low degradation of cellulose to by-products such as acetic acid during the pretreatment process. This is due to the non-derivatizing nature of the solvents. It has been observed that $ZnCl_2.4H_2O$/ Urea is an excellent solvent for biomass pretreatment due to its fractionation abilities. This is as a result of the interaction of the chloride anion (Cl^-) and the zinc cation (Zn^{2+}) of the solvent with the hydrogen atoms (H) and oxygen atoms (O) of the cellulose hydroxyl groups, respectively and the hydrogen bond formation with cellulose [16]. The relatively high lignin recovery in the solid fraction shows that only a small fraction of lignin was solubilised under the low pretreatment temperature conditions which caused minimal formation of low molecular weight lignin compounds. This gives some insight into the mechanism of the MHS pretreatment which shows that although delignification occurs, it is not as prominent in this type of pretreatment.

3.1.2 Liquid fraction

Depending on the pretreatment solvents, some of the cellulose and hemicellulose fractions will form sugar monomers in the liquid fractions. Figure 3 shows the yield of total sugars after pretreatment.

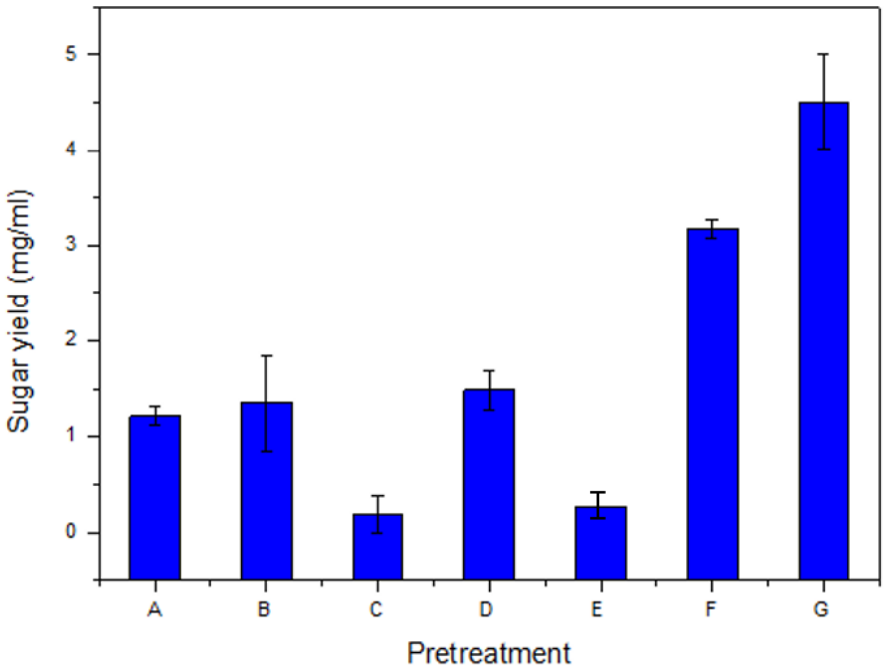

Figure 3 Yield of total sugars in liquid fraction after MHS pretreatment. Average values and standard deviations reported for duplicates. (A): $ZnCl_2.4H_2O$. (B): $LiClO_4.3H_2O$. (C): Urea. (D): $ZnCl_2.4H_2O$/Urea. (E): $LiClO_4.3H_2O$/ $ZnCl_2.4H_2O$ (F):$LiClO_4.3H_2O$/Urea. (G): $LiClO_4.3H_2O$/$ZnCl_2.4H_2O$/Urea.

249

250 The highest total sugar quantities were found in pretreatment G ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea) at levels of
251 4.5 mg/mL. For all the other pretreatment methods except pretreatment F ($LiClO_4.3H_2O$/Urea), the total sugar
252 concentrations were significantly lower in comparison. The lower sugar formation in pretreatment solvent A
253 ($ZnCl_2.4H_2O$), B ($LiClO_4.3H_2O$), C (Urea) and E ($LiClO_4.3H_2O/ZnCl_2.4H_2O$) hydrolysates correlate with the
254 results found in Figure 3 which shows these pretreatment solvents as having the highest hemicellulose recovery
255 in the solid fraction. The higher sugar formation observed in Pretreatment D ($ZnCl_2.4H_2O$/Urea), F
256 ($LiClO_4.3H_2O$/Urea) and G ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea), is mainly due to the fact that lower amounts of
257 oligomeric hemicellulose were retained in the solid fractions which shows that solubilisation of hemicellulose to
258 monomers was more severe in the liquid fraction of these pretreatment solvents. These results also indicate that
259 MHS pretreatment solvents can dissolve hemicellulose into xylose sugars, rather than degrade it into
260 fermentation inhibitors such as furfurals, unlike most acid and hydrothermal treatments.

261 **3.2 Crystallinity and surface chemistry determination**

262 **3.2.1 XRD spectra**

263 XRD analysis allows for measuring the crystallinity of the material and considers disordered cellulose,
264 hemicellulose and lignin. Cellulose accounts for the highest proportion of the crystalline structure therefore, The
265 XRD spectra is usually an indication of the cellulose crystallinity. The XRD spectra and Crystallinity index
266 (CrI) values for the effect of MHS pretreatment on corn cob are shown in Figure 4 and Table 2, respectively. In
267 this study, all samples presented the same diffraction patterns. Two typical diffraction peaks which correspond
268 to (101) and (002) peaks were observed at $2\theta = 15^{\circ}$ and 22.5°, respectively. The highest peak observed at 002
269 was observed for spectra E ($ZnCl_2.4H_2O$/ Urea) which also happens to have one of the highest cellulose
270 recoveries in Figure 2 while Urea which has one of the lowest cellulose recoveries has a low peak intensity
271 shown as spectra D in Figure 4.

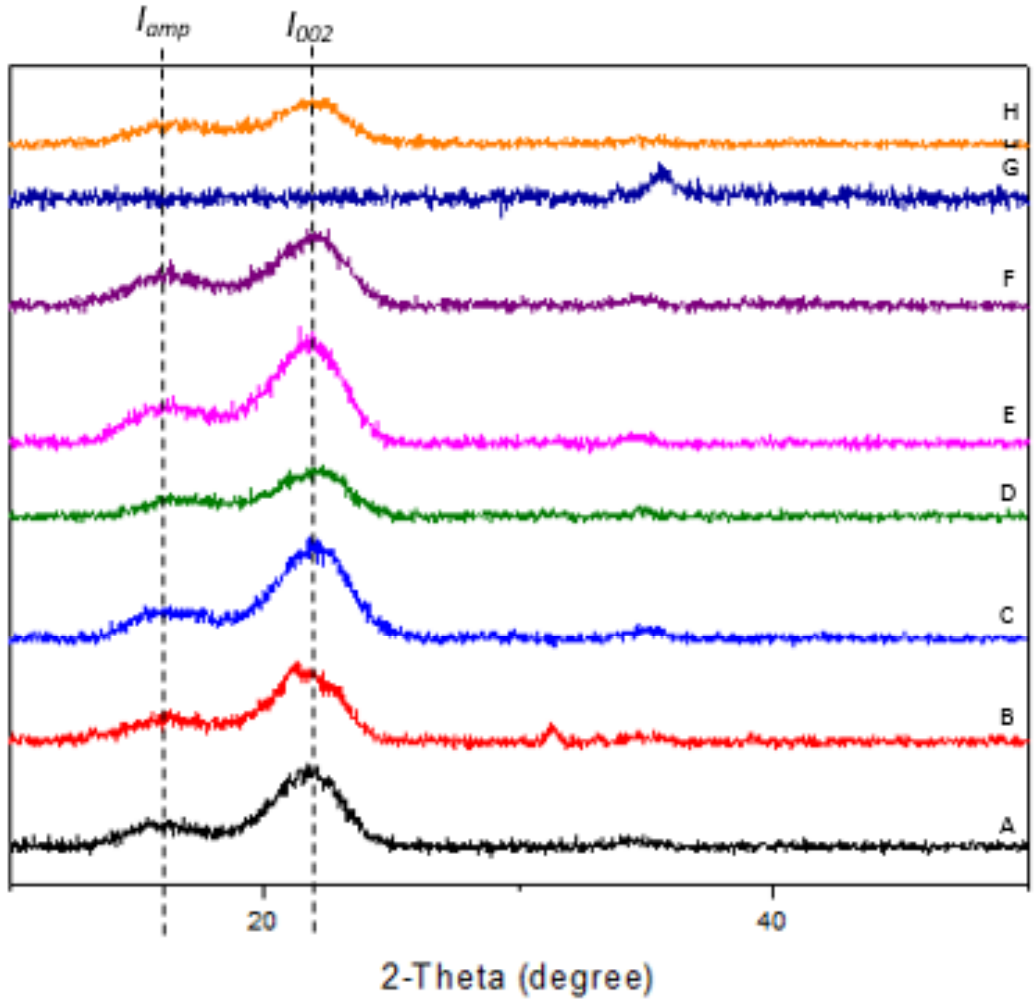

Figure 4 XRD spectra of (A): Raw (B): $ZnCl_2.4H_2O$. (C): $LiClO_4.3H_2O$. (D): Urea. (E): $ZnCl_2.4H_2O$/Urea. (F): $LiClO_4.3H_2O$/$ZnCl_2.4H_2O$ (G):$LiClO_4.3H_2O$/Urea. (H): $LiClO_4.3H_2O$/$ZnCl_2.4H_2O$/Urea

3.2.2 FTIR spectra

The FTIR technique was used to check the chemical nature of the fractions and residue after the pretreatment. The main chemical bond vibrations of lignocellulosic materials are present between 800 – 1800 cm^{-1}. The characteristic absorption bands characteristic of holocellulose components of both hemicellulose and cellulose are 1112–1120, 1161 and 897 cm^{-1}. Other cellulose characteristic bands are found at 1376, 1061 and 1025 cm^{-1} while bands observed at 1251, 1046 and 990–996 cm^{-1} are hemicellulose characteristic bands. The lignin absorption bands are at 1718, 1702, 1654, 1508, 1597, 1458, 1420, 1261, 1242, 1224 and 840 cm^{-1}.

