Fractionation of Lignocellulosic Biomass for Production of Materials and Chemicals

by

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Declaration

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Abstract

The development and application of biorefineries is the promising effective use of lignocellulose in the substitution of fossil fuel-based chemicals, materials and fuels. The biorefineries are attractive as they will utilise all lignocellulose components (cellulose, hemicellulose and lignin) for production of various products. The biorefineries require efficient lignocellulose fractionation methods, which are able to overcome the recalcitrance of lignocellulose to dissolution and chemical conversion, giving high yield, purity and preferably polymeric forms of all three major fractions.

The aim of the study was to compare organosolv and ionic liquid fractionation methods on sugarcane bagasse (SCB) and *Eucalyptus grandis* (*E. grandis*), in terms of extraction and separation efficiencies, as well as operational challenges. Effects of alkaline extraction of hemicellulose on efficiencies of organosolv and ionic liquid fractionation methods were also studied.

The choice of feedstocks was based on the availability and industrial processing in the Southern Hemisphere. Alkaline pre- and extractions of hemicelluloses were carried out using sodium hydroxide solutions. Organosolv fractionation of both SCB and *E. grandis* was carried out using aqueous ethanol and conditions adapted from Huijgen et al. (2012). The ionic liquid used was 1-ethyl-3-methylimidazolium acetate, [EMIM]OAc. Central composite design of experiments was used to optimise aqueous ionic liquid fractionation, and obtained desirable conditions were used for comparison study. The mass balances and quality of fractions were used to compare organosolv and ionic liquid fractionations in terms of extraction and separation efficiencies.

The results obtained showed that alkaline pre-extraction method extracted and preserved hemicellulose (xylan) in polymeric form. Alkaline pre-extraction solubilised significant amount of hemicelluloses, and that preserved reasonable amount of hemicellulose from degradation by either organosolv or ionic liquid process. When alkaline post-extraction was coupled with organosolv and ionic liquid fractionation methods, the recovered hemicelluloses had lower molecular weight, and were accompanied by very low xylan balances. The alkaline pre-extraction combinations with organosolv and ionic liquid fractionation methods outweighed alkaline post-extraction combinations.

Ionic liquid fractionation yielded highly digestible solid residues (≥98 %), while organosolv yielded less digestible solid residues (≤63 %). Both organosolv and ionic liquid fractionation

methods resulted in high quality lignin. Ionic liquid fractionation outweighed organosolv process in terms of hemicellulose preservation. In terms of robustness to different feedstocks, ionic liquid fractionation was more robust than organosolv process. In terms of separation efficiencies, ionic liquid fractionation produced fractions which were easily separated than organosolv process (particularly hemicellulose-lignin mixture). Although ionic liquid fractionation outweighed organosolv process, ionic liquid fractionation has the challenge/limitation of scalability due to high cost of ionic liquids. In order to address this challenge, several scale-up tests, as well as optimisation studies for recovery and recycling of ionic liquids have to be carried out. Following that, techno-economic models can be developed for ionic liquid fractionation process, and be compared to organosolv techno-economic models.

Opsomming

Die ontwikkeling en ingebruikstelling van bioraffineerders is ’n belowende en effektiewe gebruik van lignosellulose as plaasvervanger vir fossiel-gebaseerde chemiese stowwe, materiaal en brandstowwe. Die bioraffineerders is aantreklik aangesien hulle alle lignosellulose komponemte (sellulose, hemisellulose en lignien) in die produksie van verskillende produkte gebruik. Die bioraffineerders vereis doeltreffende metodes vir die afbreking van die lignosellulose wat moeilik oplos en nie maklik chemies omsitbaar is nie. Die opbrengs moet hoog en suiwer wees en verkieslik in polimeer vorm.

Daar is tydens die studie gepoog om die doeltreffendheid van afbrekingsmetodes op saamgepersde suikerriet (SCB) en Eucalyptus grandis (E. grandis), in ten die operasienele uitdagings en terme van ektraksie en en die doeltreffendheid van skeiding te toets. of extraction and separation efficiencies, as well as operational challenges. Effects of alkaline extraction of hemicellulose on efficiencies of organosolv and ionic liquid fractionation methods were also studied.

Die keuse van voerstowwe is baseer op die beskikbaarheid en industriële prossessering in die Suidelike Halfrond. Alkaliese pre- en post-ektraksie van hemiselluloses is uitgevoer met die gebruik van natrium hidroksied oplossings. Organosolv afbreking van SCB and E. grandis is met die gebruik van wateragtige etanol en volgens die metodes van Huijgen et al. (2012) uitgevoer. Die ioniese vloeistof wat gebruik is, is 1-etiel-3-metilimidazolium asetaat, [EMIM]OAc. Die Sentraal saamgestelde ontwerp van eksperimente is gebruik om die afbreking van die ionise vloeistof te optimeer en die toestande wat verkry is, is vir die vergelyking gebruik. Die balans van die massa en die gehalte van die breuke is gebruik om organosolv en ionise vloeistof afbreking in terme van ekstraksie en die doeltreffendheid van skeiding te vergelyk.

Daar is gevind dat die alkaliese voor-ekstraksie metode die hemisellulose (Xylan) en polemeer vorm bewaar, Tydens alkaliese pre-ekstraksie is groot hoeveelhede hemisellulose opgelos en dus nie tydens die organodolve of die ionise vloeistof proses degradeer nie. Toe alkalise post-ektraksie tesame met organosolv en ionise vloiestof afbrekingsmetodes gebruik is, het die hemisellulose ’n laer gewig gedra en was die oorblywende houtweefsel baie laer, die alkalise pre-ektraksie kombinasies en die ionise vloeistof afbrekings metodes is verkieslik bo die alkalisee post-ekstraksie metodes.

Ioniese vloeistof afbrekingsmetodes het hoogs verteerbare oorkiet (≥98 %) tot gevolg gehad terwyl organosolv minder verteerbare oorskiet (≤63 %) tot gevolg gehad het. Albei organosolv en ionise vloeistof afbreking het gelei tot ’n hoë gehalte lignien. Hemisellulose

is egter beter bewaar tydens die organosolv proses. In terme van robuustheid teenoor voerstowwe is ionise vloeistof afbreking meer robuust as die organosolv proses. Ioniese vloeistof afbreking het gelei tot dele wat makliker skeibaar is as die wat gevolg het op organosolv (veral die hemisellulose-lignien mengsel). Alhoewel die ionise vloeistof metode die organosolv proses oortref het, is die ionise vloeistowwe baie duur. As gevolg van hierdie probleem is baie toetse en studies gedoen wat betref die verhaal en hergebruik van ionise vloeistowwe. Tegno- ekenomiese modelle van die ionise vloeistof afbrekingsmetode kan ontwikkel word en met die organosolv tegno-ekonomiese modelle vergelyk word.

Dedication

To my beloved mother ‘M’e ‘Mapaballo Mats’eliso Makhetha.

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Abbreviations and Symbols

[BMIM]$MeSO_4$: 1-butyl -3-methylimidazolium methyl sulphonate

[BMIM]OAc: 1-butyl-3-methylimidazolium acetate

[Ch] [Arg]: cholinium argininate

[Ch] [Lsy]: cholinium lysine

[EMIM]OAc: 1-ethyl-3-methylimidazolium acetate

AIL: Acid insoluble lignin

ANOVA Analysis of variance

Da: Dalton

DMA / LiCl: dimethylacetamide / lithium chloride

DMA: dimethylacetamide

DMSO: dimethylsulphoxide

DP: degree of polymerisation

E. grandis: *Eucalyptus grandis*

ELS: evaporative light scattering

FPU: filter paper units

FTIR: Fourier transmission infra-red

GC-MS: Gas chromatography mass spectroscopy

GlcA: Glucuronic acid

HPAEC: high performance anion exchange chromatography

HPLC: high-pressure liquid chromatography

IL: ionic liquid

IR: Insoluble residue

LCC: lignin carbohydrate complex

LF: liquid fraction

Mw: molecular weight

Mn: molecular number

NMMO: N-Methylmorpholine-N-oxide

NMR: nuclear magnetic resonance

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

SCB: sugarcane bagasse

SEC/GPC: Size exclusion chromatography/ Gel permeation chromatography

S/G: Syringyl/guaiacyl ratio

1. Introduction

The society's dependence on petroleum-based resources (fuels and petroleum-based chemicals) has led to several economic and environmental challenges, in particular global warming due to the greenhouse gas emissions (Zavrel et al., 2009; FitzPatrick et al., 2010). Lignocellulose-based chemicals and materials are attractive replacements of petroleum-based products (Zavrel et al., 2009; FitzPatrick et al., 2010; Lan et al., 2011). Often people think about renewable and sustainable energy (biofuels) when considering the climatic impacts or effects of fossil resources, and that under-utilises biomass. However, biorefineries, which integrate biofuels, chemicals and materials are economically attractive, and should be given preference (FitzPatrick et al., 2010; Stark, 2011).

Biorefineries are advantageous when compared to petroleum refineries because they utilise a wide range of feedstocks and processing technologies (Carvalheiro et al., 2008; FitzPatrick et al., 2010). However, their implementation is limited by lignocellulose fractionation. Lignocellulose fractionation involves extraction and separation of lignocellulose into its constituents, and it is the major cost factor in biorefinery (FitzPatrick et al., 2010; Klein-Marcuschamer et al., 2011). Therefore, lignocellulose fractionation methods need to be optimised for high recoveries (minimal degradation) and high quality of fractions, as well as robustness to different types of feedstocks and energy efficiency (Alvira et al., 2010; FitzPatrick et al., 2010). Among several lignocellulose fractionation methods, chemical fractionation methods are the most attractive and promising fractionation processes, as they have high degree of selectivity for biomass they separate (FitzPatrick et al., 2010; Fu and Mazza, 2011b).

Although chemical fractionation methods are attractive, most of them have challenges of quite low sugar recoveries, particularly hemicelluloses, due to use of severe treatment conditions (high temperature, or acidic conditions) which increase hydrolysis and degradation reactions (FitzPatrick et al., 2010; Fu and Mazza, 2011b). High hemicellulose degradation is undesirable in biorefineries as it leads to under-utilisation of hemicellulose (biopolymers have high value applications compared to monomers and degradation products). Therefore, alkaline and near-neutral fractionation methods, like organosolv and ionic liquid fractionation methods need to be studied and tested further for the viability in biorefineries.

Alkaline extraction of hemicelluloses from lignocellulosic biomass uses basic aqueous solutions like sodium hydroxide, calcium hydroxide, ammonia, and green liquor (comprising of sodium carbonate and sodium sulphite) (Carvalheiro et al., 2008; Kumar et al., 2009; Harmsen et al., 2010; Agbor et al., 2011; Menon and Rao, 2012; Yang et al., 2012; Testova et al., 2014). Alkaline extraction is attractive as it solubilises hemicelluloses in polymeric form, is accompanied by very low sugar degradation and lignin fragmentation, results in digestible cellulose, and uses milder conditions (effective even at room temperatures and pressures) (Rezende et al., 2011; Castro et al., 2013; Vena, 2013).

Organosolv (organic solvent) fractionation is one of the methods used to separate lignocellulosic biomass into its constituents. It uses organic solvents like organic acids, ketones and alcohols, with or without catalysis, at elevated temperatures and requires pressure control (Harmsen et al., 2010; Park et al., 2010; Wildschut et al., 2013). Ethanol-based organosolv is the most explored and applied process in both demonstration and commercial scale (Arato et al., 2005; Pan et al., 2005; Zhu et al., 2010; Sannigrahi and Ragauskas, 2013; Espinoza-Acosta et al., 2014; de Wild et al., 2015). Ethanol-based organosolv is attractive as the cost of solvent can be reduced by producing it on site (from fermentation of sugars obtained from hydrolysis of cellulose and hemicellulose) (Zhao et al., 2009). Typical process conditions for ethanol-based organosolv are; 50-80 % aqueous ethanol, 160-210 °C, 15-120 min, 5-30 bar, and with or without catalysts (Pan et al., 2006; Zhao et al., 2009; Huijgen et al., 2012; Wildschut et al., 2013). Organosolv fractionation process has advantages of high selectivity, in fractionating lignocellulose high purity and low molecular weight lignin, hemicellulose and digestible cellulose fractions, easy recovery of organic solvents and recycling leading to reduced chemical consumption and operating costs (Carvalheiro et al., 2008; Harmsen et al., 2010; Agbor et al., 2011; Stark, 2011; Menon and Rao, 2012; Brandt et al., 2013; Constant et al., 2015). Often the organosolv involves the use of acid catalyst, leading to hydrolysis and/or degradation of hemicellulose polymers in lignocellulose (Sun and Cheng, 2002). In order to limit hemicellulose degradation, alkaline catalysis or pre-extraction have to be considered.

Ionic liquid fractionation of lignocellulosic biomass uses ionic liquids as solvents. Ionic liquids are organic salts melting points below 100 °C, and they differ from molecular solvents by having low vapour pressure, high thermal stability and good solvation properties Stark, 2011; Vancov et al., 2012; Yinghuai et al., 2013). Use of ionic liquids in fractionation

lignocellulosic biomass is a recently developing method and has not been taken to pilot and commercial scale, due to need for optimisation of operating conditions, and solvent recovery and recycle (Brandt et al., 2013; George et al., 2015). The conditions which have been studied are: 5-100 %w/w ionic liquid concentrations (aqueous and organic solvent mixtures), 90-190 °C, 0.5-22 hours (Tan et al., 2009; Fu and Mazza, 2011a; 2011b; Li et al., 2011b; Diedericks et al., 2012b; Sun et al., 2013; Xu et al., 2013). Ionic liquids which have their anions derived from weak bases (like acetate, formate and lactate) provide near-neutral fractionation at mild conditions (high ionic liquid concentration, and low temperatures), but fractionation efficiency is low due to limited mass transfer at mild conditions (Fu and Mazza, 2011a; Liu et al., 2012). Therefore, use of co-solvents (water or organic solvents) and elevated temperatures need to be considered.

The aim of the study was to comprehensively compare organosolv and ionic liquid fractionation methods on SCB and *E. grandis*, based on extraction and separation efficiencies, as well as operational challenges. The study of the effects of alkaline extraction of hemicellulose on efficiencies of organosolv and ionic liquid fractionation methods for both feedstocks was also included. The extraction and separation efficiencies comprised of recovering cellulose, hemicellulose and lignin at high yield, purity and quality (with hemicellulose preservation being the key aspect), and ease of separating the streams following treatments. Operational challenges comprised of robustness to different feedstocks, costs (solvents and equipment), as well as scalability of the processes.

2. Literature Review

2.1. Lignocellulose

Lignocellulose is a renewable and possibly sustainable raw material for biorefineries, with capacity to produce substitutes to petroleum-based fuels, chemicals and materials (Lee et al., 2009; Fu and Mazza, 2011a; Sun et al., 2013). Lignocellulose is a rigid material in the plant cell, comprising of complex molecular (chemical) bonds, and mainly consisting of cellulose, hemicellulose and lignin, with low contents of extractives, proteins, pectin, ash and starch (Klinke et al., 2004; Stark, 2011). The lignocellulose chemical composition depends on the type and source of plant material, as shown in Table 2.1.

Table 2.1: The composition of lignocellulose in various sources (Sun and Cheng, 2002)

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste papers from chemical pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	NA	24-29
Swine waste	6.0	28	NA
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

2.1.1. Cellulose

Cellulose is the main constituent of the cell wall in lignocellulosic plants, and provides mechanical strength and chemical stability to the plant (Harmsen et al., 2010; Liu and Sun, 2010). Cellulose is the storage form of the solar or light energy in plants through photosynthesis process (Harmsen et al., 2010). It is a linear homopolymer of glucan (D-glucopyranose) bound by β-(1→4) or (β-1, 4-glucosidic) linkages (Fengel and Wegener, 1989; Harmsen et al., 2010; Liu and Sun, 2010). The molecular formula of cellulose is

$(C_6H_{10}O_5)_n$ and upon hydrolysis it gives glucose monomers $n(C_6H_{10}O_6)$, where n is the degree of polymerisation (DP) of cellulose (Harmsen et al., 2010; Liu and Sun, 2010). The structure of single cellulose molecule is shown in Figure 2.1.

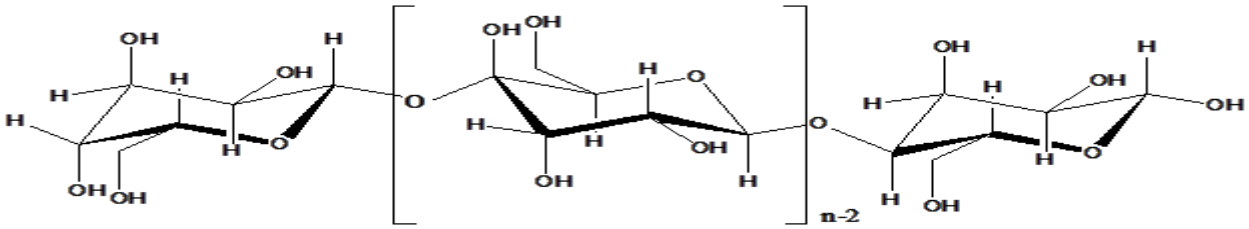

Figure 2.1: The structure of single cellulose molecule, Redrawn from (Harmsen et al., 2010; Liu and Sun, 2010).

DP and molecular weight (Mw) of anhydroglucose (glucose anhydride) unit define the average molecular mass of cellulose (Liu and Sun, 2010). DP is dependent on the source of cellulose, ranges from 200 to 17000 units, and decreases with the processing of biomass (like pulping) (Bledzki and Gassan, 1999; Harmsen et al., 2010; Liu and Sun, 2010; Agbor et al., 2011; Cateto, Hu and Ragauskas, 2011; Keskar, 2011; Almengor et al., 2012). The DP and Mw distributions in different cellulose sources are shown in Table 2.2.

Table 2.2: Distribution of degree of polymerisation and molecular weight in cellulose sources (Pulp & Paper Resources & Information Site, n.d.)

Substance	DP	Mw (Da)
Native cellulose	>3500	>570000
Purified cotton	100-3000	150000-500000
Wood pulp	600-1000	90000-150000
Commercial regenerated cellulose (e.g. Rayon)	200-600	30000-150000
β-cellulose	15-90	3000-15000
γ-cellulose	<15	<3000
Dynamite nitro-cellulose	3000-5000	750000-875000
Plastic nitro-cellulose	500-600	125000-150000
Commercial cellulose acetate	175-360	45000-100000

The strong intra- or inter-molecular hydrogen bonding (O-H) leads to the linkages of cellulose molecules, forming the microfibrils (Fengel and Wegener, 1989; Zykwinska et al., 2005). The cellulose microfibrils can form highly ordered (crystalline) or less ordered (amorphous) structures (Harmsen et al., 2010; Liu and Sun, 2010). The crystallinity of cellulose varies with the source, for example grasses have 70-80 % and native cellulose (from

cotton) have 89-96 % of crystalline cellulose, with the rest being amorphous (Fengel and Wegener, 1989).

High DP and crystallinity render cellulose to be insoluble in water, acids, organic solvents and aqueous salts at ambient temperatures and pressures. However, it can dissolve under pressure (5-48 bar) and temperatures between 100 °C and 250 °C, or through heterogeneous conversions into esters or ethers (Fengel and Wegener, 1989; Pan et al., 2005; Harmsen et al., 2010; Agbor et al., 2011). The two fundamental approaches for cellulose dissolution are use of derivatizing and non-derivatizing solvents (Heinze and Petzold, 2008; Keskar, 2011). Derivatizing solvents dissolve cellulose through chemical reactions, which result in unstable esters, ethers and acetal derivatives (Heinze and Petzold, 2008; Keskar, 2011). The non-derivatizing solvents dissolve cellulose through physical interactions, which are due to disruption of intermolecular hydrogen bonds (Heinze and Petzold, 2008; Keskar, 2011). Derivatizing solvents include dimethyl sulfoxide (DMSO), N,N-dimethyl formamide (DMF), tetrahydrofuran (THF), while non-derivatizing include solutions of inorganic compounds and salts, non-aqueous solvents like N-methylmorpholine-N-oxide (NMNO), and some alkyl substituted imidazolium ionic liquids (Heinze and Petzold, 2008; Liu and Sun, 2010; Gericke et al., 2011; Keskar, 2011; Rinaldi, 2011).

Cellulose isolation from the lignocellulose matrix is often accomplished by selective removal of lignin, hemicelluloses and other non-cellulosic substances (Liu and Sun, 2010). Cellulose isolation methods that follow this approach include alkaline extraction, hydrothermal aquasolv extraction (liquid hot water extraction, steam explosion, etc.), acid extraction, organosolv treatment, biological treatment, carbon dioxide explosion, ammonia fibre explosion and ionic liquids treatment (Harmsen et al., 2010; Liu and Sun, 2010; Agbor et al., 2011).

Cellulose has been used intensively in viscose fibre industries, and pulp and paper industries, and is gaining applications in biomaterials production (composites, nano-composites, hydrogels, high-strength and low mass automobile parts, etc.), biofuels and fuel additives (ethanol, butanol, etc.), organic acids and solvents (Bledzki and Gassan, 1999; Kamm and Kamm, 2004; Pan et al., 2005; Dufresne, 2008; Heinze and Petzold, 2008; Liu and Sun, 2010; Stark, 2011; Yang et al., 2013).

2.1.2. Hemicellulose

Hemicellulose is the family of homo- and heterogeneous pentosans $(C_5H_8O_4)_n$ and hexasans $(C_6H_{10}O_5)_n$, that include arbino-xylans, gluco-mannans, galactans, glucuronic acid, galacturonic acid and others (Fengel and Wegener, 1989; Harmsen et al., 2010; Ren and Sun, 2010). Hemicelluloses are branched (lacking crystalline structure), while their polydispersity, polydiversity and polymolecularity are high (Harmsen et al., 2010; Ren and Sun, 2010). The main constituents of hemicellulose are shown in Figure 2.2, and the representation of hemicellulose (of arborescent plants) is shown in Figure 2.3.

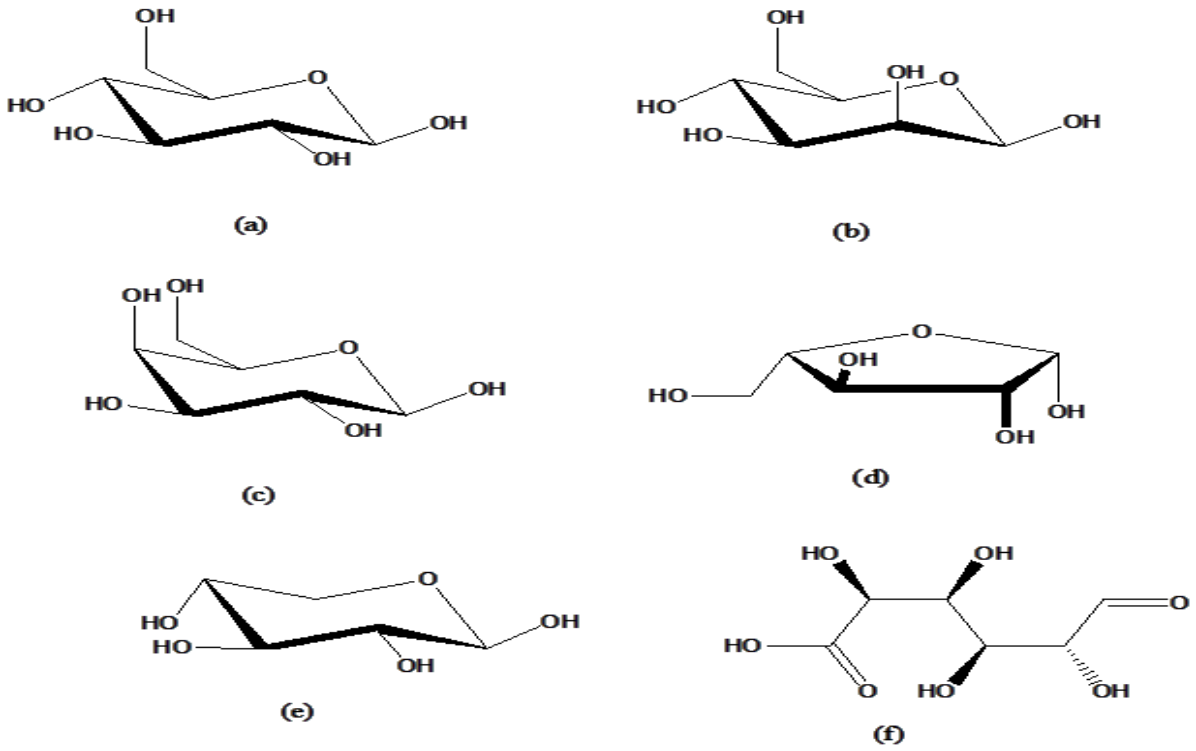

Figure 2.2: Main constituents of hemicellulose, (a) is D-Glucopyranose, (b) is D-Mannopyranose, (c) is D-Galactopyranase, (d) is L- Arabinofuranose, (e) is D-xylopyranose and (f) is D-Glucuronic acid, redrawn from (Ren and Sun, 2010).

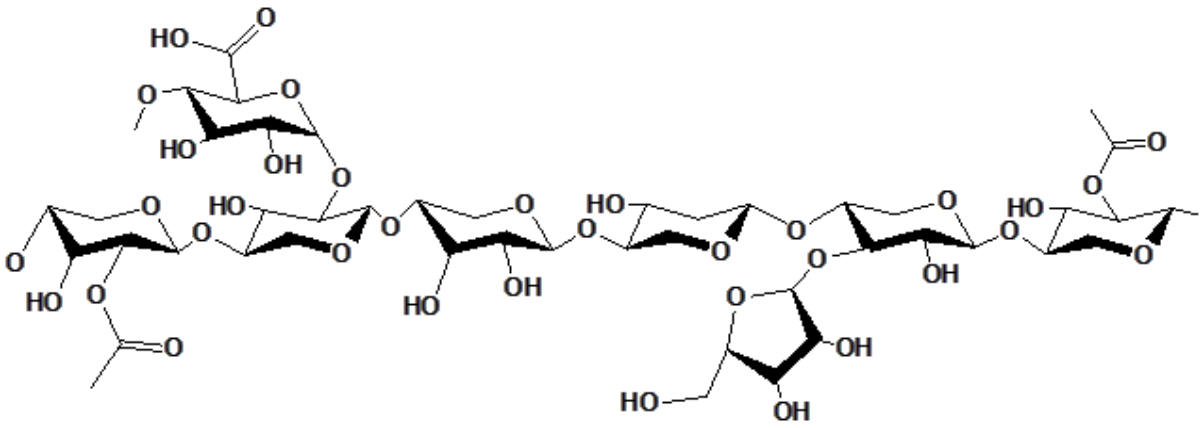

Figure 2.3: Hemicellulose backbone, redrawn from (Harmsen et al., 2010).

The linkages between hemicelluloses and cellulose are inter- and intra-molecular hydrogen bonds, whereas those between hemicelluloses and lignin, acetyl units, and hydroxycinnamic acids are covalent bonds (Sun et al., 2005; Ren and Sun, 2010). The composition of hemicellulose varies with the species type, with xylan as the predominant hemicellulose in hardwoods, annual plants and cereal (grains and straws), and glucomannan as predominant hemicellulose in softwoods (Fengel and Wegener, 1989; Ren and Sun, 2010). The hemicelluloses composition and properties for several lignocellulosic materials are shown in Table 2.3.

Table 2.3: The types and compositions of hemicellulose for several lignocellulose plants (Girio et al., 2010)

Polysaccharide type	Biological origin	Percentage (%) dry biomass	Backbone	Side-chains	Linkage	DP
Arabinogalactan	Softwoods	1-3; 35*	β-D-Galp	β-D-Galp α-L--Araf β-L-Arap	β-(1→6) α-(1→3) β-(1→3)	100-600
Xyloglucan	Hardwoods, grasses	2-25	β-D-Glcp β-D-Xylp	β-D-Xylp β-D-Galp α-L-Araf α-L-Fucp Acetyl	β-(1→4) α-(1→3) β-(1→2) α-(1→2) α-(1→2)	
Galactoglucomannan	Softwoods	10-25	β-D-Manp β-D-Glcp	β-D-Galp Acetyl	α-(1→6)	40-100
Glucomannan	Softwoods and hardwoods	2-5	β-D-Manp β-D-Glcp			40-70
Glucuroxylan	Hardwoods	15-30	β-D-Xylp	4-O-Me-α-D-GlcpA Acetyl	α-(1→2)	100-200
Arabinoglucuroxylan	Grasses, cereals and softwoods	5-10	β-D-Xylp	4-O-Me-α-D-GlcpAβ-L-Araf	α-(1→2) α-(1→3)	50-185
Arabinoxylans	Cereals	0.15-30	β-D-Xylp	α-L-ArafFeruloy	α-(1→2) α-(1→3)	
Glucuronoarabinoxylans	Grasses and cereals	15-30	β-D-Xylp	α-L-Araf 4-O-Me-α-D-GlcpA Acetyl	α-(1→2) α-(1→3)	
Homoxylans	Algae		β-D-Xylp			

Note: Xylp-xylopyranose, Galp-galactopyranose, Glcp-glucopyranose, Manp-mannopyranose, Araf-arabinofuranose, Arap-arabinopyranose, Me-α-D-GlcpA- glucuronic acid, * Larchwood

Hemicelluloses properties are influenced by DP, side chain substitutions, and bonds (hydroxyl and carboxyl) with other components of the cell wall (Ren and Sun, 2010). Although most hemicelluloses are insoluble in water at ambient temperature, they dissolve in hot water, dimethylsulfoxide (DMSO), *N,N*-dimethylacetamide/LiCl (DMA/LiCl), *N,N*-dimethylformamide/LiCl (DMF/LiCl), alkali and ionic liquids (Höije et al., 2005; Harmsen et al., 2010; Ren and Sun, 2010; Lan et al., 2011). Hemicellulose is the most sensitive (prone to degradation) lignocellulose component, and currently there is no isolation method that results in 100% yield (Ren and Sun, 2010). Some of the hemicelluloses isolation methods that have been developed include mild alkaline extraction, hydrothermal aquasolv extraction (hot water extraction, steam explosion, etc.), mild acid extraction, organosolv treatment and ionic liquids treatment (Chimphango, 2010; Ren and Sun, 2010; Lan et al., 2011; Peng et al., 2012). The isolation methods yield hemicelluloses at different sizes; mild alkaline extraction yields polymeric hemicelluloses, hydrothermal aquasolv extraction yields oligomeric and monomeric hemicelluloses, mild acid extraction yields oligomeric and monomeric hemicellulose, organosolv treatment yields oligomeric and monomeric hemicelluloses, and ionic liquids treatment yields polymeric, oligomeric and monomeric hemicelluloses (depending on acidity/basicity of ionic liquids and severity of treatment) (Carvalheiro et al., 2008; Harmsen et al., 2010; Agbor et al., 2011; Stark, 2011; Menon and Rao, 2012; Brandt et al., 2013).

In biorefinery approach, all the lignocellulose components have to be recovered in good quality in order to ensure effective utilisation of fractions, which will lead to good economics. As compared to polymeric hemicelluloses, oligomers and monomers in the extraction liquor are susceptible to further hydrolysis and forming degradation products, leading to increased separation challenges and under-utilisation (Carvalheiro et al., 2008; Harmsen et al., 2010; Agbor et al., 2011; Stark, 2011; Menon and Rao, 2012; Brandt et al., 2013). Alkaline pre-extraction of hemicelluloses has been studied in pulp and paper production, and the approach has been observed as valuable step towards biorefinery applications in pulp and paper industries (Chimphango, 2010; Gomes, 2012; Postma, 2012; Vena, 2013; Joubert, 2015). Coupling pre-treatments with alkaline post-extraction of hemicelluloses has been studied, but coupling with alkaline pre-extraction has not been investigated in pre-treatment/fractionation approaches Lan et al., 2011; Sun et al., 2013; Xu et al., 2013; Yang et al., 2013). The extracted hemicelluloses are further removed from the liquid fractions (which also contains solubilised lignin) by anti-solvent precipitation (ethanol, methanol, acetone or other organic

solvents), ammonium sulphate precipitation, iodine-complex precipitation, supercritical anti-solvent precipitation, membrane separations (ultra- and nano-filtration), and column chromatography (Chimphango, 2010; Ren and Sun, 2010; Peng et al., 2012).

The applications of hemicellulose polymers, oligomers and monomers include use in food industry (emulsifiers, stabilisers, binders and nutritional supplements), medical applications, cosmetic applications, biofuels, biomaterials (coating, films, hydrogels and thermoplastics), furfural, xylitol, and monomeric sugars like xylose (Carvalherio et al., 2008; Edlund and Albertsson, 2008; Spiridon and Popa, 2008; Chimphango, 2010; Haimer et al., 2010; Deutschmann and Dekker, 2012; Peng et al., 2012; Egüés, Eceiza and Labidi, 2013).

2.1.3. Lignin

Lignin is non-polysaccharide heteropolymer with phenylpropane (phenolic monomers) units as the main building blocks, and phenylpropane units having different substitutions on the aromatic ring (Chang, 2007; Robinson and Mansfield, 2009; Harmsen et al., 2010). The phenylpropane units are collectively called monolignols, of which the most commonly encountered are p-coumaryl, coniferyl and sinapyl alcohols (Fenger and Wegener, 1989; Harmsen et al., 2010; Brandt et al., 2013; Yinghuai et al., 2013), shown in Figure 2.4. Once the monolignols, p-coumaryl, coniferyl and sinapyl alcohols are integrated into lignin polymer, they are identified as p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), respectively (Klinke et al., 2004; Chang, 2007; Brandt et al., 2013; Yinghuai et al., 2013), as shown in Figure 2.4.

S:G:H ratio is often used to indicate lignin composition in either feedstocks or extracted lignin samples. The lignin content and distribution of the monolignols in the plant differs with the plant type, as shown in Table 2.4. As shown in Table 2.4, H-type lignin structures can be very low in most materials, leading to S- and G-type lignin structures being mostly detected with wet chemical methods, like thioacidolysis, permanganate oxidation and nitrobenzene oxidation (Robinson and Mansfield, 2009; Nunes et al., 2010). S/G ratio is used as reactivity indicator; high S-type lignin structures in feedstocks corresponds to high reactivity during fractionation and in extracted lignin corresponds to high reactivity in production of phenolic chemicals (Nunes et al., 2010; Wang et al., 2012a).

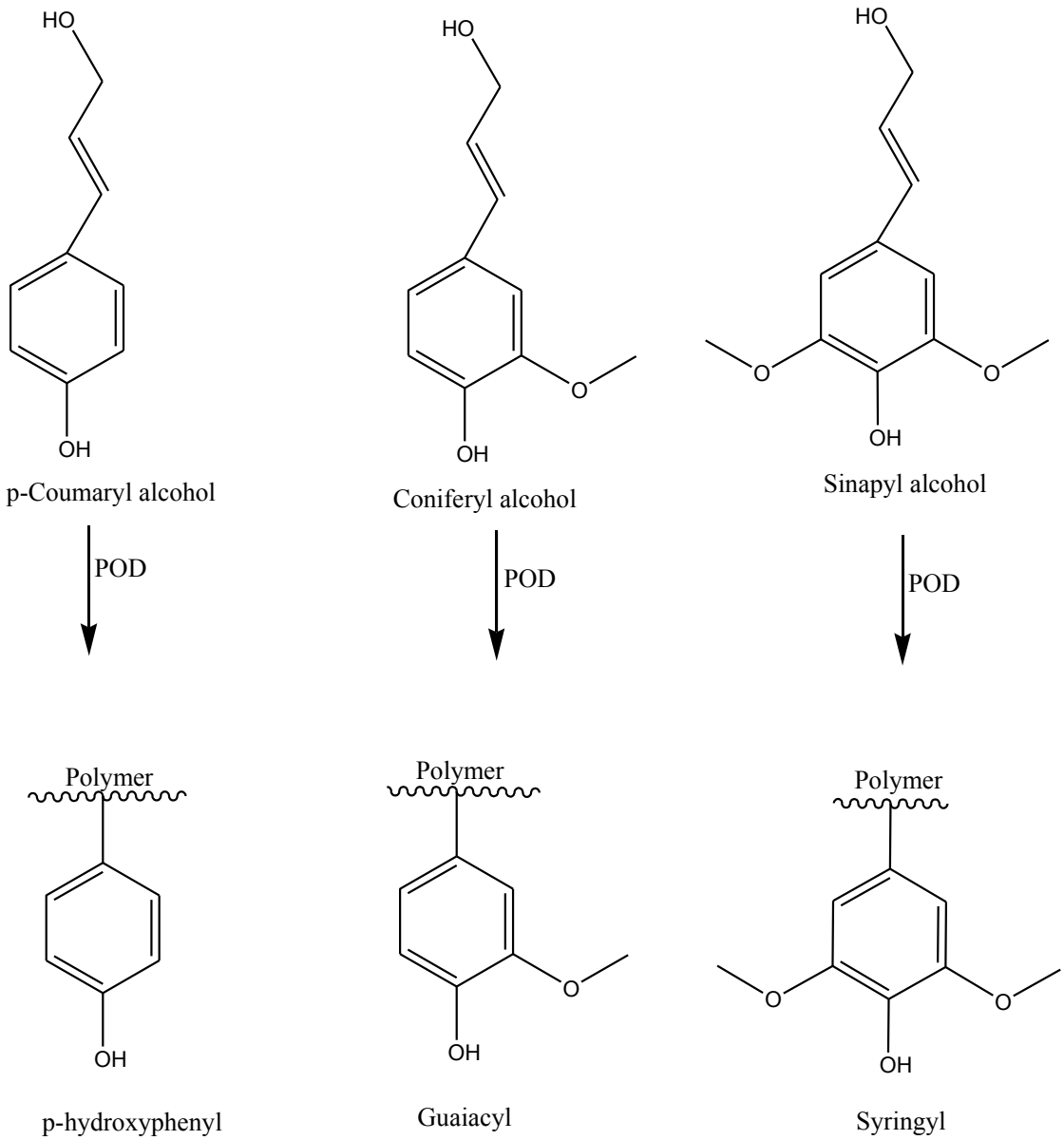

Figure 2.4: Lignin monomers (Redrawn from Chang, 2007; Brandt et al., 2013)

Table 2.4: Lignin content, and monolignols distribution in the plants Adapted from (Pinkert et al., 2011; Yinghuai et al., 2013).