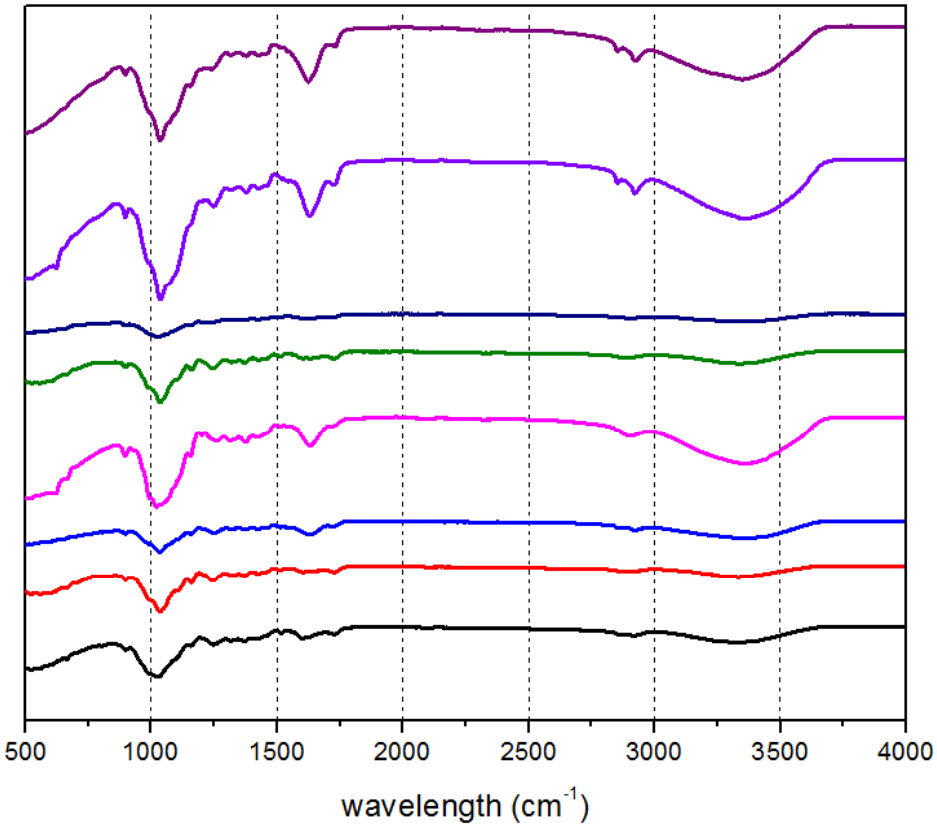

Figure 5 FTIR Spectra of (A) Raw, (B): $ZnCl_2.4H_2O$. (C): $LiClO_4.3H_2O$. (D): Urea (E): $ZnCl_2.4H_2O$/Urea (F): $LiClO_4.3H_2O/ZnCl_2.4H_2O$ (G):$LiClO_4.3H_2O$/Urea. (H): $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea

The band at a wavelength of around 1730 cm^{-1} in Figure 5 was assigned to an unconjugated C=O stretch of ester, acetyl or carboxylic acid groups in hemicellulose components. In this peak, linkages between lignin and carbohydrates may exist. FTIR intensities at this wavenumber showed increased peaks after pretreatment G ($LiClO_4.3H_2O$/Urea) and H ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea). These peaks are diminished in the raw samples (A). The peaks at wavelength 1730 cm^{-1} showed slight changes in the hemicellulose content after $ZnCl_2.4H_2O$/ Urea (E) pretreatment and a high hemicellulose removal after pretreatment using G ($LiClO_4.3H_2O$/Urea) and H ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea). These spectra correspond to the results in Figure 2 where the highest percentages of hemicellulose lost were found in samples treated with $LiClO_4.3H_2O$/Urea and $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea. This also indicates breakage of the carbohydrate and lignin linkages.

The major functional group of lignin is an aromatic compound (phenolic hydroxyl group) with a benzene ring. Structural changes in lignin and loss of aromatic compounds were shown by the changes in intensities at the 1600 cm^{-1} band. Pre-treated samples E ($ZnCl_2.4H_2O$/ Urea), G ($LiClO_4.3H_2O$/Urea) and H ($LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea) increased the intensity of the 1,600 cm^{-1} band. These spectra correlate with the results shown in Figure 2, whereby corn cob pretreatment, $ZnCl_2.4H_2O$/Urea, $LiClO_4.3H_2O$/Urea and $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea all showed similar lignin recovery when compared to one another.

The peaks at around 2900 cm^{-1} as shown in Figure 5 indicate C-H stretching vibration which shows an alkane functional group and indicates the rupture of methyl/methylene group of cellulose. After pretreatment in D, G and H, the peaks of the FTIR spectrum increase. This result shows that the methyl (CH_3) groups of the samples were disrupted. This characteristic reduction of band intensities at 2900 cm^{-1} wavelength was also found by Ciolacu et al. [18] and Kumar et al. [19] who concluded that the samples became more amorphous.

There were noticeable changes in the peaks at the 3100-3600 cm^{-1} wavelength range for most of the pre-treated samples. This corresponds to alcohols (O-H) stretching vibration which gives significant information regarding the hydrogen bonds. These changes occur as the molten hydrate salt pretreatment solvents become bound to the lignocellulose structure causing hydrogen bond breakage between O-H bonds. This also indicates possible crystalline cellulose disruption. This implies that highly ordered hydrogen bonds in the corn cob samples were disrupted through cellulose dissolution in all seven MHS pretreatment.

3.3.3 Crystallinity Index

Using data from FTIR, LOI and TCI peak ratios have been found and are tabulated in Table 2. XRD data gave information on CrI calculated using Equation (2) and the results were also tabulated in Table 2.

Table 1: FTIR crystallinity ratio and XRD Crystallinity index of raw and pre-treated biomass

	FTIR crystallinity ratio		XRD Crystallinity index	
		$\alpha_{1430}\ cm^{-1}/$ $A_{898}\ cm^{-1}$	$\alpha_{1372}\ cm^{-1}/$ $A_{2900}\ cm^{-1}$	$(I_{200}-I_{am})/I_{200}$
Pretreatment		LOI	TCI	CrI
Raw		1.224	0.935	48.044
$LiClO_4.3H_2O$	1.038		0.975	71.260
$LiClO_4.3H_2O$/Urea	1.027		0.994	77.389
$LiClO_4.3H_2O/ZnCl_2.4H_2O$	1.130		0.958	54.134
Urea	1.043		0.973	67.747
$ZnCl_2.4H_2O$	1.033		0.988	62.081
$LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea	1.075		0.954	63.626
$ZnCl_2.4H_2O$/ Urea	1.140		0.979	72.764

LOI: Lateral order index or crystallinity index based on FTIR; TCI: Total crystallinity index based on FTIR; CrI: Crystallinity index based on XRD

Higher values of TCI and LOI are characteristic of biomass samples with more ordered structures and higher crystallinity. LOI decreased after MHS pretreatment as shown in Table 2. TCI was relatively constant thus in this case, LOI was more sensitive to predict the crystallinity of the samples. In addition, it has been shown in previous literature that the LOI is a better indicator of crystallinity changes than the TCI for lignocellulosic samples [20]–[22]. The results of LOI showed the transformation of the cellulose from a highly crystalline structure to a more amorphous structure in the corn cob after pretreatment with MHS. LOI decreased from 1.224 in the raw sample to the lowest value (1.027) found in the samples treated with $LiClO_4.3H_2O$/Urea. Decrease in crystallinity of switchgrass [23] and straw [24], have also been reported after pretreatment with other cellulose solvents such as ionic liquids. Awosusi et al. (2017) reported a significant difference in the crystallinity indices as well as surface morphologies between the raw and $ZnCl_2.4H_2O$ treated biomass.

CrI values of corncob were shown in Table 2. It was notable that the CrI values of corncob residues changed approximately proportionally to the amount of cellulose recovered. Crystallinity is strongly influenced by the biomass composition. It was initially hypothesized that the crystallinity of the biomass would decrease due to the pretreatment, however since XRD measures all components and not just cellulose, there were interferences from the other amorphous components (hemicellulose, lignin). Observation of this data shows a good correlation between the CrI and cellulose content recovery (Figure 2) in the analysed material. The raw sample presented the lowest crystallinity (CrI), because it has a higher content of hemicellulose and lignin, which are amorphous [26]. The results thus show an increase in crystallinity with pretreatment and also show the pretreatment which retained the highest amount of cellulose (%) with one of the highest crystallinity index of 72,769 ($ZnCl_2.4H_2O$/ Urea) as a result of the removal of the lignin and amorphous hemicellulose fractions. The samples with lower content of lignin and hemicellulose in the compositional analysis were the samples that presented higher crystallinity, as can be noted for the pretreatment (C, D and E). Due to the lack of regular crystal structure in hemicellulose and lignin the interpretation of the crystallinity of cellulose through XRD analysis is possibly hindered by the hemicellulosic and lignin fractions. FTIR and XRD results suggest that pretreatment using MHS can reduce the crystallinity of cellulose in corn cob. A mechanism was proposed which bases the reduction of cellulose crystallinity on the ability of cellulose molecules to compete for the water molecules of the coordination sphere of a specific ion and once dissolution takes place, the hydroxyl groups of cellulose will substitute part of the coordinated water. It was suggested by Li et al. (2009) that the decrease of CrI, was due to the water rapidly precipitating, which prevented the dissolved lignocellulose from rearranging into the original crystalline structure, this resulted in biomass with more amorphous structure.

3.4 Morphological analysis for evaluation of biomass

To understand the significant changes in corn cob after pretreatment, the morphology of the untreated and pre-treated corn cob samples was investigated by SEM, as presented in Figure 6.

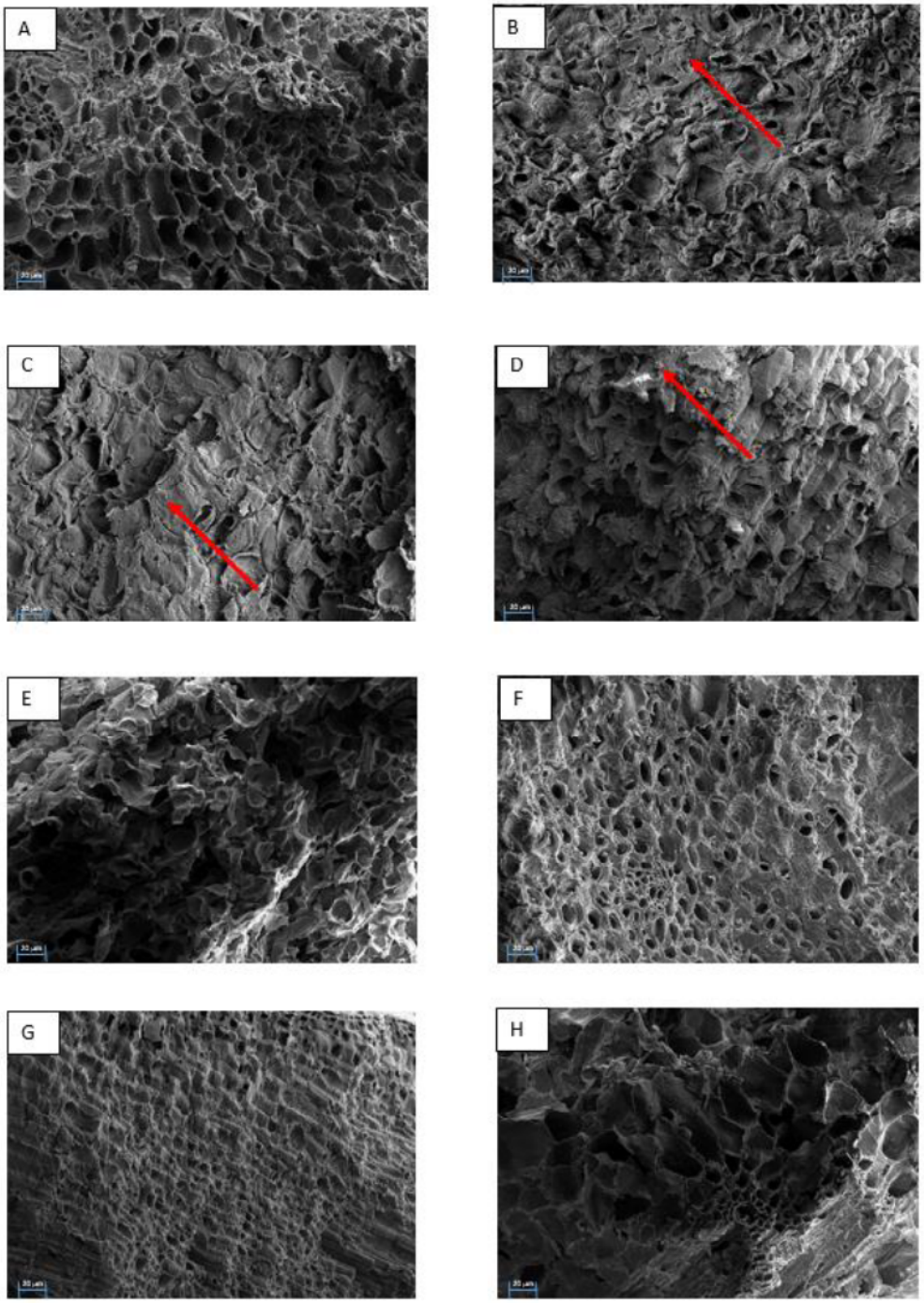

Figure 6 Scanning Electron micrograph of untreated and pretreated corn cob. (A) untreated (B) $ZnCl_2.4H_2O$ treated (C) $LiClO_4.3H_2O$ treated (D) Urea treated (E) $ZnCl_2.4H_2O$/Urea treated (F) $LiClO_4.3H_2O$/Urea treated (G) $LiClO_4.3H_2O/ZnCl_2.4H_2O$ treated (H) $LiClO_4.3H_2O/ZnCl_2.4H_2O$/Urea treated at 500X Magnification. Red arrows indicate globular structures.

SEM results for the raw samples shows an intact lamellar structure where hemicellulose and lignin wraps around the cellulose in a hetero-matrix. In addition, the structure of the raw sample is visibly ordered; however, the molten hydrated salt treated (Figures 6B-H) corncob samples showed disrupted lamellar structures due to the removal of hemicellulose and lignin. The samples also show distinct inner cavities of the biomass, thus

increasing the surface area. The particle size of the corn cob was also reduced in Figures 6B-H, in addition, there was a damage in the intact cell structure as well as a more exposed internal cell structure. The pretreatment certainly increased the external surface area and the porosity of the material for each of the pretreatment. The most obvious change from the original fibre can be noted in the Figure 6B. These samples were not homogenous therefore, quantification based on surface deconstruction cannot be done. The change in surface morphology is in agreement with the finding of Selig et al. (2009) which showed that there was similar change on the biomass surface structure occurs when corn stover is pre-treated and delignified using alkaline peroxidase [28].

Further proof of modifications which occurred after pretreatment and another interesting feature of some of these samples is the presence of globular structures (indicated by the red arrows). These globular structures are associated with lignin condensation that were observed on the surface of the corn cob after pretreatment. The formation of these lignin agglomerates on the surface have been observed in many other types of lignocellulosic biomass when they undergo organosolv steam-explosion and diluted acid pretreatment [29]. This droplet phenomenon has in previous studies been related to the severity of pretreatment conditions. The droplets were observed in the corn cob samples pre-treated with (B) $ZnCl_2.4H_2O$ (C) $LiClO_4.3H_2O$ (D) Urea. The more severe the pretreatment and its conditions, the more droplets formed on the surface. The lignin molecules liquefy and then coalesce. Once the lignin phase transition is reached, droplets are formed within the cell wall matrix. Due to the hydrostatic pressures within the layers of the cell wall, some of the lignin fractions are forced to the outer surface of the biomass samples. The small droplets bind to the pretreatment mass and are then re-deposited on the biomass surface when the samples are cooled.

Biomass morphology is a major factor that contributes to effective hydrolysis. The larger the surface area, the more MHS molecules can access the plant cell wall polymers, as well as being able to separate the main fibres into fibrils and strands. SEM analysis results supported the FTIR and XRD results which showed that MHS pretreatment were effective in reducing the crystallinity of the biomass overall.

4 Conclusion

All seven MHSs investigated in this study were effective in altering the biomass crystallinity. SEM images revealed a loose and disordered structure of biomass post pretreatment. FTIR analysis indicated that MHS treated biomass exhibited a significant loss of native cellulose crystalline structure. It can be concluded that the seven MHS solvents proposed in this study are promising green solvents to reduce biomass and cellulose crystallinity and alter surface chemistry and surface morphology during pretreatment step. The decrease of crystallinity and increase in surface area after the pretreatment will enhance the digestibility during enzymatic hydrolysis which will increase sugar yield for biochemical and biofuel production. Among the pretreatment solvents studied, $ZnCl_2.4H_2O$/ Urea pre-treated samples was the most efficient in terms of highest cellulose recovery, highest hemicellulose recovery in the liquid fraction and highest lignin recovery. It is expected that this pretreatment can improve the utilisation of biomass significantly due to the significant recovery yield of cellulose and lignin in the solid fraction and hemicellulose in the liquid fraction which shows that effective fractionation has occurred at mild conditions using a mild pretreatment solvent ($ZnCl_2.4H_2O$/ Urea).

Funder Information

The authors are grateful to the National Research Foundation, South Africa and University of the Witwatersrand, South Africa for support of this study.

Competing Interest: The author declares no competing interests

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Optimization of dissolution conditions during fractionation of corn-cob in $ZnCl_2.4H_2O$/Urea solvent system: A statistical approach

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Abstract

The choice of a suitable pretreatment method and the adjustment of the pretreatment parameters for efficient conversion of biomass are crucial for a successful biorefinery concept. Experimental results were analysed and appropriate predictive empirical linear models were developed for each response (cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery). For cellulose recovery, A (Time) and BC (Temperature * solvent concentration) were significant terms. Hemicellulose recovery in the liquid fraction showed significance of A (Time) and AC (Time * solvent concentration) while the significant coefficients in lignin recovery are A (Time) and B (Temperature). Using numerical optimization by desirability function, optimum pretreatment conditions obtained were: 90 min, 120 ^{o}C and concentration of 71.32%/28.68 (w/w) $ZnCl_2.4H_2O$/ Urea. At these conditions, the predicted recovery for cellulose, hemicellulose and lignin were 99.03%, 27.18% and 72.43% respectively with a desirability of 0.902. The actual recovery was 91%, 29% and 68% for cellulose, hemicellulose and lignin respectively at the same conditions. This study demonstrates optimization of a mild lignocellulosic biomass pretreatment method for the production of value-added products.

Keywords: Pretreatment, Lignocellulosic biomass, Molten hydrate salts, Response surface methodology, Solvent, Central Composite Design.

1 Introduction

To reduce our dependence on petroleum based sources and mitigate global climate change, Alternative and renewable fuels derived from lignocellulosic biomass need to be investigated [1]. To effectively utilise lignocellulosic resources, new technologies are needed to overcome the barriers associated with the conversion of biomass to chemicals and fuels [2]. These much needed breakthroughs include improvement of pretreatment processes that are able to fractionate biomass to the main components of lignin, cellulose and hemicellulose in high volumes in order to improve overall biomass utilisation [3].

The conventional method for the multifactorial system optimization uses one-factor at a time (OFAT). However, this type of method is time-consuming and fails to consider any possible interaction between the factors. Response surface methodology (RSM) is the most popular statistically-based optimization strategy which uses a quadratic polynomial model to establish optimal conditions [4]. RSM is used in investigating the effects of individual as well as interactive effects of factors in a process. An efficient alternative to factorial design is central composite design (CCD), this design is popularly used for second order response surfaces [5]. Determining the optimal operating conditions for effective pretreatment techniques can contribute to overcoming the cost barriers associated with the use of biomass by reducing the number of experimental trials needed to evaluate multiple parameters and their interactions[6]. RSM has been successfully applied in the study and optimization of chemical pretreatment by ionic liquids [7], alkaline peroxide [8], alkaline and dilute acids [9]. In addition, the central composite design (CCD) of RSM has previously been successfully applied in the optimization of several pretreatment processes [5,9]

Previously, a pretreatment solvent ($ZnCl_2.4H_2O$/Urea) was identified that effectively fractionated the corn cob into cellulose, hemicellulose and lignin with high recovery of cellulose in the solid fraction and high recovery of hemicellulose in the liquid fraction. The optimization of the solvent and the conditions under which the solvents operate are widely important, not only for the overall improvement of the process but also from an economic perspective. The improvement of the pretreatment would increase the yield and quality of products from downstream processes such as enzymatic hydrolysis and fermentation [10].