Plant type	Total lignin (%)	H	G	S
Softwood	25-31	<5	>95	Trace amount
Hardwood	20-28	0-8	25-50	46-75
Grasses	15-22	5-33	33-80	20-54

There are several resonance structures of the monolignols, which result in numerous types of linkages between the lignin polymers. The types of linkages include β-O-4, β-β, 5-5, β-5, 4-

O-5, β-1 and α-O-4 bonds (Fengel and Wegener, 1989; Zakzeski et al., 2010; Brandt et al., 2013; Yinghuai et al., 2013; Zhang, 2013). The types of linkages, their abundances and chemical structures are shown in Table 2.5 and Figure 2.5.

Table 2.5: The common types of lignin linkages in lignocellulose (Fengel and Wegener, 1989; Pandey and Kim, 2011: Patil, 2012; Brandt et al., 2013; Santos, et al, 2013; Yinghuai et al., 2013)

Name	Linkage type	Abundance (%)	
		Softwood	Hardwood
β-aryl ether	β-O-4	50	60
Phenylcoumaran	β-5	9-12	6
Resinol	β-β	2	3
Biphenyl	5-5	10-11	5
Biphenyl ether	4-O-5	4	7
Noncyclic benzyl aryl ether	α-O-4	2-8	7
1,2-Diaryl propane	β-1	7	7

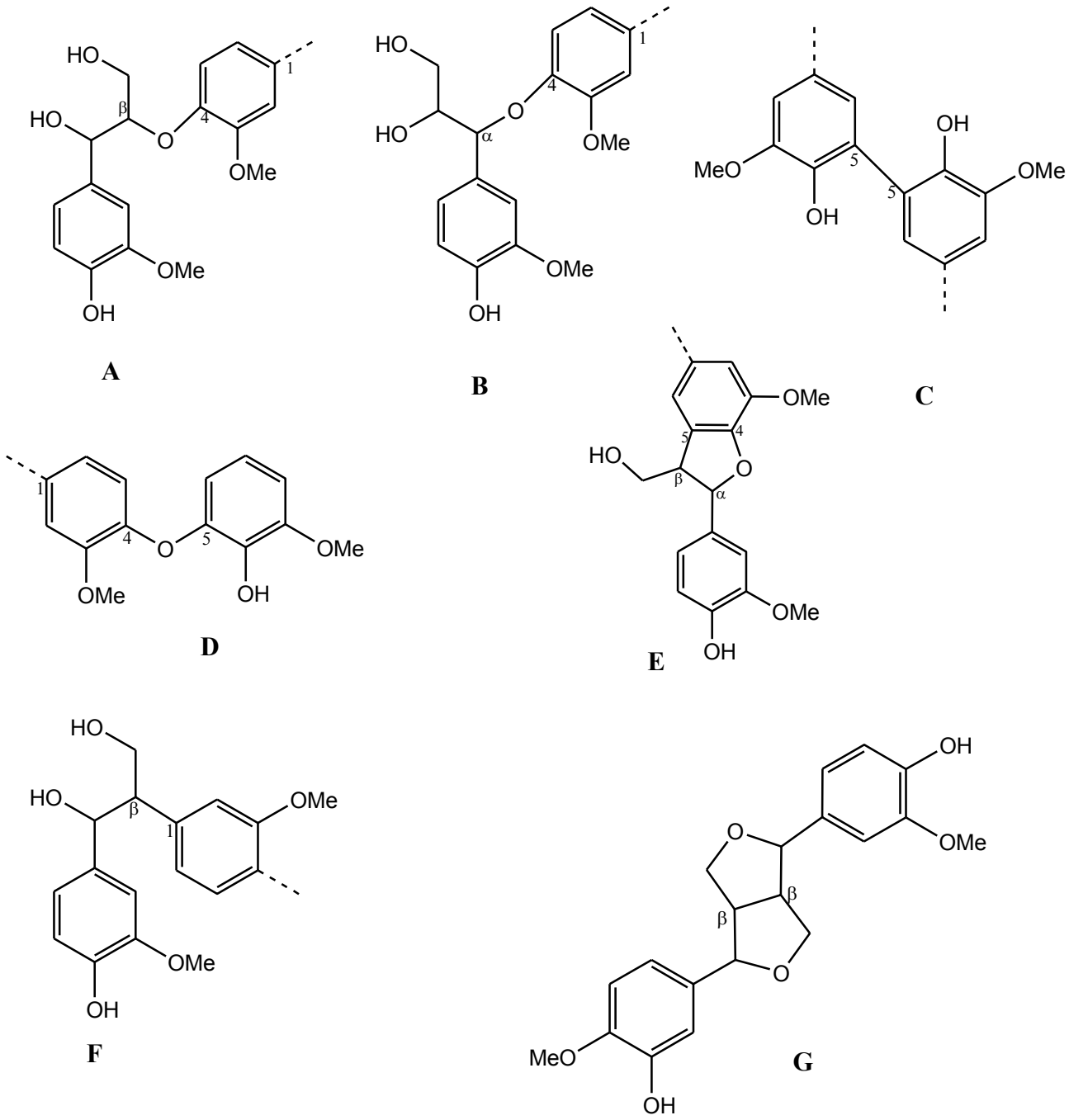

Figure 2.5: The chemical structures representing types of linkages between lignin monomers: A is β-O-4, B is α-O-4, C is 5-5, D is 4-O-5, E is β-5, F is β-1, and G is β-β (Adapted from Fengel and Wegener, 1989; Zakzeski et al., 2010; Pandey and Kim, 2011: Patil, 2012; Santos, et al, 2013; Yinghuai et al., 2013; Zhang, 2013)

Lignin acts as a linker between cellulose and hemicellulose molecules, and provides architectural support (resistance to impact compression and bending), water transport and defence (Robinson and Mansfield, 2009; Harmsen et al., 2010; Brandt et al., 2013; Yinghuai

et al., 2013). Lignin has molecular weight of more than 10000 Da, and is characterised by polydispersity (Harmsen et al., 2010; Yinghuai et al., 2013). Figure 2.6 shows lignin polymer with some of the linkages.

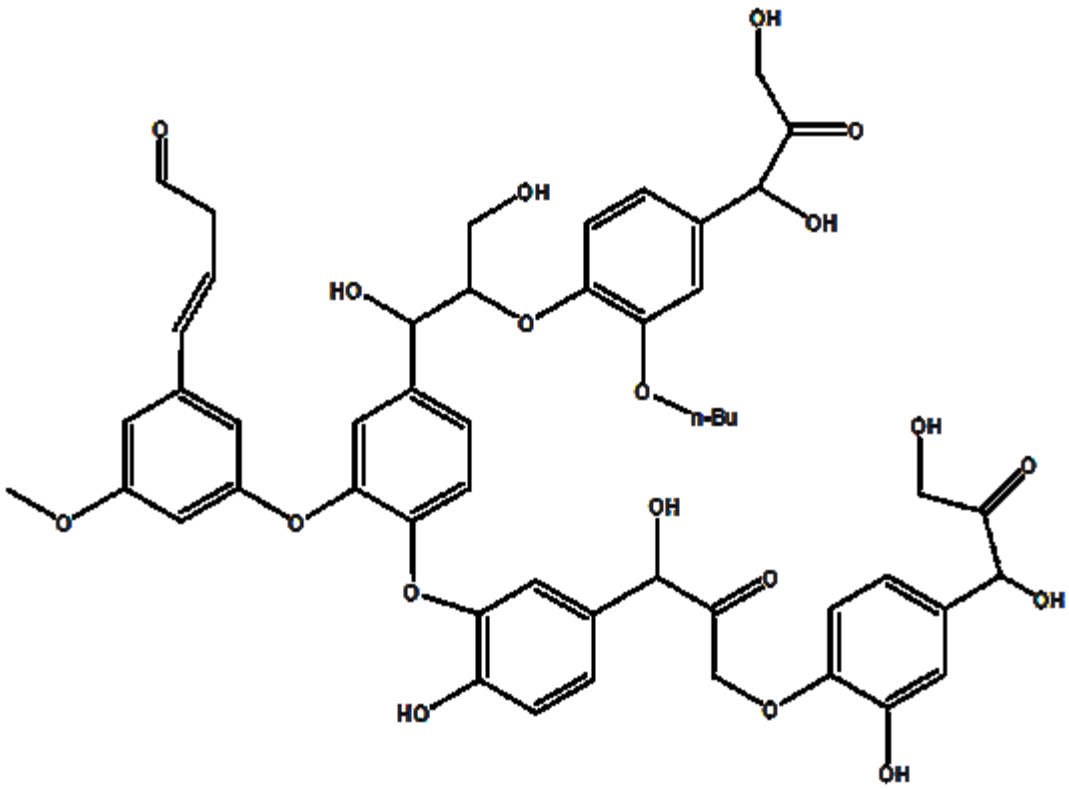

Figure 2.6: Lignin structure, redrawn from (Bozell et al., 2011)

Lignin is water insoluble but dissolves in acids, alkaline solutions, organic solvents (low molecular alcohols, dioxine, acetone, pyridine, etc.), and ionic liquids (Harmsen et al., 2010; Kline, et al, 2010; Lu and Ralph, 2010; Keskar, 2011; Brandt et al., 2013, Yinghuai et al., 2013). The isolation of lignin from the cell wall matrix is achieved through biological treatment (cellulolytic enzymes), sulphite pulping, alkaline treatment or pulping, organosolv treatment, ionic liquid treatment, carbon dioxide explosion treatment, ammonia fibre explosion, hydrothermal aquasolv, acid treatment, and oxidative delignification (Harmsen et al., 2010; Lu and Ralph, 2010; Keskar, 2011; Stark, 2011; Diedericks et al., 2012b; Brandt et al., 2013; Yinghuai et al., 2013).

Some applications of lignin include use in asphalt, cement and concrete, phenolic resins, adhesives, animal feed binders, fertilisers and other agrochemicals, paper additives, biomaterials (bioplastics, foams, polymers, etc.), fuel and fuel additives, chemicals (vanillin, cresols, catechol, resorcinol, guaiacol, etc.), dispersants, binders and stabilisers (Çetin and

Özmen, 2002; Pan et al., 2006b; Kleinert and Barth, 2008; Lora, 2008; Park et al., 2008; Stewart, 2008; Faustino et al., 2010; Lora, 2010; Lu and Ralph, 2010; Toledano et al., 2010; Zakzeski et al., 2010; Pandey and Kim, 2011; Stark, 2011; de Wild et al., 2012; Cheng et al., 2013; Wells et al., 2013; Yinghuai et al., 2013).

2.1.4. Lignin carbohydrate complexes (LCCs)

Lignin and carbohydrates (mainly hemicellulose) are not only physically bound, but also chemically bonded with covalent bonds (Cornu et al., 1994; Lawoko et al., 2005; Harmsen et al., 2010; Sakagami et al., 2010; Pasangulapati et al., 2012; Brandt et al., 2013; Du et al., 2013). The types of covalent bonds forming lignin carbohydrate complexes (LCCs) are dependent on the type of feedstock (whether grasses or woods). The LCCs in the grasses or herbaceous biomass are mainly ester bonds between ferulic acid and hemicellulose like arabinoxylans (Cornu et al., 1994; Brandt et al., 2013). The LCCs in woody biomass are mainly phenyl-glycoside bonds, ester bonds and benzyl ether bonds (Cornu et al., 1994; Lawoko et al., 2005; Yuan et al., 2011).

The LCCs affect the properties of the biomass like resistance to enzymatic hydrolysis, and lignocellulose delignification challenges (Cornu et al., 1994; Lawoko et al., 2005; Brandt et al., 2013; Du et al., 2013). The LCCs are isolated from the lignocellulosic biomass using organic solvents (Björkman LCCs isolation method), alkaline dissolution (like kraft pulping), and other treatment methods like acid, steam explosion, liquid hot water and ionic liquid treatments (Lawoko et al., 2005; Singh et al., 2005; Gáspár et al., 2007; Harmsen et al., 2010; Krishnan et al., 2010; Li et al., 2011a; Mora-Pale et al., 2011; Du, et al., 2013; Peng et al., 2014). Beside biofuels and food commodities of the components forming LCCs (carbohydrates mainly), the components forming LCCs have broad applications in pharmacology, which include; anti-tumour activities (stimulation of tumour necrosis factor production), anti-microbial activities, anti-viral activities (like anti-HIV activity), anti-parasite activities and synergistic action with vitamins (Gáspár et al., 2007; Sakagami et al., 2010).

2.2. Lignocellulose Biorefinery

The biorefinery approach is the promising substitute for petroleum refineries in the future because it utilises broad range of feedstocks (which are abundant) and processes (Kamm and Kamm, 2004; Carvalheiro et al., 2008; Alriols et al., 2010; FitzPatrick et al., 2010). The three

biorefinery platforms which are being considered in research and development are; whole crop biorefineries, green biorefineries, and lignocellulose feedstock (LCF) biorefineries (Kamm and Kamm, 2004; Carvalheiro et al., 2008). The feedstocks for whole crop biorefineries are cereals and grains, for green biorefineries are nature-wet biomass (green grasses, forage crops, immature cereal, etc.), and for LCF biorefineries are lignocellulosic materials, like wood, straw, corns over, bagasse, dry grasses, and wastes (Kamm and Kamm, 2004; Carvalheiro et al., 2008). LCF biorefineries are attractive, as they do not directly compete with food production. The summary of potential products that can be obtained in LFC biorefinery is shown in Figure 2.7.

Challenges facing the implementation of biorefineries are (i) liquefaction (incomplete dissolution of the feedstock), (ii) extraction (need for solvent inventory to achieve selective fractionation, and high economic and environmental performance), (iii) chemical conversion (the high yields and selectivities for the components are desired, so there is a need to control fractionation steps), and (iv) catalytic conversion (effect of catalyst on biomass conversion and its environmental impact) (Stark, 2011). Primary refinery involves fractionation of biomass, so lignocellulose fractionation methods need to be optimised for various types of feedstocks, high quality fractions for valourisation, and energy and chemicals efficiencies (Demirbas, 2009; Alvira et al., 2010; FitzPatrick et al., 2010), therefore more research has to be done to ensure that the primary refinery produces high quality, purity and yield constituents.

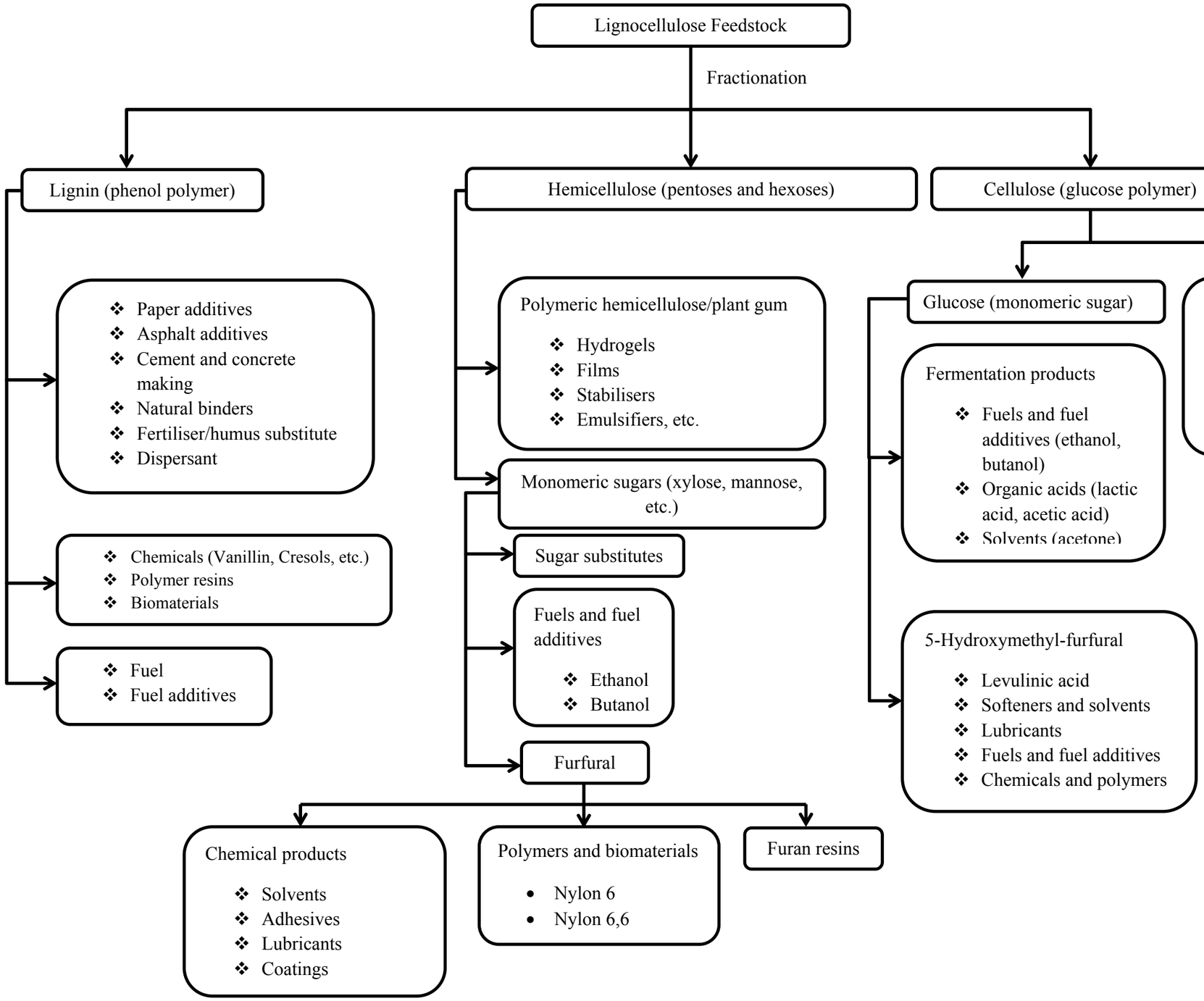

Figure 2.7: Lignocellulose feedstock biorefinery (adapted from (Kamm and Kamm, 2004; Stark, 2011))

2.3. Lignocellulose fractionation

Lignocellulose fractionation is a particular type of pre-treatment, which extracts and separates lignocellulose constituents, in good quality to allow for valourisation (FitzPatrick et al., 2010). Fractionation of lignocellulose feedstock is the fundamental process in the biorefinery (Wildschut et al., 2013). The effectiveness of lignocellulose fractionation (pre-treatment) method is assessed using this criteria; (i) ability to overcome lignocellulose recalcitrance (deconstructing lignocellulose structure and lowering cellulose crystallinity), (ii) producing high yields of cellulose, hemicellulose and lignin to sugars or chemicals applications and highly digestible cellulose, (iii) minimal degradation of carbohydrates, especially hemicellulose, (iv) minimal release of sugar and lignin degraded products, which are inhibitory toxic products, (v) allow for lignin recovery for production of chemicals and materials, and (vi) be cost effective; regeneration of energy, chemicals and enzymes, together with wide range of feedstocks, low capital and operating costs (Alvira et al., 2010; Agbor et al., 2011; Vancov et al., 2012).