In this study, the effect of pretreatment conditions (time, temperature and solvent concentration) on the use of $ZnCl_2.4H_2O$/ Urea molten hydrate was investigated and optimized for the fractionation of corn cob. RSM was adopted to investigate the effect and optimize the pretreatment conditions for fractionating cellulose, hemicellulose and lignin of corn cob. The main and interaction effects of the parameters were studied. CCD, a class of three level complete factorial design was used as a design experiment which considered pretreatment time, temperature and solvent concentration as the independent variables and the responses were % recovery of cellulose, % recovery of hemicellulose and % recovery of lignin.

2 Materials and method

2.1 *$Zncl_2.4H_2O$/ Urea Pretreatment*

The pretreatment was carried out at varying temperatures (60-90 °C) in a shaking incubator for varying times (60-120 min) using $ZnCl_2.4H_2O$/ Urea at varying concentration ratios (50-90 % w/w) (Table 1) at a constant biomass to solvent ratio of 1:10 (5 g dry biomass will be added to 50 g of each solvent) and at a constant agitation speed of 270 rpm. The slurries obtained from each pretreatment method were recovered and separated into solid and liquid fractions by vacuum filtration.

2.2 *Compositional Analysis of Solids*

The compositional analysis of biomass were analysed for hemicellulose, lignin, extractive and ash content as documented by the National Renewable Energy Laboratory (NREL) following Laboratory Analytical Procedures (LAP TP-510-42618, 42619, 42622) [11]–[13].

2.3 *Experimental Design*

The experimental design is shown in Table 1, the experimental data were analysed using RSM to fit the second order polynomial equation created by Design-Expert 7 software (Stat-Ease Inc., USA) The parameters were selected for the studies as follows: reaction temperature (60-90 °C), $ZnCl_2.4H_2O$/ Urea molar ratio (50- 90% w/w) and time (60-120 min). The variable ranges are shown in Table 1. The ranges were selected to keep the intervals within mild reaction conditions. Table 1 describes the five coded levels ($-\alpha$, -1, 0, 1, α) and un-coded independent factors, the test ranges for the parameters and the overall experimental design. The CCD design made up of 20 experiments (8 factorial points, 6 centre points and 6 axial point) were carried out with three variables and each variable varied at three levels to allow for the evaluation of the curvature [14]. The value of α is the distance from the axial points to the central point $\alpha = (2^n)^{1/4}$, in which n is the number of independent factors, for three factors α=1.68 [15]. The factorial design experiments were run in duplicates to ensure reliability; the average error was calculated and used.

2.4 *Model Formulation and Statistical Analysis*

The experimental data was used to develop a quadratic polynomial model having % cellulose recovery (Y_1), % hemicellulose recovery in the liquid fraction (Y_2) and % lignin recovery (Y_3). The general regression model (Equation (1)) was adopted.

$$Y = \alpha_0 + \alpha_1 A + \alpha_2 B + \alpha_3 C + \alpha_{12} AB + \alpha_{13} AC + \alpha_{23} BC + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{33} C^2 + \varepsilon \quad \ldots (1)$$

Where *A*, *B*, *C* represent the symbols of the independent variables temperature, time and solvent concentration respectively. The intercept term is α_0; α_1, α_2 and α_3 represent linear terms; α_{11}, α_{22} and α_{33} represent quadric terms; α_{12}, α_{13} and α_{23} are the interacting terms. All experiments were done in duplicate and data average values were calculated. The statistical analysis, plotting of response surfaces and optimization of the data were performed using "Design Expert" software (Trial version 7.0.3, Stat-Ease, Inc., Minneapolis, USA). The analysis of variance (ANOVA) was calculated. The fit quality of the polynomial equation was assessed by the coefficient of determination (R^2), the significance of the regression and statistical coefficient was checked using t-test and F-test, respectively. The predictability of the developed model was then cross validated using laboratory experiments.

2.5 *Desirability Function*

Numerical optimization by desirability function was carried out to optimize the operating conditions of the pretreatment. The desirability function technique can be used to determine the optimum conditions of a process which involves one or more responses. The desirability procedure first determines the independent variable levels that simultaneously produce the most desirable prediction for the responses, thereafter the overall desirability of the outcome is maximised based on the factors which can be controlled.

In numerical optimization, the desired goal for each factor and response were chosen. The goals defined were as follows: maximise, minimise, target, exact value (for factors only), none (for responses only) and within range. Maximum and minimum levels for each factor were chosen. The factors can also be weighted according to priority. The goals were combined to get a desirability function. The desirability function is an objective value ranging from zero (outside the goal) and one representing the goal, the program thus aims at maximising this function. The goal seeking step starts randomly from one point and makes its way to a maximal point through the steepest slope.

3 Results and discussion

3.1 *Central Composite Design Studies*

CCD was employed using the Design-Expert (Stat-Ease, Inc., Minneapolis, USA) software to study the simultaneous effects of reaction time, temperature and solvent concentration of pretreatment on cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery. The experimental conditions were obtained by a complete three-factor experimental design, which included six replications of the centre point and

six axial points and eight factorial points. Table 2 shows the series of conditions of the experiments conducted for each run.

3.2 *Composition of Solid Fraction*

The detailed recovery of the components of $ZnCl_2.4H_2O$/ Urea treated solid fraction for each run are shown in Table 2. The highest solids recoveries of cellulose for the pretreatment were obtained in run 10 (39.55 min, 75 ^oC, 70/30 % (w/w) $ZnCl_2.4H_2O$ /urea) and 12 (90 min, 49.77 ^oC, 70/30 % (w/w) $ZnCl_2.4H_2O$ /Urea) with 87.6% and 86.1%, respectively. This shows that cellulose is not less affected by the severity of the pretreatment as opposed to hemicellulose. Hemicellulose recoveries were lowest at run 8 (120 min, 90 °C, 90/10 % (w/w) $ZnCl_2.4H_2O$/Urea) and for run 4 (90 min, 49.11 °C, 70/30 % (w/w) $ZnCl_2.4H_2O$ /Urea). These severities are considered high due to longer times and higher temperatures. This is in agreement with various studies which state that hemicellulose is more susceptible to pretreatment conditions [16] . Additionally, the lowest lignin recovery for the trials were obtained during run 3 (60 min, 75, 50%), 4 (60 min, 100.23 °C, 70/30% (w/w) $ZnCl_2.4H_2O$ /Urea) 8 (120 min, 90 °C, 90/10 % (w/w) $ZnCl_2.4H_2O$ /Urea).

3.3 *Models and Statistical Analysis*

The experimental data was analysed using the Design-Expert software and a second-order polynomial equation was obtained. The equation included linear, quadratic and interactive terms for the variables tested. The polynomial equations, describing the cellulose recovery (Y_1), hemicellulose recovery (Y_2) and lignin recovery (Y_3) as a function of reaction time (A), temperature (B) and solvent concentration (C) of $ZnCl_2.4H_2O$/ Urea MHS pretreatment are shown in Equation (2), Equation (3) and Equation (4):

$$Y_1(\%) = +88.31 + 2.87A + 6.55B - 6.40C + 0.52AB - 1.81AC - 15.24BC + 0.22A^2 - 2.88B^2 - 0.15C^2 \quad \ldots \quad (2)$$

$$Y_2(\%) = +18.67 + 1.79A + 4.62B + 3.15C + 0.35AB + 1.72AC + 3.37BC + 0.075A^2 + 0.14B^2 + 1.12C^2 \quad \ldots \quad (3)$$

$$Y_3(\%) = +61.00 - 1.49A + 14.08B + 3.24C - 1.90AB + 1.02AC + 2.23BC + 0.31A^2 + 1.36B^2 + 1.77C^2 \quad \ldots \quad (4)$$

Equations (2 - 4) reveal the effect of the individual variables (linear and quadratic) or double interactions on the responses. The negative coefficients of the values show the negative effect of the factors on the responses (decrease the response), for example, solvent concentration (C) decreases cellulose recovery (Y_1), whereas the

positive coefficients of the values mean that the factors have an additive effect on the responses (increase the response).

3.3.1 *Analysis of Variance*

The significance of the developed model, the pretreatment variable (linear and quadratic) as well as the interaction effects were analysed ANOVA. ANOVA is a tool used to test the statistical significance of each variable by comparing the estimated experimental error to the mean square [17]. The *p*-value showed that some terms where significant while others were not. However, all the coefficients were considered in the design and polynomial equation to minimise the error. The results of ANOVA for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery after $ZnCl_2.4H_2O$/Urea pretreatment are presented in Table 3, Table 4 and Table 5 respectively.

The model analysis gave F-value of 10.23, 6.45 and 9.18 for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery respectively. This implies that the models are significant for each of the responses. The F-value of the model is calculated as the ratio of mean square values for individual terms to the mean square values for the residuals. The Prob > F represents the probability of the F-statistics value. This is used as a test for the null hypothesis. For a model to be considered significant, the *p*-value needs to be lower than 0.05, this value indicates that there is only a 5% chance that the models the values represent could occur due to noise [17]. The models of cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery are significant and effectively describe the responses. The cellulose recovery response model has a lower *p*-value (0.0006) than the hemicellulose recovery in the liquid fraction model (0.0037) and lignin recovery model (0.0009) indicating that the model for cellulose recovery is most significant.

In Table 3, A (Time) and BC (Temperature * solvent concentration) are significant model terms in the model Equation (2) and they significantly affect cellulose recovery, while the other coefficients do not influence cellulose recovery greatly in the range of this study. The results are in accordance with the observation that cellulose dissolution only takes place under harsh conditions such as high temperatures [18]. A (Time) and AC (Time * solvent concentration) as seen in Table 4 also have significant effects on model Equation (3), they therefore significantly affect hemicellulose recovery in the liquid fraction while the other factors do not. The solvent concentration influence is second order however there has been evidence showing the cellulose solvent (NaOH) concentrations having an impact on Hemicellulose dissolution [19,20]. For lignin dissolution, the coefficients which are significant in the model are A (Time) and B (Temperature). This is in agreement with Vancov and McIntosh (2011) who noted that in the case of delignification, even in mild alkaline solvent conditions, an increase in the temperature improves the delignification rate [21].

The "Lack of Fit F-value" of 3.06, 1.00 and 1.78 for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery respectively, implies the lack of fit is not significant when in relation to the pure error. The "Lack of Fit F value" is not significant and there is only a 10.73% (Table 3), 47% (Table 4) and 25% (Table 5) chance that the large values of F could have occurred as a result of noise.