Chemical pre-treatment methods are more promising for application in biorefineries (FitzPatrick et al., 2010; Fu and Mazza, 2011b). The comparison of some chemical pre-treatment methods is shown in Table 2.6, and the degree of hydrolysis of hemicellulose is desirable for downstream processing. As shown in Table 2.6, acidic treatments (dilute acid, wet oxidation, and acidic ionic liquid treatments) yield mainly monomeric hemicelluloses. In order to achieve higher yields of polymeric and oligomeric hemicelluloses, acidic solvents and acidic catalysts should be avoided. Aquasolv pre-treatment methods yield oligomeric and monomeric hemicelluloses, so if they were to be considered in this study, they would have to be coupled with alkaline extraction of hemicelluloses. So in this study, auto-catalysed organosolv and ionic liquid (with basic anion) treatments, coupled with either alkaline pre-extraction or alkaline post-extraction of hemicelluloses will be considered.

Table 2.6: The comparison of chemical pre-treatment methods (Adapted from (Carvalheiro et al., 2008; Harmsen et al., 2010; Agbor et al., 2011; Stark, 2011; Menon and Rao, 2012; Brandt et al., 2013))

Pre-treatment	Mode of action	Recovered sugar	Inhibitor production	By-products formation	Need for chemical reuse	Applicable to diverse biomass	Operational costs	Pilot scale attempts	Advantages	Disadvantages	Hemicellulose quality (size)
Liquid hot water	Hemicellulose removal	High	Low	No	No	Yes	Low	Yes	Minimum degradation products Low cost of solvent Low residence time	Use of high temperature and pressures Low lignin removal pH monitoring required	Mostly oligomers
Steam explosion	Hemicellulose removal Lignin transformation	High	High	Low	No	Yes	Low	Yes	Low residence time Limited use of chemicals Low environmental impact	Use of high temperature and pressures Low lignin removal	Mostly oligomers
Dilute acid	Hemicellulose removal Lignin transformation and removal	High	High	High	Yes	Yes	Moderate	Yes	Lignin removal is achieved	High equipment cost (to resist corrosion)	Oligomers Monomers
Wet oxidation	Lignin removal Hemicellulose dissolution Cellulose decrystallisation	Moderate	Low	Low	No	—	High	—	Considerable lignin removal	High equipment costs	Monomers
AFEX	Lignin removal Minimum hemicellulose dissolution Cellulose decrystallisation	High	Low	—	Yes	Yes (with limitations on softwood)	High	—	Considerable lignin removal Minimum degradation products Moderate temperature Short residence time	Costly solvent (ammonia) High environmental impact (issues)	Oligomers
CO_2 explosion	Hemicellulose removal Cellulose decrystallisation	High	Low	Low	No	—	—	—	Cost effective (low cost of solvent, CO_2) Low temperatures High solid loading capacity Minimum degradation products	High pressures	—
Alkaline	Lignin removal Hemicellulose removal	High	Low	High	Yes	Yes	Low	Yes	Low reagents cost Low and moderate temperatures	Long residence times Irreversible salt formation Large volumes of water used for washing pulp Need for neutralising liquid fractions	Polymers
Organosolv	Considerable lignin removal Hemicellulose removal	High	Low (for auto-, alkaline- and neutral catalysis)	Low	Yes	Yes	High	Yes	Sulphur-free high purity and quality lignin Does not require significant size reduction of feedstock	Solvent recovery required Costly solvents Inherent fire and explosion hazards Environmental, health and safety concerns	Oligomers Monomers
Ionic liquids	Dissolution of biomass Use of antisolvents Selective dissolution (either lignin or carbohydrate)	High	Low	—	Yes	Yes	—	—	Solvents are thermally stable, highly polar and have negligible vapour pressure	Requirement of antisolvents Need for solvent recovery and reuse Low solid loading capacity	Polymers Oligomers Monomers (for acidic ionic liquids)

2.3.1. Alkaline treatment/extraction

The alkaline pre-treatment of lignocellulosic biomass use aqueous solutions of bases, like sodium hydroxide, calcium hydroxide, potassium hydroxide, ammonium hydroxide, sodium carbonate, and green liquor (which comprises of sodium carbonate and sodium sulphite) (Carvalheiro et al., 2008; Kumar et al., 2009; Harmsen et al., 2010; Agbor et al., 2011; Menon and Rao, 2012; Yang et al., 2012). The alkaline solution which has shown high lignocellulose dissolution and high cellulose digestibility is sodium hydroxide (Rezende et al., 2011). Alkaline pre-treatments of low severities are considered as primarily hemicellulose extraction processes, while alkaline treatments of higher severity are regarded as delignification processes (Carvalheiro et al., 2008; Kumar et al., 2009; Harmsen et al., 2010; Agbor et al., 2011; Menon and Rao, 2012). The reaction mechanisms in the alkaline hydrolysis of lignocellulosic biomass include degradation of ester crosslinking lignin and hemicellulose through solvation and saponification, removal of glycosidic (uronic and glucoronic) side chains on the hemicellulose, deacetylation of lignocellulose, and hydrogen bonds' weakening (Carvalheiro et al., 2008; Harmsen et al., 2010; Agbor et al., 2011; Rezende et al., 2011; Menon and Rao, 2012). The reaction mechanisms are shown in Figs. 2.8 to 2.11. Figure 2.8 present reaction mechanism for deacetylation of hemicelluloses (xylan) during alkaline treatment of lignocellulosic biomass.

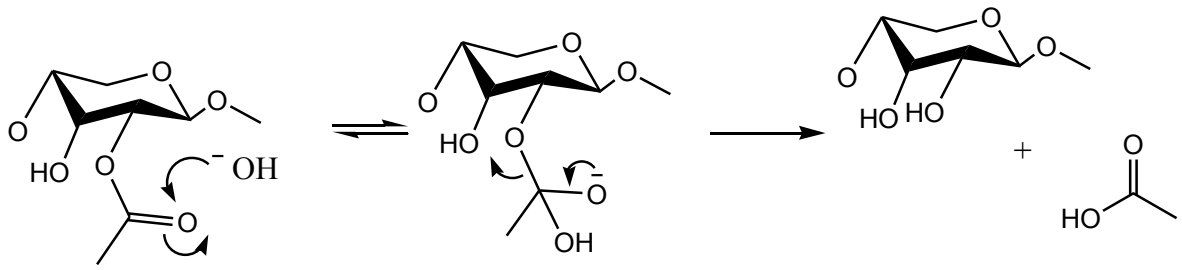

Figure 2.8: Schematic diagram showing reaction mechanism of xylan deacetylation in alkaline hydrolysis (Adapted from Patil, 2012)

Figure 2.9 presents the reaction mechanism of β-ether crosslinks (lignin reactions) in alkaline conditions. Under alkaline conditions, the β-ether crosslink in lignin rearranges into guaiacol, which rearranges further into veratrylglycerol and other phenolic compounds (McDonough, 1992; Santos et al., 2013).

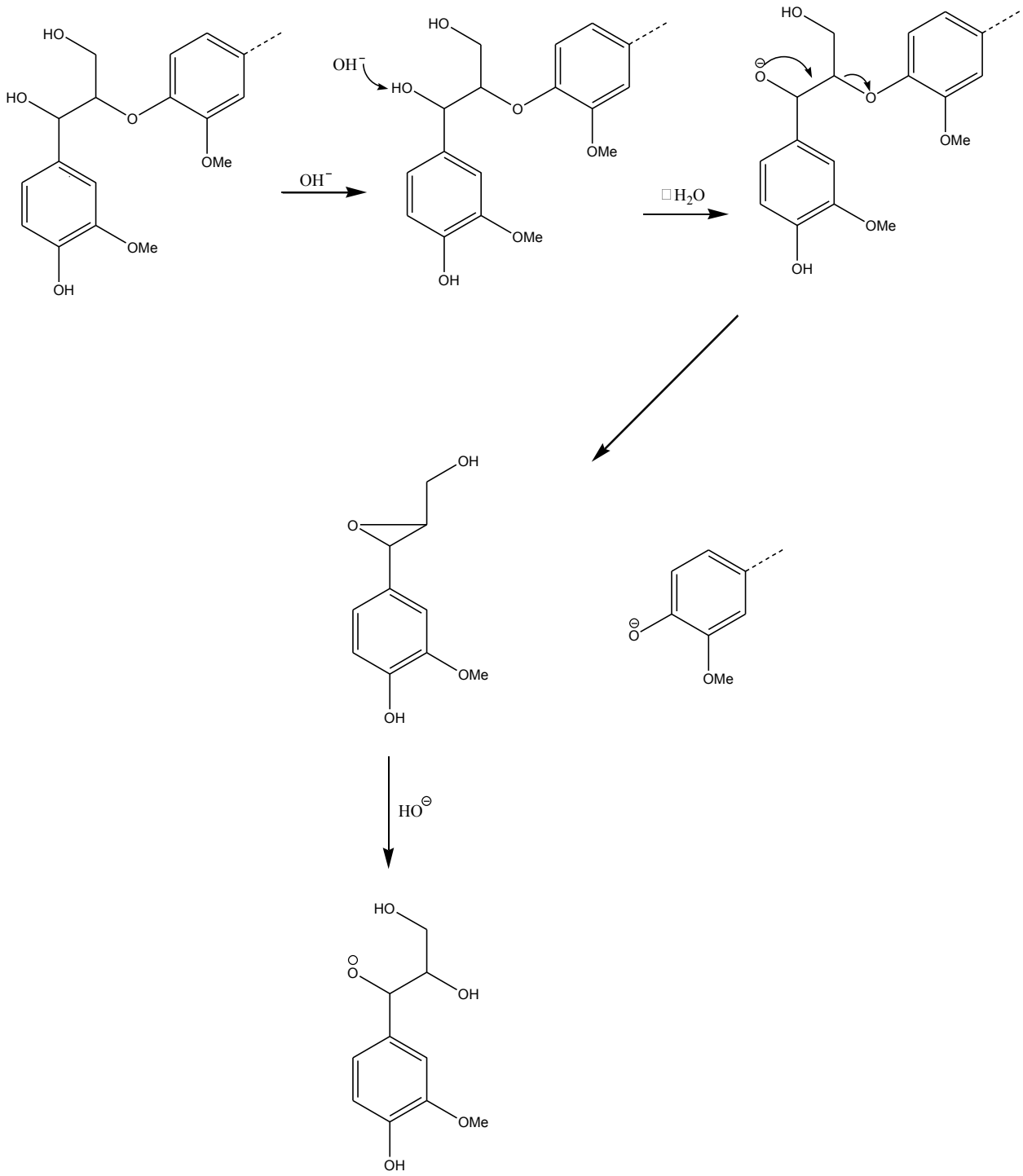

Figure 2.9: Schematic diagram showing reaction mechanism of ester and/or ether (β-O-4) crosslinks removal (Adapted from McDonough, 1992; Santos et al., 2013)

Figure 2.10 presents reaction mechanisms for β-ether crosslinks, as well. The β-ether bonds are removed through formation of enol-ether, vinyl-ether and formaldehyde, upon dehydration (McDonough, 1992; Santos et al., 2013).

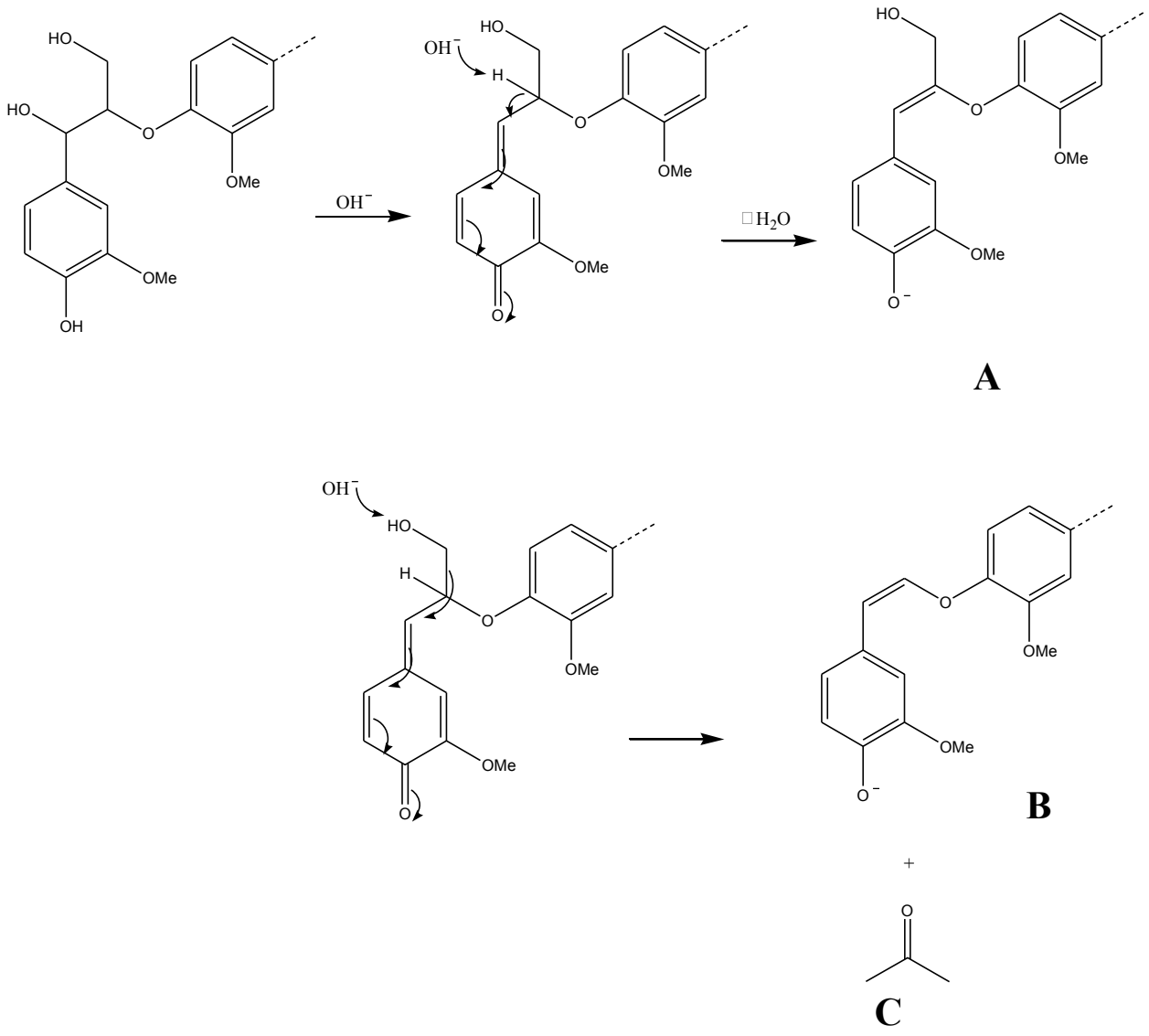

Figure 2.10: Schematic diagram showing reaction mechanism of β-O-4 reaction forming (A) enol-ether, (B) vinyl-ether and (C) formaldehyde (Adapted from McDonough, 1992; Santos et al., 2013)

Figure 2.11 presents reaction mechanisim of saponification of LCCs. The acetyl groups bonded to the carbohydrates (as well as lignin) undergoes rearrangement in alkaline conditions. Often the rearrangement result in the cleavage of acetyl bonds with ligning, resulting in acetalyted carbohydrates and deacetylated lignin (Hu and Ragauskas).

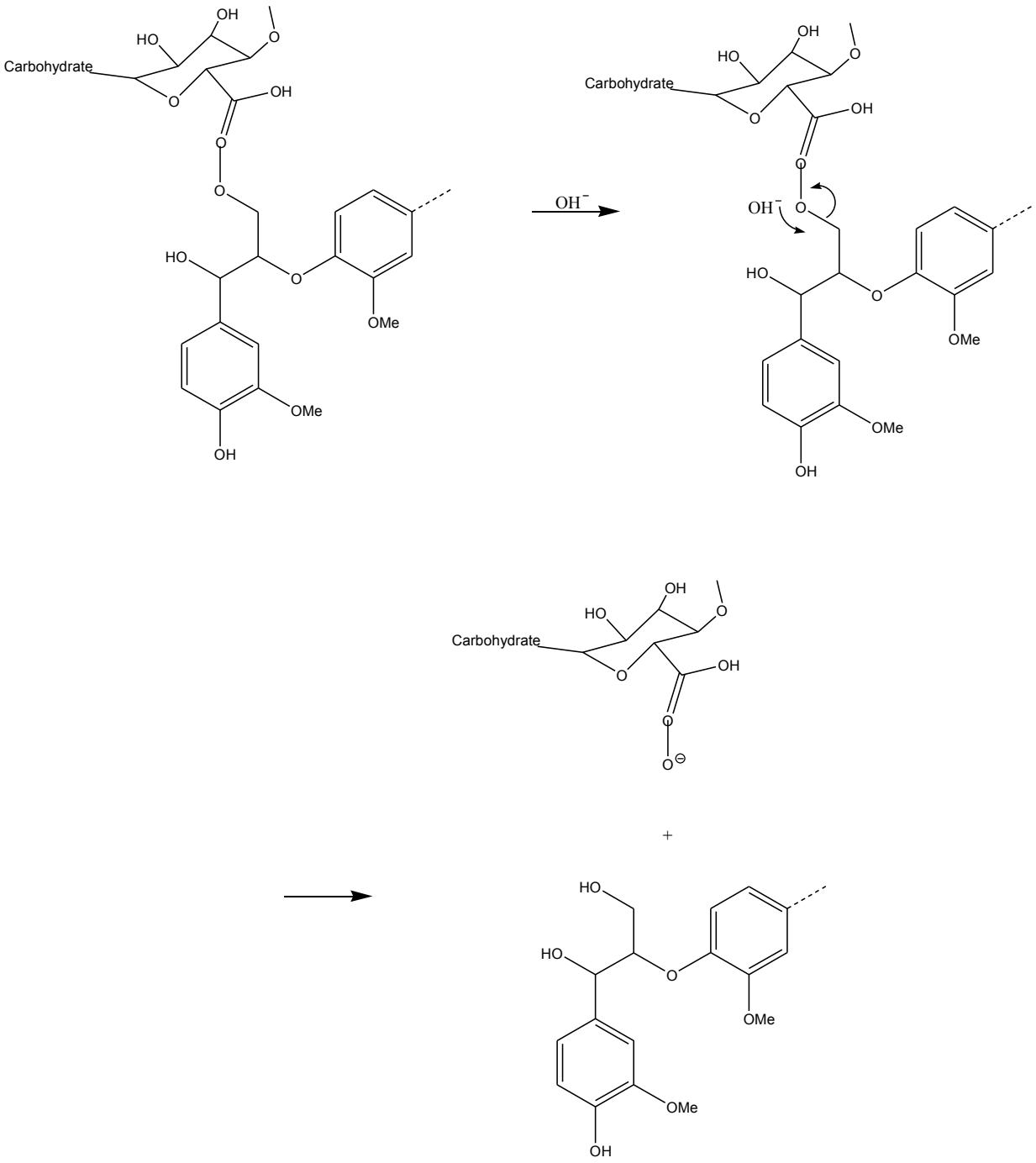

Figure 2.11: Schematic diagram showing saponification of lignin-carbohydrate complex (Adapted from Hu and Ragauskas)

As shown in Table 2.6, alkaline pre-treatment is attractive as it uses low temperatures and pressures (effective even at ambient conditions), and yields polymeric hemicelluloses of acceptable purity, with limited lignin contamination. In this study, mild alkaline extraction is preferable, as alkaline extraction is coupled with organosolv and ionic liquid fractionations. The alkaline pre-treatments of lignocellulose are presented in Tables 2.7 and 2.8 for

hardwoods and herbaceous/agricultural residues respectively, and the pretreatments shown compare the quality of fractions in relation to severity. Most researchers did not study the molecular size of extracted xylans for either hardwoods or herbaceous/agricultural residues. For hardwoods extracted xylan molecular weights of 14500 and 16000 Da were reported at severe alkaline extractions (12.5 M NaOH), and, 41000, 45000 and 53400 Da were reported at moderate alkaline extractions (Júnior et al., 2013; Vena, 2013). As for lignin extracted from alkaline treatment of hardwoods, there was no information available for S/G ratios. The recovered solids from alkaline extraction of xylan from hardwoods had poor digestibilities, with exceptions of hybrids and H_2O pre-extracted eucalypts. For herbaceous/agricultural residues, extracted xylan molecular weights' range of 32793 Da was reported (Vena, 2013). No information was available for S/G ratios of extracted lignin. As for recovered solids, in most alkaline pre-treatment of herbaceous/agricultural residues high digestibilities were reported.

Although most of the researchers did not report molecular weight of extracted xylans and quality of lignin fractions, there are some trends observed. For the alkaline treatment of hardwoods, shown in Table 2.7, cellulose retained in the solids after alkaline treatment is high (≥80% of cellulose in virgin materials). The high cellulose retention is attractive aspect for pre-treatment of lignocellulose (as sugar losses and degradation have to be minimal for effective pre-treatment). It was also observed that as the process severity increased (high alkali concentrations, high temperatures or longer residence time); cellulose dissolution/loss was increased. Cellulose digestibility for recovered solids was observed to increase with increasing severity. Both cellulose recovery and digestibility are important factors for biorefinery approach. Therefore, when treatment is carried out, there has to be the balance between the two. It was also observed that as hemicelluloses were extracted, significant amounts of lignin were solubilised as well. The similar trends were observed from alkaline treatment of herbaceous/agricultural residues, as shown in Table 2.8. The difference was that good pre-treatment was achieved at lower severities than those used in alkaline treatment of hardwoods. Both high cellulose recovery and high digestibility were obtained at mild alkaline treatment of herbaceous/agricultural residues. Furthermore, very high amounts hemicellulose (up to 96 %) and lignin (up to 89 %) were solubilised at more or less similar conditions to those applied to hardwoods.

Table 2.7: Alkaline treatment of the hardwoods

Biomass	Treatment conditions	Solid recovery (%)	Glucan yield (%)	Glucan digestibility (%)	Extracted Xylan (%)	Extracted lignin (%)	References
E. globulus (H_2O pre-extracted)	0.2 M NaOH, 1:10 solid-liquid loading, 70 °C, 1 h, 150 rpm	78.0±1.3	96.93	75±3	—	—	Castro et al., 2013
Hardwood mixture (mainly maple)	1.76 M Na_2O, 1:4 solid-liquid loading 160 °C, 1.83 h, 2 rpm	88.6	97.04	—	11.57	11.07*	Um and Walsum, 2010
Eucalyptus residues	1 M NaOH, 1:10 solid-liquid loading, 60 °C, 24 h	83.2	94.02	8.0	42.78*	17.61*	Park and Kim, 2012
	1 M KOH, 1:10 solid-liquid loading, 60 °C, 24 h	83.5	94.98	7.0	36.36*	18.60*	
	1 M Na_2CO_3, 1:10 solid-liquid loading, 60 °C, 24 h	93.3	97.61	5.2	16.04*	8.64*	
	15 % aq. NH_3, 1:10 solid-liquid loading, 60 °C, 24 h	90.1	96.41	6.0	17.65*	14.29*	
	1 M Na_2CO_3 percolation	79.1	98.33	19.0	28.88*	21.93*	
E. grandis	1 M NaOH, 1:10 solid-liquid loading, 120 °C, 1 h, 105 kPa	73.1	103.69±30.35	75	0*	4.09*	Lima et al., 2013
Hybrid *E. grandis x urophylla*	1 M NaOH, 1:10 solid-liquid loading, 120 °C, 1 h, 105 kPa	63.4	100.85±20.04	100	12.35*	40.72*	
E. globulus	2.5 M NaOH, 1:10 solid-liquid loading, 100 °C, 1 h	92.8	97.61	—	13.75*	5.41*	Júnior et al., 2013
	12.5 M NaOH, 1:10 solid-liquid loading, 100 °C, 1 h	82.8	92.19	—	33.13*	21.24*	
Sweet gum	2.5 M NaOH, 1:10 solid-liquid loading, 100 °C, 1 h	89.2	95.88	—	7.94*	5.28*	
	12.5 M NaOH, 1:10 solid-liquid loading, 100 °C, 1 h	81.9	91.30	—	33.86*	12.20*	
E. grandis	1 M NaOH, 1:10 solid-liquid loading, 90 °C, 4 h	70.08	76.25	—	8.50	8.98*	Vena, 2013
	2 M NaOH, 1:10 solid-liquid loading, 40 °C, 4 h	68.59	75.00	—	12.40	8.38*	
	2 M NaOH, 1:10 solid-liquid loading, 90 °C, 2 h	75.78	81.04	—	10.30	2.99*	
	2 M NaOH, 1:10 solid-liquid loading, 90 °C, 4 h	63.40	73.54	—	16.00	13.17*	

The * indicates values which were determined using mass differences not detected values

Table 2.8: Alkaline treatment of herbaceous/agricultural residues

Biomass	Treatment conditions	Solid recovery (%)	Cellulose (glucose) yield (%)	Cellulose digestibility (%)	Extracted hemicellulose (xylose) (%)	Extracted lignin yield (%)	References
Elephant grass	0.175 M NaOH, 1:17.5 solid-liquid loading, 100 °C, 2 h	53.93	96.9	82	20.2	31.8	Eliana et al., 2014
Corn stover	1.75 M NaOH, 1:10 solid-liquid loading, 140 °C, 0.5 h, 200 rpm	63.1	92.49	~64	23.90*	62.70*	Li et al., 2012
	2.5 M NaOH, 1:10 solid-liquid loading, 140 °C, 0.5 h, 200 rpm	53.5	91.56	~76	31.18*	84.78*	
	1.75 M NaOH, 1:10 solid-liquid loading, 160 °C, 0.5 h, 200 rpm	56.6	87.49	~59	36.29*	67.05*	
	2.5 M NaOH, 1:10 solid-liquid loading, 160 °C, 0.5 h, 200 rpm	50.7	89.67	~85	36.56*	85.13*	
Sugarcane bagasse	0.2 M NaOH, 1:10 solid-liquid loading, 121 °C, 1 h, 105 kPa	83.0	98.3	—	49.4*	63.8*	Khuong et al., 2014
	0.25 M NaOH, 1:10 solid-liquid loading, 121 °C, 1 h, 105 kPa	63.0	81.9	—	65.0*	79.5*	
	1.25 M NaOH, 1:10 solid-liquid loading, 121 °C, 1 h, 105 kPa	73.1	81.6	—	96.2*	89.1*	
Rice straw	1.29 M Na_2O, 1:6 solid-liquid loading, 140 °C, 0.5 h, 6 rpm	74.6±1.6	95.1±0.2	73.2	24.1±0.2*	41.8±0.4*	Yang et al., 2012
Sugarcane bagasse	1.5M NaOH, 1:10 solid-liquid loading, 65 °C, 1.53 h	—	94.6	—	69.1	18.7*	Vena, 2013
Corn stover	0.38 M Na_2CO_3, 1:10 solid-liquid loading, 140 °C, 0.33 h	52.3	76.0	86.2±0.2	40.2*	40.0*	Kim et al., 2014a
Rice straw	2.08 M $(NH4)_2CO_3$, 1:10 solid-liquid loading, 80 °C, 12 h	65.9	92.3	72.2	17.4*	23.9*	Kim et al., 2014b
Sugarcane bagasse (acid pre-extracted)	0.25 M NaOH, 1:10 solid-liquid loading, 120 °C, 0.67 h	72.5±2.8	~72	99.8±7.4	~16*	~68*	Rezende et al., 2011
	0.5 M NaOH, 1:10 solid-liquid loading, 120 °C, 0.67 h	68.3±2.0	~68	96.9±1.9	~18*	~72*	
	0.75 M NaOH, 1:10 solid-liquid loading, 120 °C, 0.67 h	70.7±1.8	~70	95.5±10.5	~18*	~72*	
	1 M NaOH, 1:10 solid-liquid loading, 120 °C, 0.67 h	65.9±4.7	~67	97.4±19.9	~18*	~72*	

The * indicates values which were determined using mass differences not detected values

2.3.2. Organosolv

Organosolv fractionation method uses organic solvents or aqueous organic solvents, with or without catalysts, at elevated temperatures (as high as 210 °C) and pressure (as high as 30 bar) for lignocellulose treatments (Harmsen et al., 2010; Park et al., 2010; Wildschut et al., 2013). Some organic solvents used are low boiling point alcohols (methanol and ethanol), polyols or high boiling point alcohols (ethylene glycol, glycerol, propylene glycol, and tetrahydrofuryl alcohol), ketones (methyl isobutyl ketone, and acetone), phenols, ethers, and organic acids (formic acid and acetic acid) (Zhao et al., 2009; Harmsen et al., 2010; Agbor et al., 2011). The use of alcohols, mainly low boiling point alcohols, is advantageous as they are easy to recover through distillation at low energy consumption; they are low cost and miscible with water (Zhao et al., 2009).

Organosolv achieves lignin removal through hydrolysis of internal lignin bonds, ether and 4-0-methylglucuronic acid ester bonds between lignin and hemicellulose (Zhao et al., 2009; Agbor et al., 2011; Cybulska et al., 2012). Organosolv (under acidic conditions) dissolves hemicellulose through hydrolysis of glycosidic bonds in lignocellulose (Zhao et al., 2009; Agbor et al., 2011). Lignin and hemicellulose solubilisation (which reduces enzyme absorption and adsorption to lignin and hemicellulose), and altered cellulose structure (decrystallised and depolymerised) lead to improved enzymatic digestibility of cellulose-rich residues (Pan et al., 2005; Zhao et al., 2009; Agbor et al., 2011; Mesa et al., 2011). Organosolv also produces high quality lignin (highly pure, unaltered, and less condensed), which dissolves in many organic solvents and has potential for several industrial applications (adhesives, resins, films, biodegradable polymers, etc.) (Pan et al., 2005; Brosse et al., 2009; Zhao et al., 2009; Huijgen et al., 2010; Agbor et al., 2011; Goh et al., 2011; Mesa et al., 2011; Huijgen et al., 2012; Wildschut et al., 2013).

The catalysts that can be used in organosolv process include inorganic acids (H_2SO_4, HCl, H_3PO_4), organic acids (formic, oxalic, acetylsalicylic, salicylic acids), base catalysts (NaOH, $Ca(OH)_2$), and neutral catalysts (chlorides and nitrates of magnesium, calcium and barium) (Zhao et al., 2009; Harmsen et al., 2010; Park et al., 2010; Agbor et al., 2011). However, acid catalysts (both organic and inorganic acids lower pH of the process) result in degradation of hemicelluloses into monomers which are vulnerable to acidic reactions (to furfural, HMF, and pento- and hexo-sides), and thus acid catalysts are undesirable when oligomeric and

polymeric hemicelluloses are preferred (Zhao et al., 2009; Agbor et al., 2011). Other aspects of organosolv, like vast variety of feedstocks are shown in Table 2.6.

Ethanol-based organosolv is attractive for biorefinery approach, as the solvent can be produced within the process through fermentation of sugars (pentoses and hexoses), thus reducing solvent cost (Zhao et al., 2009). Furthermore, low boiling point of ethanol enhances solvent recovery through distillation, and recycling (Zhao et al., 2009). The Figure 2.12 below shows the alcohol (methanol or ethanol) organosolv process. The biomass is treated in the presence of catalyst, below 180 °C or in the absence of catalyst at higher temperatures, ranging from 185 °C to 210 °C, and pressure control of 5-30 bar (Pan et al., 2006; Zhao et al., 2009; Huijgen et al., 2012; Wildschut et al., 2013). For ethanol-based organosolv, residence time used range from 15 min to 120 min (with 60 min being preferred), and solvent concentration of 50-80 %w/w, with 60 %w/w being the most preferred (Pan et al., 2006; Zhao et al., 2009; Huijgen et al., 2012; Wildschut et al., 2013).

As shown in Figure 2.12, cellulose rich solid residue is separated from organosolv liquor by filtration, which is followed by aqueous alcohol and water washes (Pan et al., 2005; Zhao et al., 2009). Organosolv liquor or black liquor (which is hemicellulose and lignin rich) is diluted and lignin is separated through precipitation in acidic conditions, while hydrolysed (depolymerised) hemicellulose remains in the water soluble fraction (Pan et al., 2005; Zhao et al., 2009; Koo et al., 2011a; 2011b). But further hydrolysis of hemicelluloses into degradation products is undesired in biorefinery, so the separation of hemicellulose-lignin mixture has to be done with much attention. The use of alkaline catalysts and hemicellulose selective separation methods has to be employed to minimise degradation of the hemicellulose.

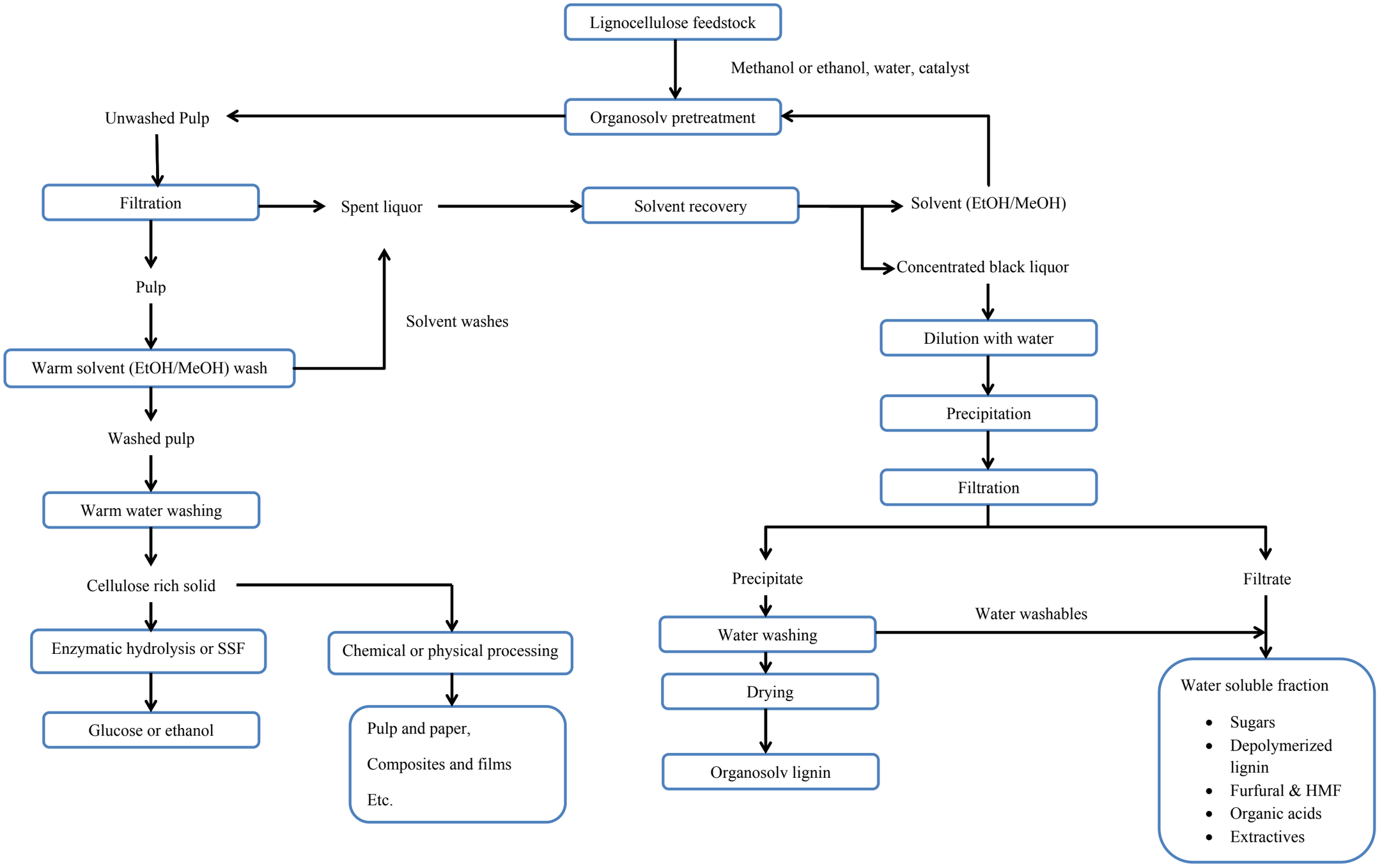

Figure 2.12: Alcohol organosolv process (Adapted from (Pan et al., 2006; Zhao et al., 2009; Huijgen et al., 2012; Wildschut et al., 2013))

The organosolv process yields monomeric and oligomeric sugars from hemicellulose. In alcohol organosolv, the sugars react with alcohols to form pento- and hexo-sides (Ferrier et al., 1968; Bouxin et al., 2014). This reaction targets α-glycosidic bonds (due to the anomeric effect). The reactions for sugars and alcohol (ethanol) are shown in Figure 2.13.