3.3.2 *Model Summary*

Table 6 shows the model summary for each response. The coefficient of variability (C.V) measures residual variations of the data in relation to the size of the mean. Higher C.V values usually indicate lower reliability while relatively low values of C.V indicate better reliability and precision of the conducted experiments. The C.V% was a low value of 5.56%, 14.03% and 5.41% for cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery respectively which indicates that the experiments conducted were reliable and precise. The value of the signal-to-noise ratio (adequate precision ratio) was found to be 12.919, 11.593 and 9.672 indicating the models have an adequate signal. The adequate precision ratio and adjusted determination coefficient (Adj R^2) exceeded 4 and 0.70, respectively for all three models. This indicates that a good correlation between input and output variables could be drawn by the model developed and it was implied that the quadratic model could be used to explore the design space, predict the responses and find the optimal conditions of this process.

3.3.3 *Pareto Analysis*

The percentage effect of each of the factors was done by the Pareto analysis [6]. The analysis calculates the percentage effect (*Pi*) of each factor on the response, according to the following equation:

$$Pi = \left(\frac{\beta_i^2}{\sum_{i=1}^{14} \beta_i^2}\right) \times 100 (i \neq 0) \quad \ldots \quad (5)$$

Where βi is the regression coefficient of each process variable.

Figure 1, Figure 2 and Figure 3 shows the Pareto plot. As seen in Figure 1 under the $ZnCl_2.4H_2O$/ Urea pretreatment, the most significant factor in cellulose recovery are B (Temperature) and C (Solvent concentration) in both linear and interactive forms. The interactive form (AB) however has the highest influence of 69.07%. Hemicellulose recovery in the liquid fraction was mainly affected by temperature (linear term), followed by interactive term (BC), solvent concentration (linear term), time (linear term) and interaction of time and temperature. Finally, lignin recovery was affected primarily and almost entirely by temperature (linear term) with 88.72% effect. Followed by solvent concentration (linear term) at 4.7%. The highest interactive term was temperature*solvent concentration (BC) with 2.2% effect on lignin recovery.

3.3.3 *Model Fitting*

The reliability of the predicted models was additionally tested by comparing the predicted and experimental response results. The observed recovery from the experiment and the predicted values are shown and represented as a parity plot in Figure 4 with the R^2 values for each response. In each case, there was satisfactory

correlation between the predicted values and observed values from the experiment which was R^2=0.9021, 0.8531, 0.8920 for Figure 4A, Figure 4B and Figure 4C, respectively. The values indicate close agreement between the experimental and predicted values for each response. In Figure 4A, Figure 4B, Figure 4C, 9.8%, 14.69% and 22.3% of the total variation, respectively, was not explained by the model.

3.4 *Influence of Pretreatment Parameters Combinations*

The three-dimensional response surface plot described by the above-mentioned polynomial Equation (2), Equation (3) and Equation (4) were fitted to the experimental data as represented in Figure 5. One of the variables was kept constant (at midpoint) while the other two variables were varied according to the experimental ranges determined previously. The shapes of the response surfaces indicate the type and amount of the interaction between the different independent variables [22]. The surface plots for the cellulose, hemicellulose and lignin recovery are shown in Figure 5A, Figure 5B and Figure 5C. Figure 5A shows that the pretreatment temperature had the major increasing effect on cellulose recovery. As seen in Figure 5A, longer times result in higher cellulose recovery only for the pretreatment at higher temperatures while the effect of longer pretreatment time was negligible at lower pretreatment temperatures. At higher solvent concentrations, cellulose recovery increased, however, as the concentration increased, the effects of increasing temperature became negligible after 77 ^{o}C. Overall, the cellulose recovery was maximum at 100.23% with only a small increase when time is increased to approximately 90 min.

The response surface plots for the hemicellulose recovery in the liquid fraction are shown in Figure 5B. The results showed that temperature also has a large influence on hemicellulose recovery in the liquid fraction. Figure 5B shows a decrease in the recovery of hemicellulose in the liquid fraction with increasing concentration until 75% where an increase is observed thereafter. The largest recovery in the liquid fraction is observed at higher concentration and longer time. In Figure 5B, it was observed that at lower concentrations, the effect of temperature is negligible. While at lower temperatures, the effect of concentration is negligible. However, at higher temperatures and concentrations, we notice an increase in the hemicellulose recovery in the liquid fraction. As expected, the recovery of hemicellulose is highly dependent on the severity of the experiment. This is in agreement with various other studies which have shown a dependence of hemicellulose on severity compared to the other components [23]. The graph of the interaction between concentration and temperature (Figure 5B) gives the maximum recovery of 24.6% at 100% concentration, 100.23 ^{o}C and time at the midpoint of 75 minutes; these are the best conditions for achieving high yields of recovered hemicellulose in the liquid fraction. This validates the results of the Pareto chart in Figure 2 where Temperature* Solvent concentration (BC) had the highest interactive influence on the response.

Figure 5C represents the effects of temperature and time on lignin recovery at a constant solvent concentration of 75%. An increase in temperature resulted in a linear increase in lignin recovery. The length of time had no influence on the lignin recovery at lower temperatures; however, the lignin recovery was maximal at shorter times. Results in Figure 5C shows that an increase in time caused a linear decrease in lignin recovery at low solvent concentrations, however at higher solvent concentrations; there is a slight decrease in lignin recovery with increasing time. Solvent concentration at shorter times resulted in a decrease in lignin recovery until

approximately 62.50% thereafter, an increase in lignin recovery was observed. Figure 5C shows the effect of temperature and concentration on lignin recovery at a constant time of 75 min. Solvent concentration has a negligible effect on the recovery of lignin at lower temperatures. There is however a slight increase in lignin recovery with increasing concentration. Temperature has a linear effect on lignin recovery as the increase in temperature results in an increase in the response at both high and low solvent concentrations. Figure 4.8C shows the highest amount of recovery (69%) and this agrees with the Pareto chart (Figure 3) where BC is shown to have the highest interactive effect on lignin recovery

3.5 *Optimization by Desirability Function.*

The mathematical model which was developed from the experimental data can be used for the effective prediction of the operating conditions which should be used for the optimization of the model responses. The strategy that was used can determine the pretreatment temperature, pretreatment time and solvent concentration which leads to maximum responses. Desirability function (DF) assigns a scale-free value to all responses in the problem by the so-called individual desirability functions and then combine these values by taking usually their geometric mean to obtain an overall desirability function as a single objective [17]. This multiple response numerical method was applied for optimization of any combination of three factors (time, temperature, concentration). Figure 6 was obtained from design expert analysis and demonstrates the desirability values of the numerical optimization procedure in which the criteria were set as follows: "in range" for time (60 min), "in range" for temperature (70-120 ^{o}C), "in range" for concentration (50-90%) the objective was to optimize (maximize) the responses, cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery to analyse economically viable optimal conditions. A variety of solutions were found however the best local maximum was shown as a desirability plot (Figure 6). The desirability plot shown in Figure 6, provides the optimized treatment conditions of 90 min, 120 ^{o}C and concentration of 71.32% (w/w) $ZnCl_2.4H_2O$/ Urea. At this condition, cellulose recovery was 99.03%, hemicellulose recovery in the liquid fraction was at 27.18% and lignin recovery was at 72.43% and desirability at 0.902. These optimum values were cross validated experimentally which resulted in 91% cellulose recovery, 29% hemicellulose recovery in the liquid fraction and 68% lignin recovery at the same conditions. These values indicate the suitability and accuracy of the model. Apparently, the recovery of cellulose needs different conditions compared to hemicellulose recovery in liquid fraction and lignin recovery. These were the best conditions to favourably optimize all three responses.

The optimized value for the solvent concentration is 71.32% (w/w) $ZnCl_2.4H_2O$/ Urea. This low solvent concentration is advantageous as it reduces the viscosity of the solvent. This reduces some challenges which are usually encountered with higher concentrations of MHS. The optimized temperature of 120 ^{o}C is higher than the glass transition temperature of both lignin and hemicellulose which are 40 ^{o}C and from 50 ^{o}C – 100 ^{o}C, respectively. This temperature is thus expected to delignify the biomass as well as increase the dissolution of hemicellulose to an extent [24]. The pretreatment time of 90 min is desirable as it is expected to reduce the degradation of the polysaccharide components (cellulose and hemicellulose) which results in an increase in polysaccharide yield and decrease in fermentation inhibitors. The obtained desirable conditions differ largely from those obtained in the pretreatment of wheat straw using ionic liquid pretreatment. The conditions found in the study performed by Fu and Mazza were 158 °C, 49.5% ionic liquid concentration and 216 min [25]. The large difference in the optimal conditions is caused by the responses for the optimization being based on the

recovery of fermentable sugars in the pretreatment of wheat straw as opposed to whole fractions of the components as calculated in this study [25].

4. Conclusion

The effect of dissolution parameters (solvent concentration, reaction time and temperature) on the pretreatment efficiency. Appropriate predictive empirical linear models were developed for each response (cellulose recovery, hemicellulose recovery in the liquid fraction and lignin recovery). The effects of the individual variables and their interactions were found to be statistically significant. The results showed that the quadratic terms among the investigated variables were statistically insignificant. Time was shown to influence all the responses while the temperature * solvent concentration interaction and time * solvent concentration interaction were shown to influence cellulose recovery and hemicellulose recovery, respectively. Optimization resulted in 91% cellulose recovery, 29% hemicellulose recovery in the liquid fraction and 68% lignin recovery at mild conditions of 90 minutes, 120 °C and 71%/29% (w/w) $ZnCl_2.4H_2O$/ Urea. This study demonstrates optimization of a mild lignocellulosic biomass pretreatment method that enables the production of value-added products from cellulose, hemicelluloses and lignin.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The authors are grateful to the National Research Foundation, South Africa and University of the Witwatersrand, South Africa for support of this study.