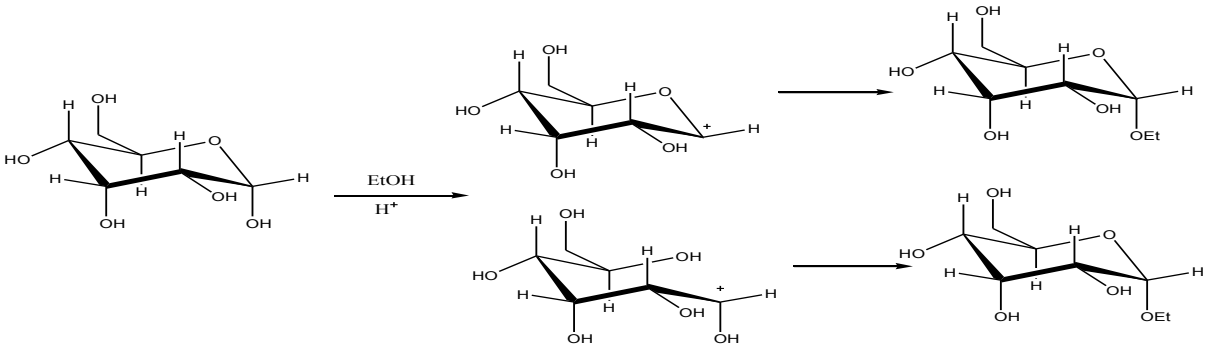

Figure 2.13: Schematic illustration of formation of carbohydrates (both pentoses and hexoses follow similar substitution) glycosides (Ferrier et al., 1968; Bouxin et al., 2014)

Lignin undergoes several reactions during organosolv: alcohol organosolv treatments enhance hydrolysis of benzyl ether linkages, cleavage of lignin-carbohydrates (ether and 4-O-methylglucuronic acid ester linkages), and lignin condensation reactions (McDonough, 1992; Zhao et al., 2009). Acid catalysed organosolv or acid-based organosolv, hydrolysis of α-ether bonds and β-aryl ether linkages are dominant for lignin dissolution McDonough, 1992; Zhao et al., 2009).

Tables 2.9 and 2.10 present organosolv treatments of herbaceous/agricultural residues and hardwoods. As shown, ethanol organosolv has been intensively studied in lignocellulose pre-treatment. Most researchers did not study the molecular size of extracted xylans for both hardwoods and herbaceous/agricultural residues. For herbaceous/agricultural residues, no xylan molecular weights were reported for the results presented in Table 2.9. The S/G ratios of extracted lignin for the organosolv treatments presented in Table 2.9 were reported as 0.47-0.55, with lower ratios obtained at temperatures ≥200 °C, while higher ratios were obtained at treatment temperatures ≤190 °C (Wörmeyer et al., 2011; Hu et al., 2012; Huijgen et al., 2014). As for recovered solids, in most alkaline pre-treatment of herbaceous/agricultural residues high digestibilities were reported. For hardwoods' extracted xylan no molecular weights were reported. Similarly, for lignin extracted from organosolv treatment of hardwoods, there was no information available for S/G ratios.

There are also important trends observed in organosolv processes shown in Tables 2.9 and 2.10. Autocatalysed organosolv treatment of herbaceous/agricultural residues (shown in

Table 2.9) resulted in high cellulose recoveries, but low cellulose digestibility (~50 %). Autocatalysed organosolv process is observed to use higher temperatures. When acid catalysed organosolv process is used, high cellulose recovery is accompanied by high cellulose digestibility (≥80 %). High cellulose digestibility (89 %) was observed even when very low concentration of acid (0.29 %) was used. Up to 93 % xylan dissolution and 94 % lignin dissolution have been reported for acid catalysed organosolv treatment of herbaceous/agricultural residues. The high hemicellulose and lignin dissolutions were obtained at severe conditions (2.5 % acid catalyst), and were accompanied by significant cellulose dissolution and loss. Acid catalysed organosolv treatment cleaves lignin-hemicellulose bonds, solubilises carbohydrates (cellulose to some extent) through hydrolysis of glycosidic bonds, and degrades monomeric sugars, as well as enhancing lignin condensation (Zhao et al., 2009). Although acid catalysed organosolv treatment yields highly digestible solids, high hemicellulose dissolution and degradation, as well as lignin condensation are its drawbacks. On the other hand, alkaline catalysed organosolv has shown similar results as autocatalysed organosolv. The only difference is that alkaline catalysed organosolv uses lower temperatures than autocatalysed organosolv. The use of lower temperatures and alkaline catalyst cause the process conditions to be milder, thus limiting hemicellulose and lignin degradation reactions.

Table 2.10 presents organosolv treatment of hardwoods. The similar trends are observed for treatment of hardwoods, whereby autocatalysed organosolv yielded low cellulose digestibility, while acid catalysed organosolv yielded highly digestible solids. The high cellulose digestibility of acid catalysed organosolv treated solids is related to high decrystallisation and depolymerisation of cellulose enhanced by acidic conditions. Increased severity (particularly acidity and temperature) significantly solubilised and degraded hemicellulose and lignin. Acid catalysed organosolv treatment of both agricultural residues and hardwoods resulted in highly digestible solids, high hemicellulose solubilisation and degradation, as well as high lignin solubilisation. As for autocatalysed organosolv treatment of hardwood, the process had to be coupled with pre-hydrolysis in order to that of agricultural residues. The difference can be related to different types of feedstocks, agricultural residues are easier to fractionate than hardwoods.

Table 2.9: Organosolv treatment of herbaceous/agricultural residue biomass

Biomass	Treatment conditions	Solid recovery (%)	Glucan yield (%)	Glucan digestibility (%)	Extracted Xylan (%)	Extracted lignin (%)	References
Wheat straw	60 % EtOH, 10 % solid-liquid loading, 200 °C, 1 h	57.90	95.56	51	0.45	67.41*	Huijgen et al., 2012
Pre-extracted wheat straw	60 % EtOH, 10 % solid-liquid loading, 200 °C, 1 h	51.7	94.88	67	8.85	59.26*	
Wheat straw	50 % acetone, 7.04 % solid-liquid loading, 190 °C, 1 h	56.0	91.77	72	1.94	71.76*	Huijgen et al., 2010
	50 % acetone, 7.04 % solid-liquid loading, 205 °C, 1 h	48.7	92.05	87	0.46	78.83*	
Miscanthus x giganteus	65 % EtOH, 11 % solid-liquid loading, 170 °C, 1 h, 0.5 % H_2SO_4	79.50	81.70	—	11.72	16.29	Brosse et al., 2009
	65 % EtOH, 11 % solid-liquid loading, 170 °C, 1 h, 0.9 % H_2SO_4	67.43	97.39	—	22.28	43.13	
Pre-extracted	65 % EtOH, 11 % solid-liquid loading, 170 °C, 1 h, 0.9 % H_2SO_4	48.39	98.33	—	66.07	58.28	
Wheat straw	60 % EtOH, 10 % solid-liquid loading, 190 °C, 1 h	67.7	97.0	37.2	3.7	46.3	Wildschut et al., 2013
	60 % EtOH, 10 % solid-liquid loading, 200 °C, 1 h	63.2	96.8	44.4	7.8	58.6	Huijgen et al., 2014
	60 % EtOH, 10 % solid-liquid loading, 190 °C, 1 h, 0.294 % H_2SO_4	43.0	91.0	89.4	29.5	85.4	
	50 % EtOH, 10 % solid-liquid loading, 210 °C, 1.5 h	48.6	94.8	85.9	2.0	83.1	
Rye straw	50 % EtOH, 10 % solid-liquid loading, 170 °C, 1 h	81.7	92.3	28.9	8.2	—	Perez-Cantu et al., 2013
	50 % EtOH, 10 % solid-liquid loading, 170 °C, 3 h	75.1	91.5	35.9	19.9	—	
	50 % EtOH, 10 % solid-liquid loading, 180 °C, 2 h	68.4	93.7	41.8	28.3	—	
	50 % EtOH, 10 % solid-liquid loading, 190 °C, 1 h	63.8	92.1	47.8	38.4	—	
Rye straw	50 % EtOH, 10 % solid-liquid loading, 167 °C, 0.58 h, 2.45 % H_2SO_4	43.3	87.3	76.0	92.5	93.78*	Ingram et al., 2011 Wörmeyer et al., 2011
Empty palm fruit bunch	65 % EtOH, 11 % solid-liquid loading, 180 °C, 1 h, 0.50 % H_2SO_4	68.46	84.73	87.20	36.66	53.00*	Goh et al., 2011
Kanlow switch grass	75 % EtOH, 11 % solid-liquid loading, 180 °C, 1 h, 0.90 % H_2SO_4	58.7	94.0	98.0	62.0*	60.5*	Cateto et al., 2011 Hu et al., 2012
Sugarcane bagasse	50 % EtOH, 17 % solid-liquid loading, 175 °C, 1.5 h, 1.5 % NaOH	73.33	88.25	26.05	1.02	20.76*	Mesa et al., 2010
	50 % EtOH, 17 % solid-liquid loading, 175 °C, 1 h, 1. 5 % NaOH	60.37	70.90	22.88	1.09	44.31*	
	50 % EtOH, 17 % solid-liquid loading, 175 °C, 1.5 h, 1.25 % NaOH	79.00	83.77	17.64	0.21	18.00*	
	50 % EtOH, 17 % solid-liquid loading, 175 °C, 1 h, 1.25 % NaOH	90.27	102.53	29.39	3.78	9.44*	
Rice straw	75 % EtOH, 11 % solid-liquid loading, 150 °C, 0.5 h, 1.0 % H_2SO_4	78.3	88.49	—	41.81*	35.06*	Amiri et al., 2014
	75 % EtOH, 11 % solid-liquid loading, 150 °C, 1 h, 1.0 % H_2SO_4	76.8	85.54	—	45.03*	39.46*	
	75 % EtOH, 11 % solid-liquid loading, 180 °C, 0.5 h, 1.0 % H_2SO_4	72.1	86.31	—	48.39*	51.65*	
	75 % EtOH, 11 % solid-liquid loading, 180 °C, 1 h, 1.0 % H_2SO_4	66.9	84.71	—	48.68*	56.32*	

The * indicates values determined using mass differences not detected values

Table 2.10: Organosolv treatment of hardwoods

Biomass	Treatment conditions	Solid recovery (%)	Glucan yield (%)	Glucan digestibility (%)	Extracted Xylan (%)	Extracted lignin (%)	References
Hybrid poplar	50 % EtOH, 10 % solid-liquid loading, 180 °C, 1 h, 1.25 % H_2SO_4	52.72	88.17	97	52.49	74.13	Pan et al., 2006a
Buddleja davidii (semi-hardwood)	50 % EtOH, 10 % solid-liquid loading, 180 °C, 1 h, 1.25 % H_2SO_4	61.95	84.95	~80	45.50	23.50	Hallac et al., 2010b
	50 % EtOH, 10 % solid-liquid loading, 180 °C, 0.67 h, 1.75 % H_2SO_4	56.07	83.18	~100	41.49	40.58	
	65 % EtOH, 10 % solid-liquid loading, 195 °C, 1 h, 1.5 % H_2SO_4	47.05	86.06	~100	32.83	74.58	
E. globulus Prehydrolysed	60 % EtOH, 11 % solid-liquid loading, 175 °C, 1 h	75.4	95.70	39.4	69.37*	53.56*	Romaní et al., 2011
	60 % EtOH, 11 % solid-liquid loading, 200 °C, 1 h	68.6	95.01	54.1	43.76*	70.72*	
Liriodendron tulipifera (poplar)	50 % EtOH, 10 % solid-liquid loading, 120 °C, 0.83 h, 1.0 % H_2SO_4	78.3	ND	—	1.3	22.98*	Koo et al., 2011a
	50 % EtOH, 10 % solid-liquid loading, 130 °C, 0.83 h, 1.0 % H_2SO_4	69.8	ND	—	3.0	32.77*	
	50 % EtOH, 10 % solid-liquid loading, 140 °C, 0.83 h, 1.0 % H_2SO_4	60.7	ND	—	5.5	43.40*	
Liriodendron tulipifera (poplar)	50 % EtOH, 10 % solid-liquid loading, 140 °C, 0.83 h, 1.0 % NaOH	74.9	109.34	65.6	21.21*	27.98*	Koo et al., 2011a; 2011b
	50 % EtOH, 10 % solid-liquid loading, 150 °C, 0.83 h, 1.0 % NaOH	73.8	107.74	65.9	24.81*	30.93*	
	50 % EtOH, 10 % solid-liquid loading, 160 °C, 0.83 h, 1.0 % NaOH	73.2	105.56	67.8	25.76*	31.49*	

The * indicates values determined using mass differences not detected values

2.3.3. Ionic liquids

Ionic liquids are organic salts that are liquid at temperatures below 100°C, have large organic cations and smaller organic or inorganic anions (FitzPatrick et al., 2010; Stark, 2011; Vancov et al., 2012). Ionic liquids differ from other molecular solvents by their distinct physic-chemical properties like negligible vapour pressure, non-flammability, good thermal stability, solvation properties and phase behaviour (Stark, 2011; Vancov et al., 2012; Yinghuai et al., 2013). The ionic liquids are classified into (i) task-specific or multi-functional ionic liquids, especially imidazolium, (ii) chiral ionic liquids, that have chiral centres (on anion, cation or both), (iii) protic ionic liquids, which have exchangeable proton and they are very acidic (thus are called destructive ionic liquids), (iv) switchable polarity ionic liquids, which equilibrate between a higher polarity and lower polarity depending on the reaction conditions, and (v) metallic salts ionic liquids, which mainly act as catalysts (Yinghuai et al., 2013).

Ionic liquids are said to be environmental benign, as they have low toxicity and no explosive gas releases during use (Mäki-Arvela et al., 2010; Holm, and Lassi, 2011; Stark, 2011). However, some limitations or challenges with ionic liquids include the high viscosities, corrosiveness, expensive prices, and energy-intensive recycling of pure ionic liquids (Mäki-Arvela et al., 2010; Fu and Mazza, 2011a; Stark, 2011). To address the issue of corrosiveness, halogen-free ionic liquids like [EMIM]OAc and 1,3-dimethylimidazolium dimethylphosphate, [MIM](MeO_2)PO_2 have to be considered for ionic liquid fractionation (Zavrel et al., 2009). High viscosity of ionic liquids can lead to mass and phase transfer limitations, which can be overcome by dissolution at high temperatures (this has challenges of side-reactions and stability problems of ionic liquids) and/or use of co-solvents (Mäki-Arvela et al., 2010; Fu and Mazza, 2011a; Sun et al., 2013; Xu et al., 2013). Some co-solvents which have been studied in lignocellulose treatment with ionic liquids include water, DMSO, DMF, DMAc, methanol, acetone, and 1,4-dioxane (Mäki-Arvela et al., 2010; Fu and Mazza, 2011a; 2011b; Pinkert et al., 2011; Sun et al., 2013; Xu et al., 2013). The other aspect that affects the dissolution efficiencies of ionic liquids is the impurities in the ionic liquids like halides and volatiles (Zavrel et al., 2009; Holm and Lassi, 2011; Brandt et al., 2013).

The ionic liquids are used to deconstruct (decrystallise) the lignocellulose, and their utilisation in the pre-treatment of lignocellulose can be in three ways, which are (i) selective lignin dissolution, (ii) selective hemi(cellulose) dissolution, and (iii) complete dissolution of the biomass (Mäki-Arvela et al., 2010; Stark, 2011; Brandt et al., 2013; Yinghuai et al.,

2013). The lignin can be selectively dissolved using [C_nMIM]-based ionic liquids that contain medium hydrogen bond acceptor (HBA) strength anions like methyl sulphate $[MeSO_4]^-$ and alkyl-benzylsulphonate mixed with xylenesulphonate $[ABS]^-$, and DBU-salt based ionic liquids with anions like tosylate, trifluoroacetate, lactate, hydrogen sulphate, trifluoromethane-sulphonate and thiocynate (Lee et al., 2009; Tan et al., 2009; Stark, 2011; Brandt et al., 2013). [EMIM]OAc is one of the ionic liquids which have been reported to be effective solvent for delignification of straws (Fu et al., 2010). The partial delignification has been achieved using mixtures of [EMIM]OAc with co-solvents like water, DMF, DMSO, DMAc (Fu and Mazza, 2011a; 2011b; Sun et al., 2013), and the mixture of 1-butyl-3-methylimidazolium acesulfamate, [BMIM]Ace, with 1,4-dioxane, DMSO, methanol, acetone and DMF (Pinkert et al., 2011, Xu et al., 2013).

The complete dissolution of carbohydrates without dissolution of lignin can be achieved using ionic liquids with high hydrogen bond acidity strength with auxiliaries like [BMIM]Cl, with aqueous hydrochloric acid or CF_3CO_2H, and $[BMIM]HSO_4$ (Stark, 2011). However, this dissolution approach leads to degradation (depolymerisation) and is limited by challenging removal of low molecular weight oligosaccharides (Stark, 2011).

Lignocellulose is completely dissolved using ionic liquids with high hydrogen bond acidity strength like chloride or acetate, followed by selective precipitation of cellulosic materials using the anti-solvents like water, acetone, acetone-water mixture, acetonitrile, ethanol, methanol and dichloromethane (Zhu et al., 2006; Fort et al., 2007). Some lignin and hemicellulose remain in the ionic liquid and have to be precipitated or separated out. Often, solubilised lignin is separated from ionic liquid fraction using acid precipitation, while solubilised hemicelluloses are not recovered (Lan et al., 2011; da Costa Lopes et al., 2013a; Yang et al., 2013). Acid precipitation of lignin degrades hemicelluloses. Acid soluble lignin and hemicellulose degradation products contaminate ionic liquid, and can increase costs of recovery and lower recycling efficiency (Tan et al., 2009).

The ionic liquid fractionation process is shown in Figure 2.14. The carbohydrate-rich residue can be further extracted with alkaline solution in order to dissolve hemicellulose and residual lignin (Lan et al., 2011; Yang et al., 2013). Some conditions which have been explored are: 5-100 %w/w ionic liquid concentrations (aqueous and organic solvent mixtures), 90-190 °C, 0.5-22 hours (Tan et al., 2009; Fu and Mazza, 2011a; 2011b; Li et al., 2011b; Diedericks et al., 2012b; Sun et al., 2013; Xu et al., 2013).

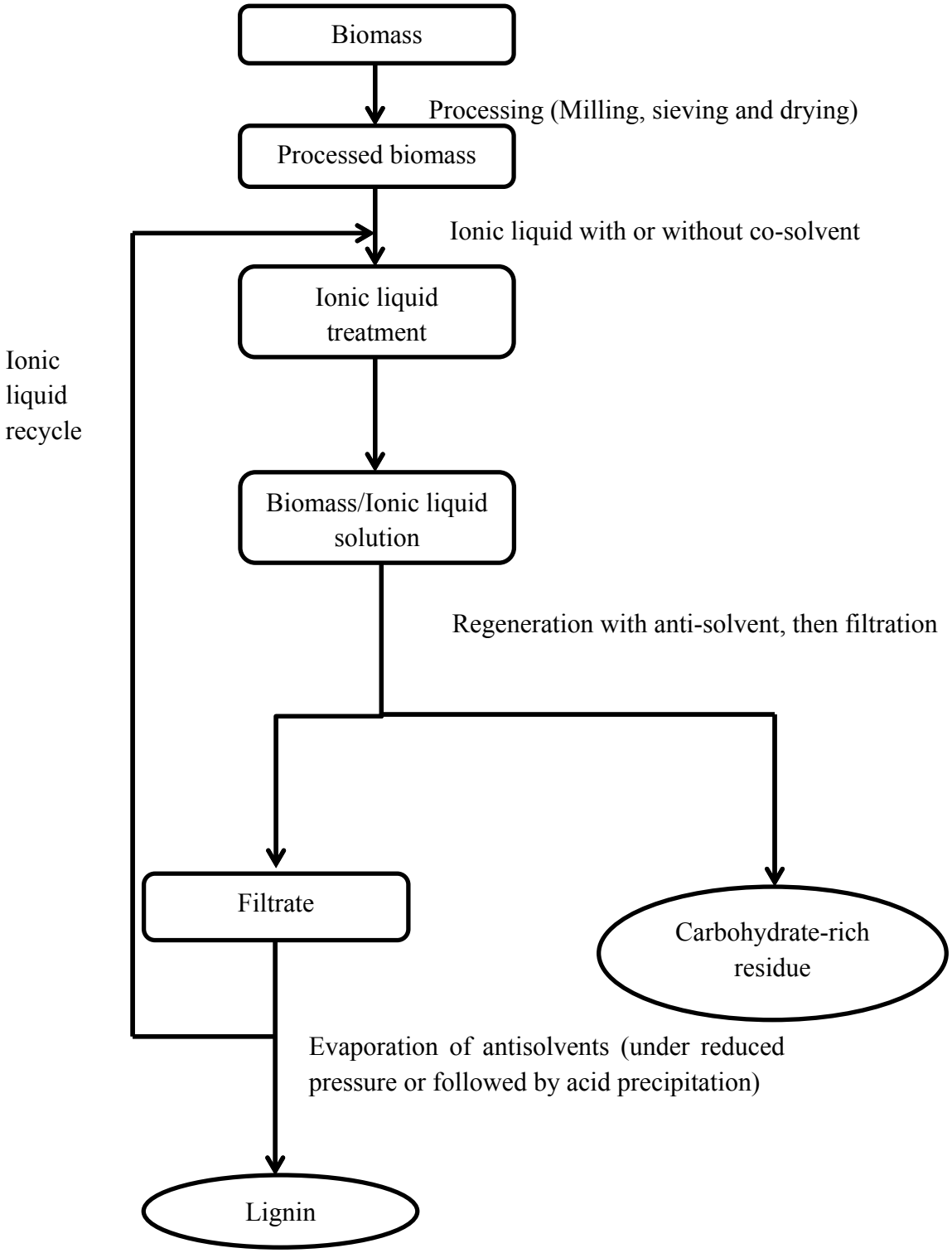

Figure 2.14: Ionic liquid treatment of biomass (Lan et al., 2011; Yang et al., 2013; Yinghuai et al., 2013).

Ionic liquids solubilise plant polymers through various reactions. The ionic liquid treatments follow a trend of using high temperatures or long treatment times to effect solubilisation of plant polymers (Brandt et al., 2013). Acidic or protic ionic liquids (those with halogens, sulphurous anions, etc.) degrade plant polymers (Yinghuai et al., 2013; Brandt et al., 2013; Leskinen et al., 2014). The ionic liquid treatment can lead to depolymerisation (fragmentation) of lignin through cleavage of β-O-4 ether bonds and oxidative cleavage of α-β carbon bonds (Kubo et al., 2008; Jia et al., 2010; Cox et al., 2011; Brandt et al., 2013; Cox and Ekerdt, 2013; Yinghuai et al., 2013; Lee et al., 2014; Leskinen et al., 2014). The β-O-4 ether bond cleavage reaction in ionic liquid treatment is dependent on the anion in the ionic liquid: strongly hydrogen bond-basic anions (like halogens and sulphurous anions) tend to yield high cleavage products than weakly hydrogen bond-basic anions (Brandt et al., 2013; Yinghuai et al., 2013; Leskinen et al., 2014). In ionic liquid treatment, lignin also undergoes dehydration (especially in basic ionic liquids like acetate based ones) and condensation reactions (Brandt et al., 2013; Yinghuai et al., 2013). The dehydration reaction of lignin in ionic liquids is shown in Figure 2.15.

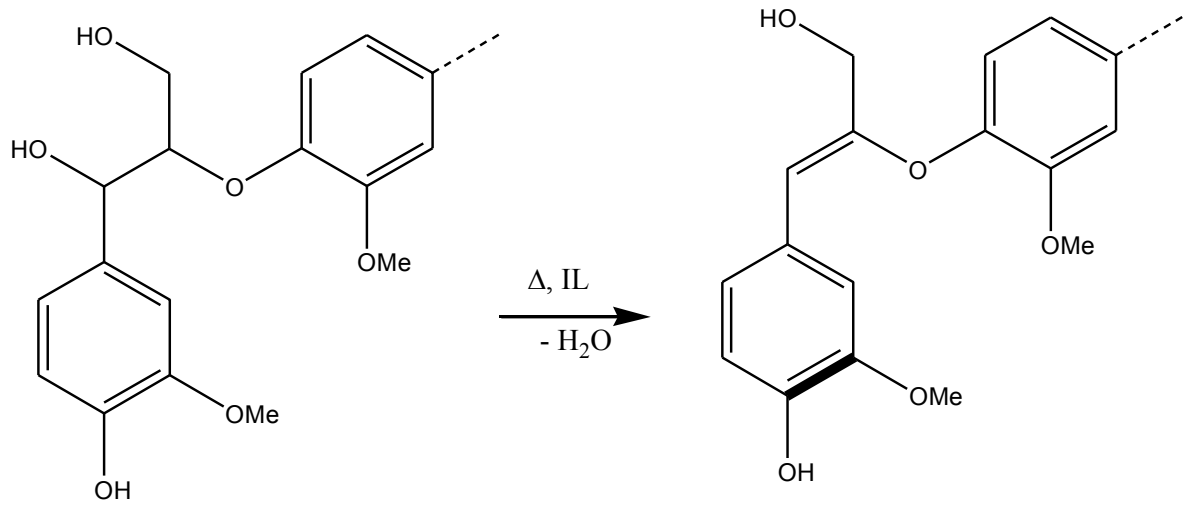

Figure 2.15: Lignin dehydration in ionic liquids (Kubo et al., 2008)

Polysaccharides (cellulose and hemicellulose) undergo several reactions in ionic liquid treatments. Cellulose hydrolysis occurs in acid ionic liquid treatments, and is undesirable (Kupiainen, 2012; Leskinen et al., 2014). Hemicellulose dissolution occurs through depolymerisation of hemicellulose polymers and deacetylation of hemicellulose-acetyl bonds (Brandt et al., 2013; Lee et al., 2014; Leskinen et al., 2014).

Moreover, all the lignocellulose components (cellulose, hemicellulose and lignin) are prone to esterification in ionic liquid treatments, and three major reactions being acetylation,

benzoylation and carboxylation (Liebert and Heinze, 2008; Karatzos et al., 2012a; Yinghuai et al., 2013; Leskinen et al., 2014). The esterification reactions are undesirable, especially on the cellulose (Leskinen et al., 2014).

The undesirable side reactions in ionic liquid treatments can be avoided by use of mild conditions, and addition of possible catalyst and co-solvents (Leskinen et al., 2014). The co-solvents have been highlighted earlier in this section. However, aqueous ionic liquid (IL/H_2O) seems to be the most attractive option, and has shown good fractionation properties in grass-type feedstocks (Fu and Mazza, 2011a; 2011b; Brandt et al., 2011; Brandt et al., 2013; Leskinen et al., 2014).

Beside the challenges of undesirable side reactions, high viscosities, and high costs of ionic liquid, the scale-up and commercialisation of ionic liquid treatment (fractionation) of biomass is limited by low solid loading. As shown in Table 2.6, most researchers used the biomass solid loading of 3-5 % in order to minimise gel phase formation during regeneration with antisolvents (Dibble et al., 2011; Li et al., 2013). However, the gel phase formation and separation are not dependent on solid loading only, but also on the volumes and compositions of antisolvents used (Dibble et al., 2011; Li et al., 2013). The use of large volumes of antisolvents (mainly ketone-alcohol mixtures) is considered to avoid gel phase formation, but it increases solvent expenditure and other operating costs (Li et al., 2013). The other alternative for working with the gel phase formed during high solid loading ionic liquid treatment is use of cheaper antisolvents (like water) coupled with mechanical breakdown of gels with blenders or homogenisers (Li et al., 2013).

Some ionic liquid pre-treatments of lignocellulose are shown in Tables 2.11 and 2.12 for herbaceous/agricultural residues and hardwoods respectively. Most researchers did not study the molecular size of extracted xylans for both hardwoods and herbaceous/agricultural residues. For both herbaceous/agricultural residues and hardwoods, no xylan molecular weights and S/G ratios were reported for the results presented in Tables 2.11 and 2.12.

As it can be seen from Tables 2.11 and 2.12, ionic liquid treatment has been applied on different types of feedstocks (herbaceous/agricultural residues and hardwoods). Most studies carried out on ionic liquid pre-treatment used pure ionic liquids. Since ionic liquids are viscous, the mass transfer challenges are overcome by using low solid loadings. The use of low solid loading was also considered in order to limit gel formation during ionic liquid

treatment (Dibble et al., 2011; Li et al., 2013). In most treatments presented in Tables 2.11 and 2.12 low solid loadings (≤10 %) were used. Those low solid loadings form part of the major factors affecting the scalability of ionic liquid treatment, and needs to be optimised (Brandt et al., 2013; Li et al., 2013; George et al., 2013). The use of co-solvents (like water) has also been studied in order to address viscosity challenges, and has proven to achieve satisfactory fractionation (as shown in Tables 2.11 and 2.12). However, treatments using ionic liquids with co-solvents still need to be studied for various (higher) solid loadings.

Ionic liquids used in Tables 2.11 and 2.12 have basic anions, which allow those ionic liquids to buffer the treatment conditions and preserve carbohydrates (cellulose and hemicellulose) (Brandt et al., 2011). That was reflected by high cellulose recoveries (≥80 %). When ionic liquid treatments were tested at low temperatures, they had to be coupled with long residence times, in order to achieve good fractionation. The high cellulose recoveries, high cellulose digestibility, preservation of hemicelluloses, high quality lignin, as well as robustness to different types of feedstocks indicate that ionic liquid treatment is good fractionation method.

Table 2.11: Ionic liquid treatment of herbaceous/agricultural residues

Biomass	Treatment conditions	Solid recovery (%)	Glucan yield (%)	Glucan digestibility (%)	Extracted Xylan (%)	Extracted lignin (%)	References
Corn stover	[EMIM]OAc, 3 % solid-liquid loading, 160 °C, 3 h	53.3	94.8	~100	89.4*	82.8*	Li et al., 2011a
Switch grass	[EMIM]OAc, 3 % solid-liquid loading, 160 °C, 3 h	49.3	84.5	96	81.5*	69.2*	Li et al., 2010
Switch grass	[EMIM]OAc, 3 % solid-liquid loading, 120 °C, 3 h	85	97.4	~94	21.7*	34.6*	Arora *et al.*, 2010
	[EMIM]OAc, 3 % solid-liquid loading, 160 °C, 3 h	56	79.5	~97	82.6*	65.4*	
Miscanthus x giganteus	80 % aqueous [EMIM]OAc, 5 % solid-liquid loading, 120 °C, 22 h	69.3	96.1	~74	56.8*	56.2*	Brandt et al., 2011
Switch grass	[EMIM]OAc, 15 % solid-liquid loading, 160 °C, 3h	55.3	87.5	96.8	32.6	77.2*	Li et al., 2013
Sugarcane bagasse	[EMIM]OAc, 5% solid-liquid loading, 150°C, 1.5 h	66.2	100	~87	54.8	60.1*	Karatzos et al., 2012b
Sugarcane bagasse	[EMIM]OAc, 10 % solid-liquid loading,100 °C, 1 h	90.5	93.9	~45	2.1	4.2	Diedericks et al., 2012b
	[EMIM]OAc, 10 % solid-liquid loading, 100 °C, 2 h	88.9	94.4	~58	2.5	9.0	
	[EMIM]OAc, 10 % solid-liquid loading, 125 °C, 2 h	73.1	92.3	~80	9.1	20.1	
Wheat straw	50 % aqueous [EMIM]OAc, 5% solid-liquid loading, 150°C, 3h, 150 rpm	58.9	80.4	92.5	37.3*	68.4*	Fu and Mazza, 2011b
Triticale straw	[EMIM]OAc, 5 % solid-liquid loading, 150°C, 1.5h	51.2	86.1	101.6	64.4*	76.3*	Fu and Mazza, 2011a
	50 % aqueous [EMIM]OAc, 5 % solid-liquid loading, 150 °C, 1.5 h	63.2	95.2	93.9	28.6*	62.9*	
	25 % aqueous [EMIM]OAc, 5 % solid-liquid loading, 150 °C, 1.5 h	71.9	97.5	75.0	14.9*	44.5*	
	5 % aqueous [EMIM]OAc, 5 % solid-liquid loading, 150 °C, 1.5 h	78.6	97.9	37.8	4.8*	21.6*	
Miscanthus x giganteus	[EMIM]OAc, 4 % solid-liquid loading, 70 °C, 44 h	86.6	98	100	8.6	34.1	Shill et al., 2011
Triticale straw	[EMIM]OAc, 5 % solid-liquid loading, 90 °C, 24 h	66.0	92.9	97.6	30.7*	45.5**	Fu et al., 2010
	DMEAF, 5 % solid-liquid loading, 90 °C, 24 h	80.4	93.6	28.9	7.6*	10.1**	
	DMEAA, 5 % solid-liquid loading, 90 °C, 24 h	81.6	90.4	25.1	9.0*	7.0*	
	DMEAG, 5 % solid-liquid loading, 90 °C, 24 h	80.0	89.5	27.9	10.8*	6.7*	
	DMEAS, 5 % solid-liquid loading, 90 °C, 24 h	83.6	92.9	20.9	9.1*	8.1*	
Typha capersis	[BMIM]OAc, 5.2 % solid-liquid loading, 110 °C, 6 h	94.4	99.6	84.57	11.2*	11.8	Audu et al., 2012
Mixed biomass: flour	[EMIM]OAc, 10 % solid-liquid loading, 160 °C, 3 h, 150 rpm	64.9	89.1	~96	66.1*	34.9*	Shi et al., 2013
: pellets	[EMIM]OAc, 10 % solid-liquid loading, 160 °C, 3 h, 150 rpm	63.1	90.4	~96	64.7*	35.7*	
Corn stover	[EMIM]OAc, 4.8 % solid-liquid loading, 125 °C, 1 h	73.5	86.4	—	33.5*	43.5	Wu et al., 2011
	[EMIM]OAc, 9.1 % solid-liquid loading, 125 °C, 1 h	73.0	87.3	—	33.9*	33.7	
	[EMIM]OAc, 13 % solid-liquid loading, 125 °C, 1 h	74.3	88.4	—	29.7*	25.2	
	[EMIM]OAc, 23 % solid-liquid loading, 125 °C, 1 h	76.7	90.2	—	20.1*	19.3	
	[EMIM]OAc, 33 % solid-liquid loading, 125 °C, 1 h	80.7	92.8	—	13.6*	15.1	

	[EMIM]OAc, 41 % solid-liquid loading, 125 °C, 1 h	82.0	90.1	—	15.6*	12.7	
	[EMIM]OAc, 50 % solid-liquid loading, 125 °C, 1 h	85.3	90.4	—	15.8*	8.2	
	[BMIM]OAc, 33 % solid-liquid loading, 125 °C, 1 h	81.6	89.2	~78	15.0*	14.6	
	[BMIM]OAc, 41 % solid-liquid loading, 125 °C, 1 h	84.2	89.2	~65	18.6*	11.4	
Switch grass	[EMIM]OAc, 33 % solid-liquid loading, 125 °C, 1 h	83.9	92.8	~73	15.4*	18.7	
Rice straw	[Ch] [Lys], 5 % solid-liquid loading, 90 °C, 24 h	55.9	92.2	86.7	55.1*	60.4	Hou et al., 2012
	[Ch] [Gly], 5 % solid-liquid loading, 90 °C, 24 h	51.6	80.5	79.0	59.1*	59.9	
	[Ch] [Ala], 5 % solid-liquid loading, 90 °C, 24 h	55.6	84.2	81.5	48.9*	58.3	
	[Ch] [Ser], 5 % solid-liquid loading, 90 °C, 24 h	61.4	95.6	86.5	38.9*	54.7	
	[Ch] [Thr], 5 % solid-liquid loading, 90 °C, 24 h	60.9	93.0	84.7	40.9*	53.1	
	[Ch] [Met], 5 % solid-liquid loading, 90 °C, 24 h	58.7	88.9	82.8	60.9*	55.2	
	[Ch] [Pro], 5 % solid-liquid loading, 90 °C, 24 h	60.5	91.3	82.1	44.1*	52.9	
	[Ch] [Phe], 5 % solid-liquid loading, 90 °C, 24 h	70.4	88.7	70.2	10.8*	41.5	
Switch grass	90 % aqueous [$FurEt_2NH$] [H_2PO_4], 10 % solid-liquid loading, 160 °C, 3h	62.8	94.5	~90	48.8	20.0	Socha et al., 2014
	90 % aqueous [$VanEt_2NH$] [H_2PO_4], 10 % solid-liquid loading, 160 °C, 3h	77.9	94.1	~60	33.9	3.9	
	90 % aqueous [*p*-$AnisEt_2NH$] [H_2PO_4], 10 % solid-liquid loading, 160 °C, 3h	56.7	89.1	~95	51.4	43.0	
	90 % aqueous [EMIM]OAc, 10 % solid-liquid loading, 160 °C, 3 h	58.0	92.3	~98	44.8	52.4	
Rice straw	[Ch] [Arg], 6.7 % solid-liquid loading, 90 °C, 12 h	47.7	90.1	74.6	63.2*	80.6	An et al., 2015
Wheat straw	[Ch] [Arg], 6.7 % solid-liquid loading, 90 °C, 12 h	49.2	78.2	60.7	55.6*	78.0	
Sugarcane bagasse	[Ch] [Arg], 6.7 % solid-liquid loading, 90 °C, 12 h	52.2	78.5	63.1	15.9*	73.5	
Corncob	[Ch] [Arg], 6.7 % solid-liquid loading, 90 °C, 12 h	57.2	94.8	75.2	24.7*	68.7	
Sugarcane bagasse	5 % aqueous [Ch] [Lsy], 5 % solid-liquid loading, 90 °C, 6 h	72.8	96.8	72.8	5.5*	26.9	Hou et al., 2013
	20 % aqueous [Ch] [Lsy], 5 % solid-liquid loading, 90 °C, 6 h	64.4	91.6	76.7	7.4*	32.2	
	50 % aqueous [Ch] [Lsy], 5% solid-liquid loading, 90°C, 6h	59.6	93.4	83.1	11.8*	38.6	
	80 % aqueous [Ch] [Lsy], 5 % solid-liquid loading, 90 °C, 6 h	59.1	91.4	83.8	14.0*	45.0	
	[Ch] [Lsy], 5 % solid-liquid loading, 90 °C, 6 h	58.3	89.6	82.1	20.5*	44.7	