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Figures/Illustration

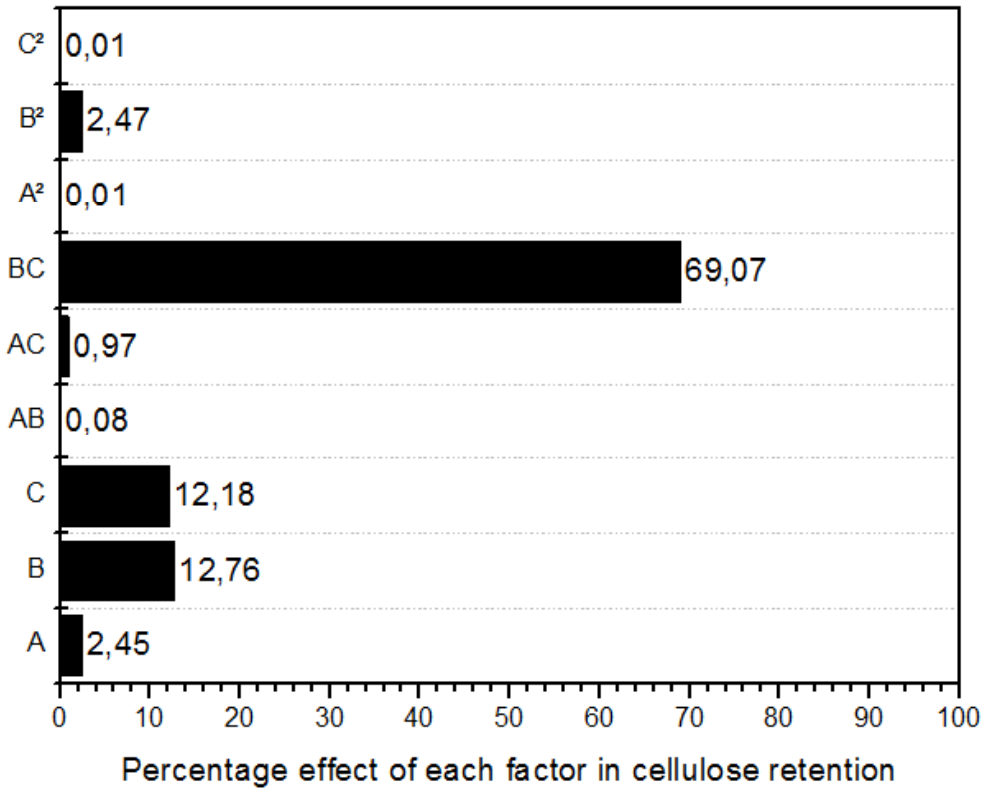

Figure **1**. Pareto chart showing the percentage effect of each factor in cellulose recovery

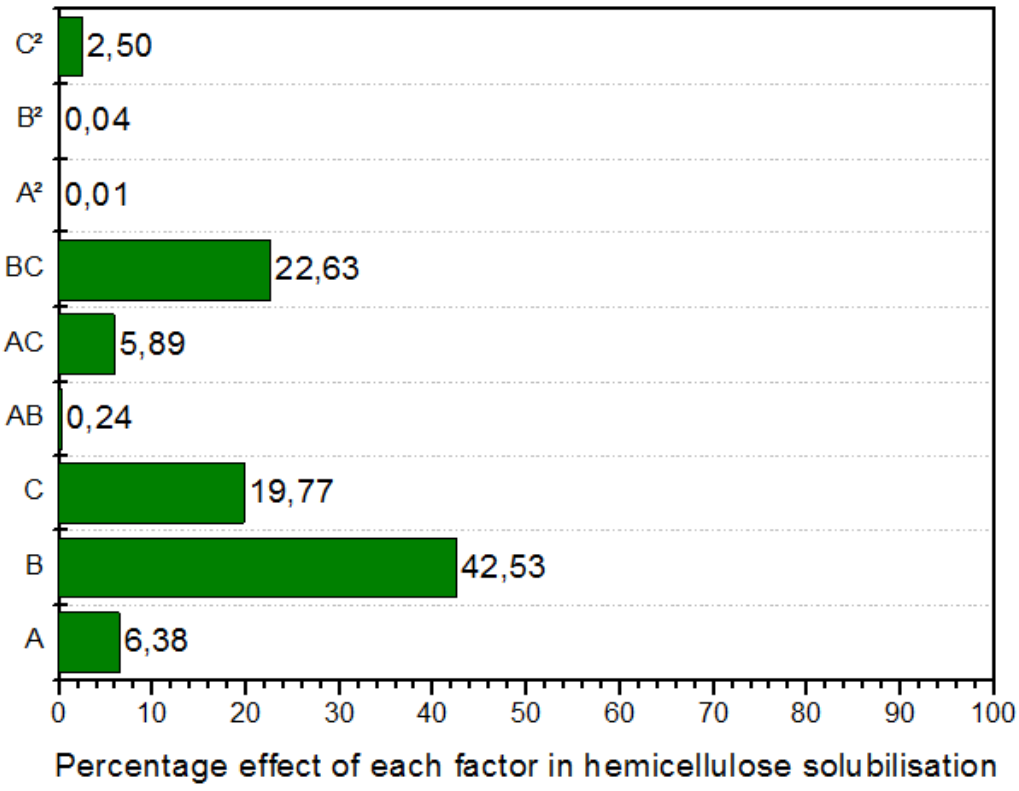

Figure **2**. Pareto chart showing the percentage effect of each factor on hemicellulose recovery in the liquid fraction

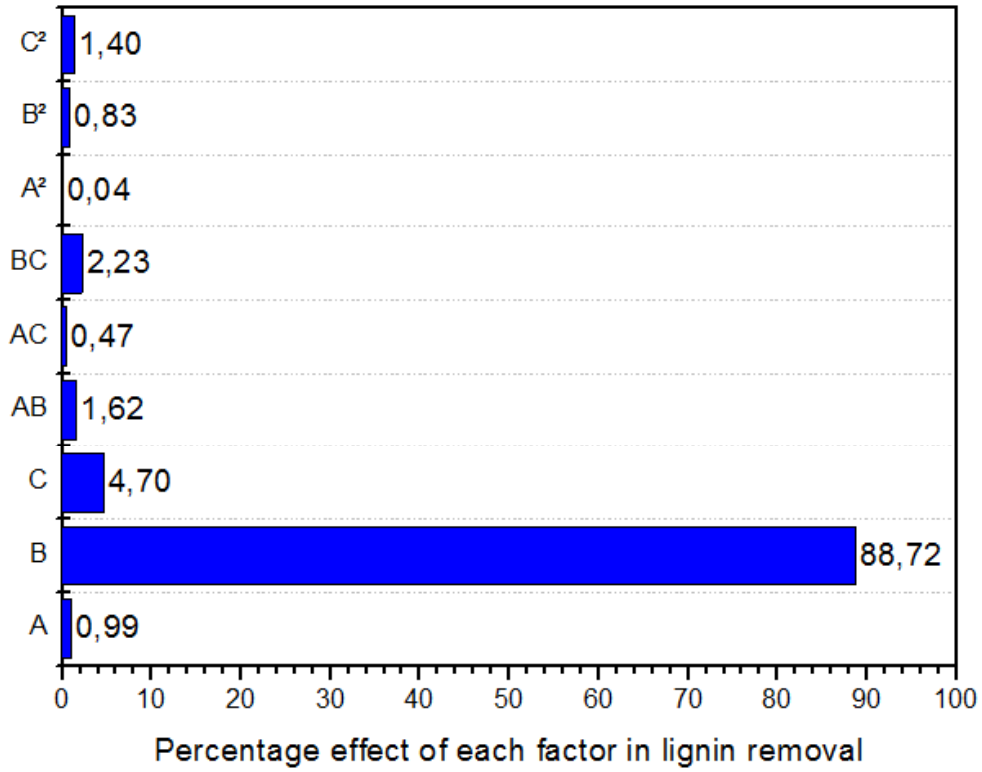

Figure **3**. Pareto chart showing the percentage effect of each factor on lignin recovery

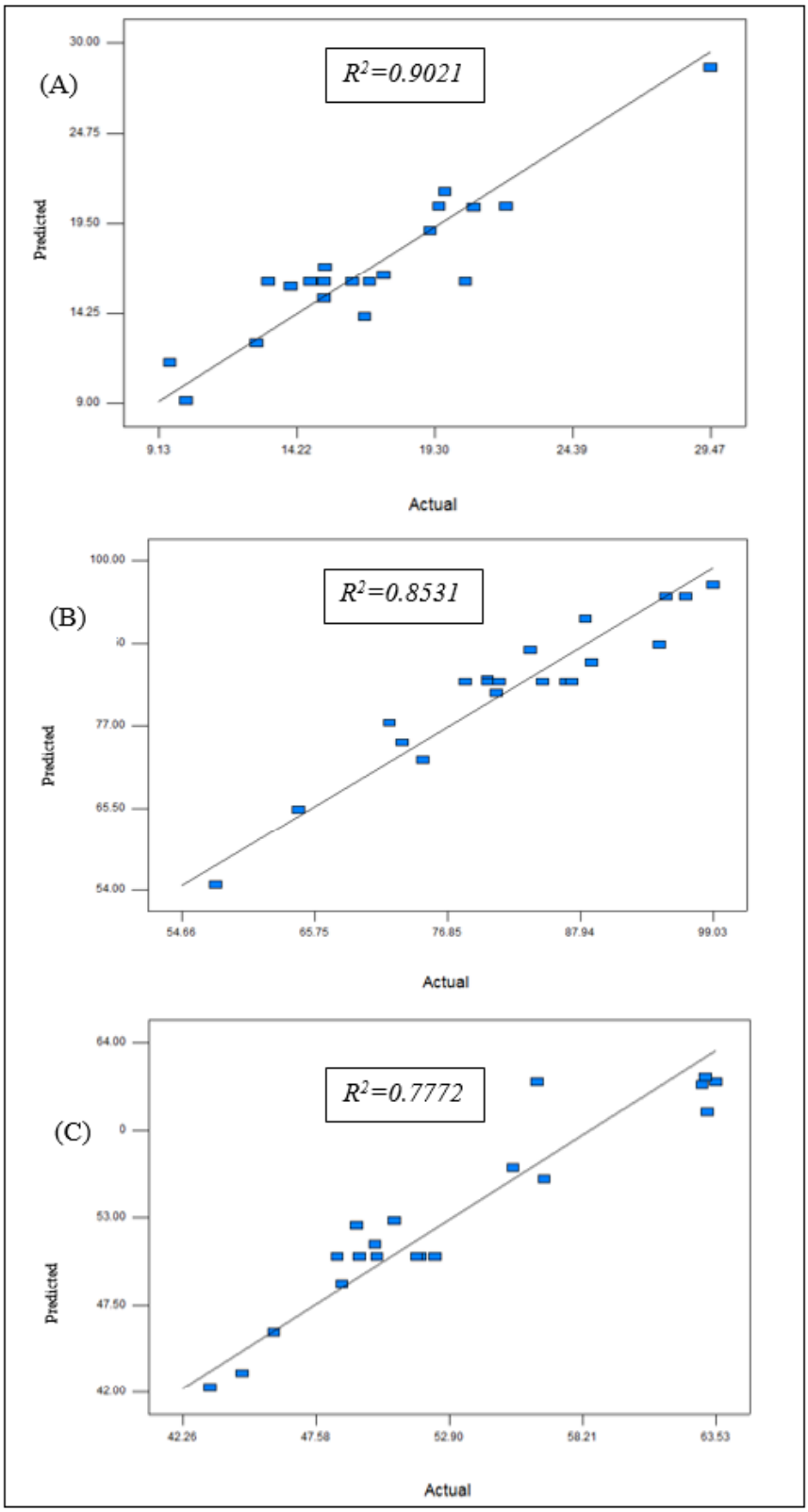

Figure **4**. Parity plot showing distribution of predicted vs. actual values of responses. (A) Cellulose recovery. (B) Hemicellulose recovery in the liquid fraction. (C) Lignin recovery.

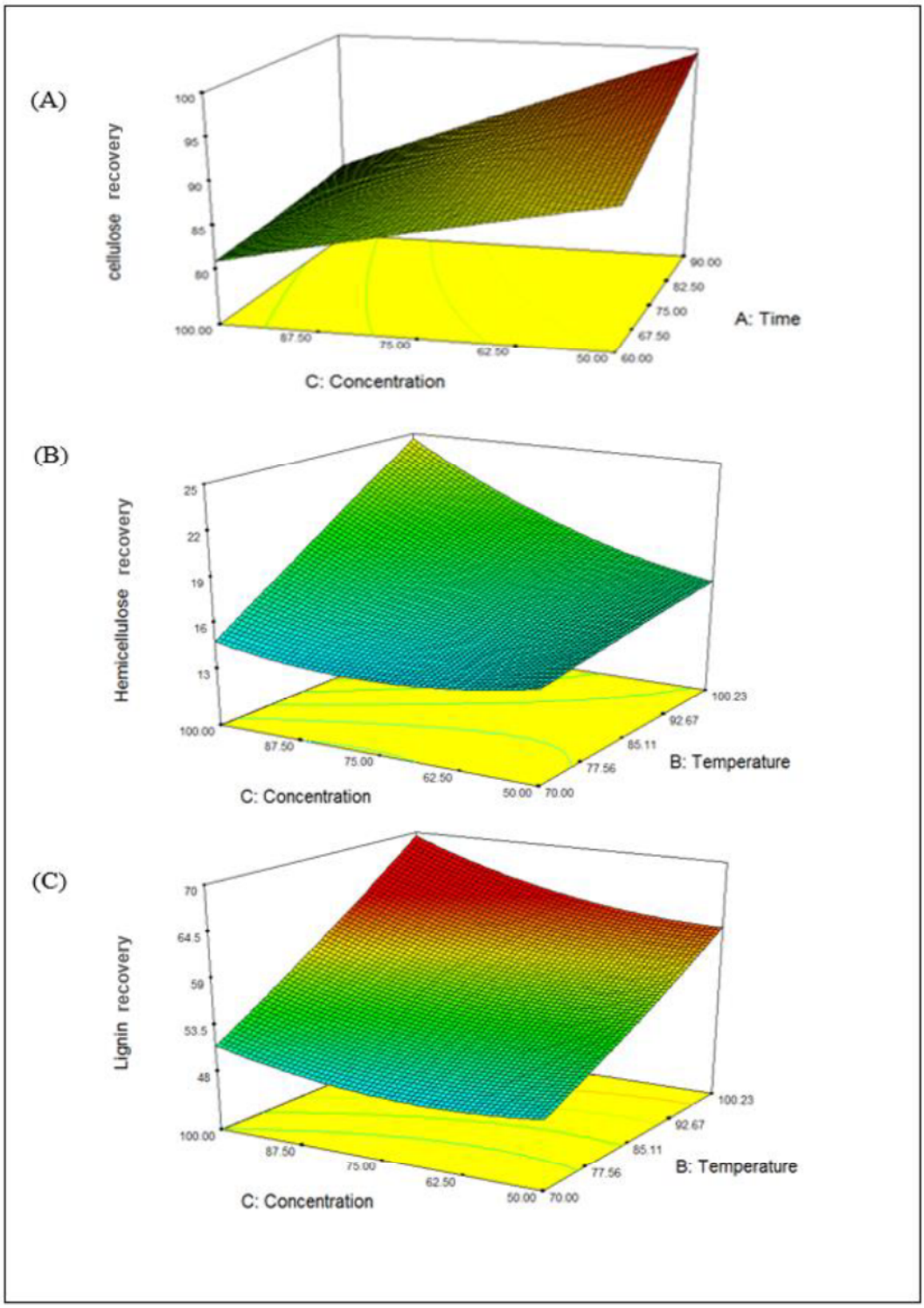

Figure **5**. Response surface plot showing effect of pretreatment conditions on (A) Cellulose recovery. (B) Hemicellulose recovery (C) Lignin recovery. Midpoint conditions 75%/25% (w/w) $ZnCl_2.4H_2O$/ Urea, 75 °C, 75 min

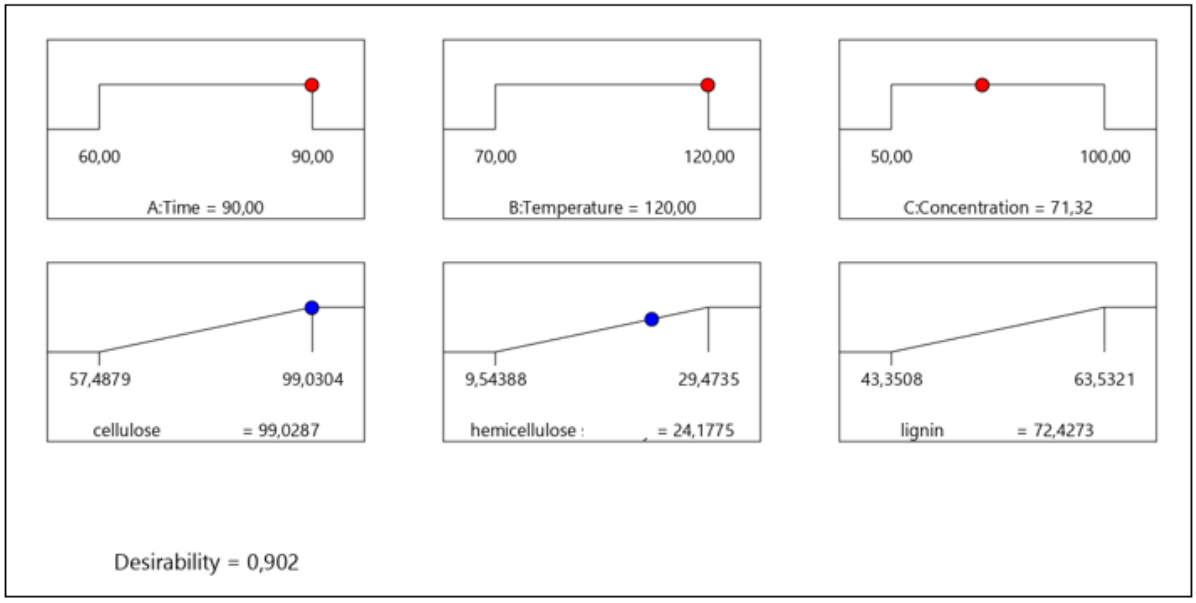

Figure **6**. Desirability ramp of cellulose recovery, hemicellulose recovery in liquid fraction and lignin recovery

Tables and captions

Table **1.** Independent variables and their levels in the experimental design

Independent variable	Symbols	Unit	Code Levels				
			-1.68 (-α)	-1	0	1	1.68 (+α)
Time	A	min	39.55	60	90	120	140.45
Temperature	B	°C	49.77	60	75	90	100.23
Solvent concentration	C	%	36.36	50	70	90	103.64

Table **2**. Experimental responses of the dependent variables after pretreatment using central composite design

Run	Variable			Response		
	A: Time (min)	B: Temperature (°C)	C: Concentration (%)	Y_1: Cellulose recovery (%)	Y_2: Hemicellulose recovery (%)	Y_3: Lignin recovery (%)
1	60	60	50	57.487	15.220	45.903
2	120	60	50	74.779	16.727	48.612
3	60	75	50	88.874	17.418	63.182
4	90	100.23	70	95.097	21.928	63.532
5	60	60	90	80.921	10.150	44.640
6	120	60	90	83.765	19.137	50.701
7	60	75	90	100.220	15.265	62.986
8	120	90	90	88.359	29.473	63.112
9	39.55	75	70	73.056	12.741	49.193
10	140.45	75	70	99.030	19.682	56.694
11	90	49.77	70	64.434	9.5438	43.350
12	90	100.23	70	96.729	19.459	56.409
13	90	75	36.36	71.993	14.009	49.943
14	90	75	103.64	94.561	20.753	55.441
15	90	75	70	80.192	14.728	49.319
16	90	75	70	78.353	16.903	50.018
17	90	75	70	81.149	20.438	52.334
18	90	75	70	84.803	16.269	48.437
19	90	75	70	86.734	13.168	51.740
20	90	75	70	87.210	15.220	51.597

Table **2**. ANOVA Analysis for Responses Y_1 [Cellulose recovery (%)]

Source	Sum of squares	Degree of freedom	Mean squares	*F-values*	*p*-value *Prob > F*
Model	1934.95	9	214.99	10.23	0.0006
A	71.26	1	71.26	3.39	0.0953
B	34.03	1	34.03	1.62	0.2319
C	62.64	1	62.64	2.98	0.1149
AB	2.26	1	2.26	0.11	0.7495
AC	49.57	1	49.57	2.36	0.1555
BC	316.45	1	316.45	15.06	0.0031
A^2	10.84	1	10.84	0.52	0.4889
B^2	18.75	1	18.75	0.89	0.3671
C^2	0.14	1	0.14	6.547E-003	0.9371
Residual	210.10	10	21.01		
Lack of Fit	140.97	4	35.24	3.06	0.1073
Pure error	69.13	6	11.52		
Total	2145.05	19			

Table **3**. ANOVA Analysis for Response Y_2 [Hemicellulose recovery in the liquid fraction (%)]

Source	Sum of squares	Degree of freedom	Mean squares	*F-values*	*p-value* *Prob > F*
Model	326.75	9	36.31	6.45	0.0037
A	27.84	1	27.84	4.95	0.0503
B	16.96	1	16.96	3.01	0.1132
C	15.20	1	15.20	2.70	0.1313
AB	1.04	1	1.04	0.18	0.6768
AC	44.91	1	44.91	7.98	0.0180
BC	15.42	1	15.42	2.74	0.1288
A^2	1.28	1	1.28	0.23	0.6438
B^2	0.045	1	0.045	7.919E-003	0.9308
C^2	7.18	1	7.18	1.28	0.2851
Residual	56.27	10	5.63		
Lack of Fit	22.48	4	5.62	1.00	0.4761
Pure error	33.79	6	5.63		
Total	383.02	19			

Table **5**. ANOVA Analysis for Response Y_3 [Lignin recovery (%)]

Source	Sum of squares	Degree of freedom	Mean squares	F-values	p-value *Prob > F*
Model	674.73	9	74.97	9.18	0.0009
A	19.38	1	19.38	2.37	0.0504
B	157.40	1	157.40	19.27	0.0014
C	16.11	1	16.11	1.97	0.1905
AB	30.88	1	30.88	3.78	0.0805
AC	15.90	1	15.90	1.95	0.1932
BC	6.75	1	6.75	0.83	0.3847
A2	20.94	1	20.94	2.56	0.1404
B2	4.17	1	4.17	0.51	0.4912
C2	18.00	1	18.00	2.20	0.1685
Residual	81.67	10	8.17		
Lack of Fit	44.35	4	11.09	1.78	0.2508
Pure error	37.32	6	6.22		
Total	756.41	19			

Table **6**. Model summary for responses

Response	Standard deviation	Mean	C.V.%	PRESS	Adeq-precision
Cellulose recovery (%)	4.58	82.39	5.56	1460.56	12.91
Hemicellulose recovery (%)	2.37	16.91	14.03	300.85	11.59
Lignin recovery (%)	2.86	52.86	5.41	497.61	9.67

Development of a Non-Derivatizing molten hydrate salt pretreatment system for South African corn cob: A preliminary investigation

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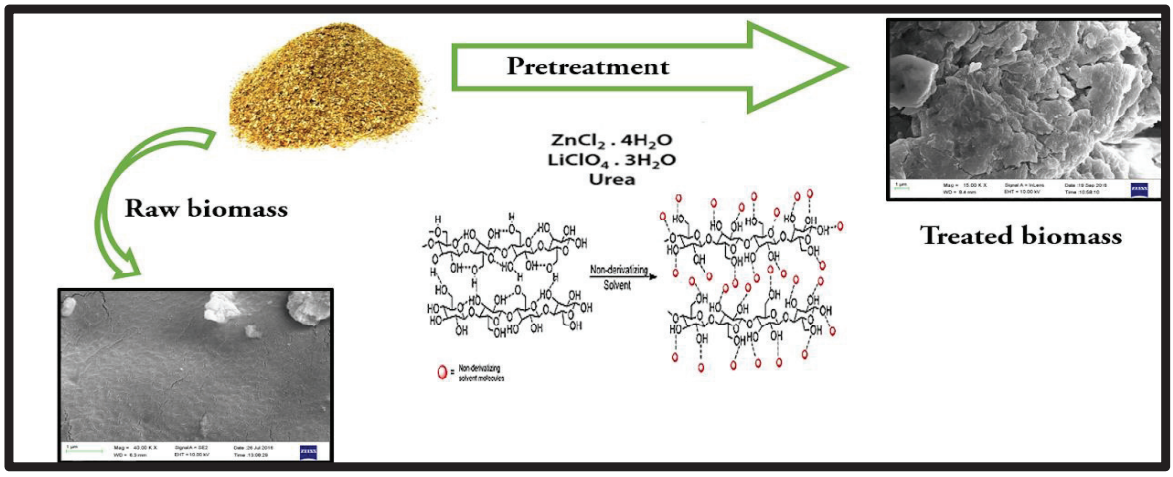

Figure 1: Scanning electron micrographs of raw biomass and $ZnCl_2.4H_2O$ treated biomass

Abstract

The future widespread production of biomass-derived fuels, chemicals, and materials requires cost-effective processing of sustainable feedstock. Pretreatment is arguably the most expensive processing step in the processing of lignocellulosic biomass. Seven molten salt hydrate systems namely, unary, binary and ternary mixtures are proposed in this study. The study is aimed at determining a novel pretreatment process which is less energy intensive, generates less waste and is both environmentally and economically more attractive than conventional routes. Outcome of the preliminary investigations show favourable amounts of polysaccharide fractions (68%) in the corn cob waste. In addition, pretreatment with the unary complex ($ZnCl_2.4H_2O$) was able to deconstruct the biomass. Although in its preliminary stage, the results show the feasibility of the study in the determination of a novel pretreatment process for corn cob waste. However, further research effort is needed to expand on the feasibility.

1 Introduction

There is currently a global energy crisis which has resulted from the increase in demand for energy from rapidly industrializing nations, the dwindling petroleum and natural gas supplies as well as the detrimental effects of carbon dioxide emissions from fossil fuels on climate change. To supply the changing world demands without further damaging the environment, the current oil-based economy must be switched to a bio-based economy. Bio-based products can be developed to replace existing petroleum-based product. Lignocellulosic biomass (LCB) which is abundant, cheap and does not contribute to net CO_2 emission is and alternative source for the biorefinery to produce energy and platform chemicals currently produced from oil refinery [1]. However, the natural recalcitrance of LCB is a major challenge to achieving enzymatic hydrolysis. Therefore, there is a need to develop efficient pretreatment technologies that will be economical and environmentally friendly to do this.

Examples of LCB are agricultural residues, such as corn stover as well quickly grown grasses or plants, such as switchgrass. In South Africa, there is immense potential in the beneficiation of corn cob waste as large quantities of the waste are discarded annually. Corn cob waste often causes environmental pollution due to lack of its effective utilization or disposal. The conversion of the waste corn cobs to valuable chemical products is therefore extremely important. Due to the recalcitrant nature of lignocellulose, however; it is very challenging to process the biomass into chemicals for value-added products [2]. As a result of the low solubility of the cellulose in common solvents, it is often required to use environmentally hazardous and/or costly systems to dissolve cellulose and separate the lignocellulosic components. A variety of studies have been done on the dissolution of cellulose, however, few studies have been done on the use of cellulose solvents on the pretreatment of lignocellulosic material. Awosusi *et al.* investigated the effect of water of crystallization of $ZnCl_2$ on the pretreatment of biomass and reported that the

highest overall yield of fermentable hexose sugars was achieved using $ZnCl_2.4H_2O$ pre-treatment of the cob samples at conditions of 70 °C for 1 h and 4-d enzymatic digestion using cellulase [3]. Finding environmental friendly and economically feasible solvent systems, such as molten salt hydrate systems, which are able to pre-treat lignocellulosic biomass efficiently is of extreme importance. This study therefore seeks to compare the efficiency of seven different molten hydrate salt solvent systems on the pretreatment of South African corn cobs towards developing an efficient environmentally benign solvent for LCB treatment. The efficiency will be assessed as the ability for the pretreatment to retain polysaccharide fractions, and lignin removal post-pretreatment as well as the physical deconstruction of the biomass.

2 Method/Experiment/Body

The raw corn cob waste is subjected to seven different pretreatment solvent systems namely unary: $ZnCl_2.4H_2O$, $LiClO_4.3H_2O$, and Urea binary: $LiClO_4.3H_2O$/Urea and $ZnCl_2.4H_2O$/Urea and ternary: $LiClO_4.H_2O/ZnCL_2.4H_2O$/Urea at 70°C for 60 minutes. Physicochemical properties of the corn cob were determined prior to and post pretreatment in order to determine the efficiency of the solvent systems during the pretreatment. The chemical characterisation done by compositional analysis of the solid and liquid fraction. The important mass balances used for analysing the efficiency of pretreatment processes include glucose, xylose and lignin balances. The efficacy of the pretreatments will be determined according to the amount of polysaccharides retained in the solid fraction, the solubilisation of hemicellulose and the removal of lignin.

For the physicochemical characterisation, the treated biomass will be analysed using Scanning Electron Microscopy (SEM), Light Microscopy (LM), X-ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and a rheometer. SEM and LM analysis will be used to observe any effect of the pretreatments on the surface morphology of the biomass. XRD analysis will be conducted to see whether the pretreatments had any effect towards the crystallinity of cellulose while FTIR analysis will be done to determine the presence and absence of important functional groups of the biomass. In addition, the rheometer will be used rheological properties of the biomass slurry after pretreatment.

3 Results/ Discussion

Compositional analysis of the corn cob prior to pretreatment shows hemicellulose with the highest percentage at 42% followed by cellulose at 26% and lignin which is a combination of AIL (Acid insoluble lignin) and ASL (Acid soluble lignin) at 23%. The extractives which consist of chlorophyll, waxes, fats, resin acids, terpenoids, phenolic substances, and gums makes up 9% of the biomass composition. These results show a favourable amount of the polysaccharide fractions which is promising for the fermentation to biochemical and biofuels.

Preliminary investigations into the feasibility of using molten hydrate salts as a pretreatment method for corn cob waste shows that the unary complex ($ZnCl_2.4H_2O$) pretreated biomass undergoes a structural change to a more roughened and compacted surface morphology compared to the SEM of the untreated corncob which revealed a smooth, intact surface with ordered fibres (figure 1). These changes in the supramolecular structures can be attributed to the molten salt solubilizing the cellulose by the interaction between ionic species and hydroxyl groups which breaks the hydrogen-bonding network and separates the lignocellulosic components.

4 Conclusions

Corn cob waste was treated using a mixture of various molten hydrate salt systems and the resulting residues characterised.

The following conclusions were drawn from the study.

1) As a whole, corn cobs are promising resources for biofuel and biochemical production. The compositional analysis shows that there is an adequate amount of carbohydrates in the corn cob samples which is indicative of high theoretical fermentable sugar yields.

2) Based on the SEM analysis, the deconstruction of the biomass cell wall is achieved by the pretreatment of the biomass at 70°C for 60 minutes in $ZnCl_2.4H_2O$

These results correlate with previous work done by Awosusi *et al.* The pretreatment process is however still in the infant stages, and more efforts are needed to further characterise the pretreatment residues and optimize the operation as well determine a kinetic model of dissolution.

Acknowledgments

The authors acknowledge the financial support received from the National Research foundation.

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