% aqueous ionic liquid implies the ionic liquid content in the aqueous solution

The * indicates values determined using mass differences not detected values

Table 2.12: Ionic liquid treatment of hardwoods

Biomass	Treatment conditions	Solid recovery (%)	Glucan yield (%)	Glucan digestibility (%)	Extracted Xylan (%)	Extracted lignin (%)	References
Willow (hardwood)	80 % aqueous [EMIM]OAc, 5 % solid-liquid loading, 120 °C, 22 h	70.8	78.4	~60	61.9*	17.4*	Brandt et al., 2011
Maple wood flour	[EMIM]OAc, 5 % solid-liquid loading, 110 °C, 1.5 h	80	84.5	90	15.8*	44	Lee et al., 2009
	[EMIM]OAc, 5 % solid-liquid loading, 130 °C, 1.5 h	73	84.5	95	26.3*	63	
	[EMIM]OAc, 5 % solid-liquid loading, 90 °C, 1.5 h	83	87.4	80	12.3*	28	
	[EMIM]OAc, 5 % solid-liquid loading, 90 °C, 5 h	80	85.4	91	14.0*	42	
	[EMIM]OAc, 5 % solid-liquid loading, 90 °C, 8 h	81	85.4	91	12.3*	44	
Maple wood flour	[EMIM]OAc, 33 % solid-liquid loading, 125 °C, 1 h	82.8	85.6	~74	23.5*	13.5	Wu et al., 2011
Poplar	[EMIM]OAc, 33 % solid-liquid loading, 125 °C, 1 h	87.4	90.4	~68	16.2*	10.1	
Red oak	[EMIM]OAc, 5 % solid-liquid loading, 110 °C, 16 h	—	—	—	—	34.9*	Sun et al., 2009
Maple wood flour	[BMIM]OAc, 5 % solid-liquid loading, 90 °C, 6 h	—	—	64	—	25	Doherty et al., 2010
	[BMIM]OAc, 5 % solid-liquid loading, 90 °C, 12 h	—	—	74	—	35	
	[BMIM]OAc, 5 % solid-liquid loading, 90 °C, 24 h	—	—	74	—	49	
	[EMIM]OAc, 5 % solid-liquid loading, 90 °C, 6 h	—	—	59	—	26	
	[EMIM]OAc, 5 % solid-liquid loading, 90 °C, 12 h	—	—	65	—	32	
	[EMIM]OAc, 5% solid-liquid loading, 90°C, 24h	—	—	70	—	37	
Eucalyptus	[Ch] [Arg], 6.7 % solid-liquid loading, 90 °C, 12 h	65.5	82.9	58.0	38.3*	54.7	An et al., 2015

% aqueous ionic liquid implies the ionic liquid content in the aqueous solution

The * indicates values determined using mass differences not detected values

2.3.4. Separation methods in lignocellulose fractionation

The main challenge in the selected fractionation methods (organosolv and ionic liquids) is obtaining separate, high purity streams of lignin and hemicellulose from their liquid mixture. In both processes, the cellulose rich residue is separated from the liquid hemicellulose-lignin mixture by filtration (Pan et al., 2005; Zhao et al., 2009; Lan et al., 2011, Yang et al., 2013; Xu et al., 2013). There are several methods which can be used to obtain hemicellulose fractions with minimal degradation from the hemicellulose-lignin liquid mixture, including: (i) miscible organic solvent precipitation of hemicellulose, (ii) membrane technology, (iii) size exclusion chromatography (or anion exchange chromatography), and (iv) sub/supercritical anti-solvent precipitation of hemicellulose.

The miscible organic solvent precipitation has been applied mainly with ethanol, but methanol, acetone, and other organic solvents have been used (Ren and Sun, 2010; Lan et al., 2011; Yang et al., 2013; Xu et al., 2013). However, this precipitation is not very efficient as some of the hemicellulose is not recovered, the improvement can be achieved through slow precipitation and adding several volumes of organic solvents (Ren and Sun, 2010; Lan et al., 2011; Yang et al., 2013; Xu et al., 2013). As mentioned, the precipitation pH has to be near neutral (≥7.00) to ensure that hemicelluloses are not degraded.

The liquid solution of hemicellulose-lignin can undergo treatments like (i) microfiltration, which removes high molecular species, (ii) ultrafiltration, which pre-concentrates hemicellulose, (iii) diafiltration (or nanofiltration), which gives separate streams of hemicellulose and lignin, or (iv) reverse osmosis (Ren and Sun, 2010; Toledano et al., 2010; Peng et al., 2012). The membranes act as selective barriers (are permeable for some components and impermeable for some) and their efficiencies depend on configuration and molecular weight cut-off, temperature and pressure (Ren and Sun, 2010; Toledano et al., 2010). The membrane methods with their applications and conditions are shown in Table 2.13.

In their work (Alriols et al., 2010) used a set of tubular ceramic membranes with cut-offs of 5-15 kDa in separation of organosolv black liquor, obtaining four fractions (those less than 5 kDa, 5-10 kDa, 10-15 kDa and above 15 kDa).

Table 2.13: Common applications and operating conditions of different membrane separation techniques (Toledano et al., 2010)

Process	Membrane type and pore size	Membrane material	Driving force (bar)	Applications
Microfiltration	Symmetric microporous (0.1-10μm)	Ceramics, metal oxides (aluminium-, titanium-, zirconium-), graphite, polymers (cellulose nitrate or acetate, PVDF, polyamides, polysulfone, PTFE)	1-5	Sterile filtration, clarification
Ultrafiltration	Asymmetric microporous (1-10nm)	Ceramics, polysulfone, polypropylene, nylon 6, PTFE, PVC, acrylic copolymer	1-10	Separation of macromolecu-lar solutions
Nanofiltration	Thin-film membranes	Cellulosic acetate and aromatic polyamide	10-30	Removal of hardness and desalting
Reverse osmosis	Asymmetric skin-type (0.5-1.5nm)	Polymers, cellulosic acetate, aromatic polyamide	Up to 200	Separation of salts and microsolutes from solutions

Anion exchange chromatography is a method based on the ion exchange mechanisms, and is more likely to give pure hemicellulose fraction (Peng et al., 2012). The chromatographic methods (anion exchange) are good in removal of non-polysaccharide substances like lignin and lipophilic extractives (Willför et al., 2008). The other chromatographic method that can be used to fractionate or separate the hemicellulose-lignin mixture is size exclusion chromatography or gel permeation chromatography, based on the molar mass and hydrodynamic volume (Willför et al., 2008).

Supercritical carbon dioxide, which has attractive physic-chemical properties at critical temperature and pressure, has gained attraction as alternative solvent for precipitating

biopolymers like hemicellulose and proteins (Haimer, et al, 2008; 2010). One of the commonly used hemicellulose solvents is DMSO, which has been found suitable for several hemicellulose purification protocols (Ebringerova and Heinze, 2000; Sun and Tomkinson, 2003; Sun et al., 2005; Xu et al., 2008). DMSO solvent and supercritical carbon dioxide (green anti-solvent) are used in precipitation of hemicellulose, but the mass transfer resistance can be increased by using DMSO/water mixtures instead of pure DMSO (Haimer et al., 2010). In this precipitation, the factors which can be varied to give desired type of hemicellulose are DMSO: water proportion, precipitation pressure and temperature (Haimer et al., 2010).

2.4. Study motivation

Economic and environmental challenges related to fossil fuels are dominant, and use of biomass has potential to eradicate the current challenges. Most attempts in addressing petroleum related problems using biomass have been focused on biofuels only, which under-utilises the biomass. Biorefineries are possible solution to these problems.

In biofuels production from lignocellulose, which has been studied extensively, the main objective of pre-treatment was to recover highly digestible solids with minimal degradation (inhibitory) products, but not paying attention to the quality of other lignocellulose components. In biorefineries, which focuses not only on biofuels production but utilisation of other lignocellulose components as well, the pre-treatment/fractionation methods need to be reconsidered in order to isolate and preserve hemicelluloses and lignins of good quality, along with production of cellulose-based products. Near-neutral and alkaline fractionation methods; like auto- and base-catalysed organosolv, ionic liquid fractionation, and alkaline extraction, need to be optimised for various types of feedstocks and high extraction and separation efficiencies.

As mentioned in Chapter 1, organosolv process has already been optimised, and tested at pilot and commercial scale. However, ionic liquid fractionation is recent, and has some problems limiting its application in biorefineries. The challenges facing scale up of ionic liquid fractionation method is cost of solvent and high viscosity (which presents mass transfer limitations). Often researchers used very low solids loading (≤5 %), as shown in Section 2.3.3. Low solids loading affect economic viability of ionic liquid fractionation process. Use of various co-solvents, in order address current challenges is being explored. However,

optimisation of fractionation using ionic liquids with co-solvents has not been extensively studied for different types of feedstocks, and has not been benchmarked with other fractionation methods, which are used at pilot and commercial scale biorefineries.

The current challenges motivated for ionic liquid fractionation optimisation in this study. Water was selected as co-solvent (as it is cheaper when compared to organic solvents), in order to reduce cost of fractionation.

2.5. Research aims and objectives

The aim of the study was to compare organosolv and ionic liquid fractionation methods on SCB and *E. grandis*, in terms of extraction and separation efficiencies, as well as operational challenges. With the study of the effects of alkaline extraction of hemicellulose on efficiencies of organosolv and ionic liquid fractionation methods for both feedstocks being considered.

The objectives of the study included:

i. To study the effects of coupling organosolv fractionation with alkaline extraction on SCB and *E. grandis*, based on yield, purity and quality of fractions.
ii. To study the effects of coupling ionic liquid fractionation with alkaline extraction on SCB and *E. grandis*, based on yield, purity and quality of fractions.
iii. To assess the efficiencies of organosolv and ionic liquid fractionation methods (with or without alkaline extractions) on SCB and *E. grandis*, in terms of mass closure.

The key deliverable of the study was to select the best method which is suitable for application in biopolymer-based biorefineries. The parameters used to assess the method included high yield, purity and quality fractions, scalability and operational costs. Alkaline extractions were expected to extract large quantities of polymeric hemicellulose (preservation of hemicellulose). Organosolv was expected to yield high quality lignin and digestible cellulose. Coupling organosolv with alkaline extractions was expected to improve hemicellulose preservation. Ionic liquid fractionation was also expected to yield highly digestible cellulose and high quality. Coupling ionic liquid fractionation with alkaline extractions was expected to improve hemicellulose preservation.

The experimental framework of this study involved use alkaline extraction of hemicellulose, organosolv fractionation and ionic fractionation methods on local feedstocks. The next

chapters will look into materials, methods and equipment used to carry out the experiments. While Chapters 4 and 5 will present and discuss the results, in particular organosolv and ionic liquid fractionation methods will be comprehensively compared.

3. Experimental Design, Materials and Methods

3.1. Introduction

The characterisations of lignocellulosic feedstocks and fraction (obtained from fractionation processes) were carried out using the methods outlined in section 3.2. The sample preparation was done prior to compositional analysis and fractionations. The methodology overview for the fractionation processes are shown in Figure 3.1.

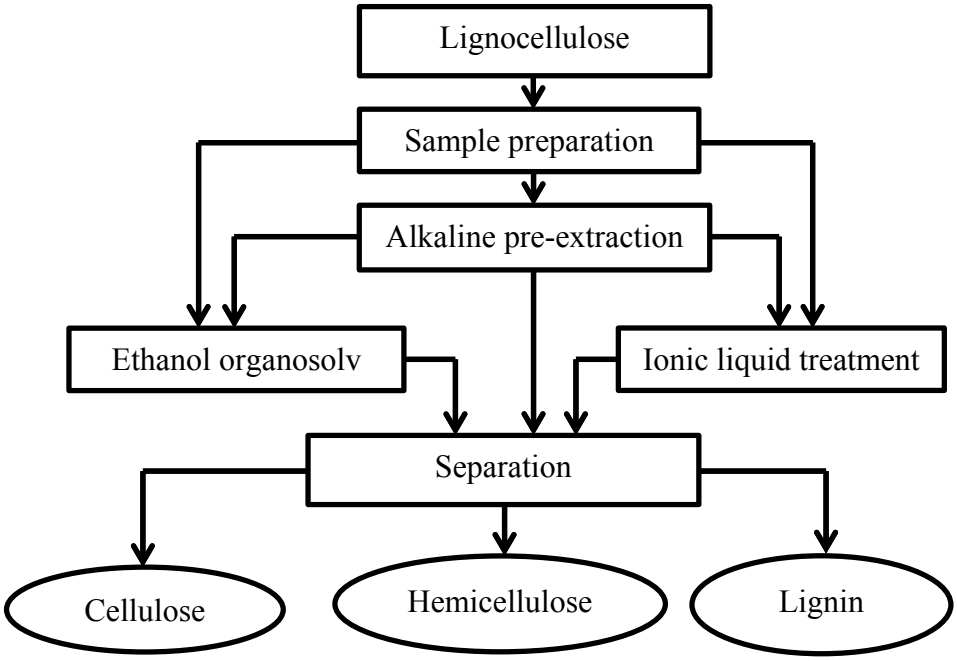

Figure 3.1: The experimental process overview

3.2. Materials and Methods

3.2.1. Materials

SCB was supplied by TSB Sugar, Mpumalanga Province, and *E. grandis* wood chips were obtained from 11-year-old *E. grandis* plantation in Tzaneen in South Africa. Lignocellulosic biomass samples were subjected to air-drying to moisture content below 10 % (w/w). The SCB was shaken to remove some sand particles. To ensure removal of sand from the bagasse, two washing approaches were employed and compared. The first washing approach (W1) involved washing before size reduction, while second washing approach (W2) involved washing after size reduction. Both feedstocks were taken through similar steps of size reduction. The samples were milled to ≤4 mm particle size using Retsch ZM100 Pilot-plant scale mill, and then screened to obtain 0.425 to 0.850 mm particle sized material using

Vibratory Shaker Retsch AS200 equipped with sieves. The oversized material was further milled using Laboratory scale Retsch ZM200 mill, and screened again.

The chemicals which were used to carry out this study are presented in in Table 3.1. Enzymes coacktails which were used are: Optiflow cellulase cocktail of 150 FPU from Genencor, and β-glucosidase (Novozyme 188) of 695 IU from Novozyme.

Table 3.1: Summary of chemicals used

Name of chemical	**Purity of chemical (%)**	**Supplied by**
Cellobiose	≥99	Sigma-Aldrich
Glucose	≥99	Sigma-Aldrich
Xylose	≥99	Sigma-Aldrich
Arabinose	≥99	Sigma-Aldrich
Mannose	≥99	Sigma-Aldrich
Galactose	≥99	Sigma-Aldrich
Potassium hydroxide pellets	≥85	Sigma-Aldrich
Sulphuric acid	72	Sigma-Aldrich
Hydrochloric acid	37	Sigma-Aldrich
Perchloric acid	60	Sigma-Aldrich
Sodium hydroxide pellets	≥97	Sigma-Aldrich
Citric acid	≥99.5	Sigma-Aldrich
Sodium citrate	≥99	Sigma-Aldrich
([BMIM]$MeSO_4$)	≥96	Sigma-Aldrich
([EMIM]OAc)	≥90	Sigma-Aldrich
Beechwood xylan	≥90	Sigma-Aldrich
Ethanol	≥96	Kimix Chemical and Laboratory Suppliers
Acetone	≥99.5	Kimix Chemical and Laboratory Suppliers
Nitrogen gas	≥99.5	Afrox

3.2.2. Methods

3.2.2.1. Determination of moisture content

The moisture content of feedstocks and fractionation residual solids was determined according to the National Renewable Energy Laboratory Analytical Procedure 510-42621 NREL/TP (Sluiter et al., 2008d). The air-dried and conditioned samples, as well as oven-dried fractionation residual solids were weighed into pre-dried and pre-weighed glass vessels. The vessels with the samples were then placed in an oven at 105 °C overnight, and the mass of vessels with dry samples were recorded (Sluiter et al., 2008d). The moisture content of the samples was calculated using Equation 3.1.

$$Moisture\ content\ (\%) = \frac{M_W - M_D}{M_D} \times 100 \qquad \text{Equation 3.1}$$

Where M_W is mass of sample before drying and M_D is mass of sample dried at 105 °C.

3.2.2.2. Determination of ash content

The ash content of the feedstocks and fractionation residues (products) were determined using NREL/TP-510-42622 (Sluiter et al., 2008b). The samples were weighed into pre-weighed ashing crucibles, and then placed in Gallenkamp Muffle Furnace at 575 ± 25°C for four hours (Sluiter et al., 2008b). The crucibles were cooled in the desiccator and weighed (Sluiter et al., 2008b). The ash content of the samples was calculated Equations 3.2.

$$Ash\ (\%) = \frac{M_{Ash}}{M_{ADW}} \times \frac{M_{AR}}{M_D} \times 100 \qquad \text{Equation 3.2}$$

Where M_{Ash} is mass of ash, M_D is mass of oven dried sample (105 °C), M_{ADW} is mass of air dried sample, and M_{AR} is mass of sample as received.

3.2.2.3. Determination of extractives content

The extractives (water and ethanol soluble) in the biomass were determined using NREL/TP-510-42619 procedure (Sluiter et al., 2008a). The lignocellulosic biomass samples of 5.000 ± 0.001g were weighed into cellulose extraction thimbles (Sluiter et al., 2008a). The extraction solvents (distilled water or ethanol) of the volume of 190 ± 5 mL were added into pre-weighed receiving flasks of the Soxhlet apparatus, which refluxed for 24hours (Sluiter et al., 2008a). Following the extraction, the evaporation of the solvents in the flasks to dryness was performed, flasks dried at 105 °C for 4hours and weighed (Sluiter et al., 2008a). The extractives in the biomass samples calculated using Equation 3.3.

$$\%\ Extractives = \frac{M_{Extr}}{M_{ODW}} \times 100 \qquad \text{Equation 3.3}$$

M_{Extr} is the mass of the extractives in the flasks and M_{ODW} is mass of oven dried sample.

3.2.2.4. Determination of carbohydrates and lignin content in the solids

The carbohydrates (monomeric sugars) and lignin content in the feedstocks and fractionation residual solids were done as per the NREL/TP-510-42618 method (Sluiter et al., 2012). The 0.30 ± 0.01 g dry extractives-free biomass samples or fractionation residual solids were weighed, and hydrolysed with 3 mL of 72 % sulphuric acid at 30 °C (in the water bath) for 60 minutes with stirring every 10minutes (Sluiter et al., 2012). The dilution of the acid to 4 % by adding 84 mL of distilled (deionised) water was done following 60minutes hydrolysis, and those were autoclaved at 121 °C for 60 minutes (Sluiter et al., 2012). For extracted hemicelluloses, 1 M sulphuric acid with 90 minutes autoclaving was used. To determine insoluble lignin, the gravimetric approach (which considers ash-free insoluble lignin) was used as adapted from NREL/TP-510-42618 method (Sluiter et al., 2012). The acid soluble lignin in the hydrolysate was determined using ultra violet-visible (UV-Vis) spectrophotometric method at 240 nm within the 6 hours after acid hydrolysis (Sluiter et al., 2012). For sugar analyses, the hydrolysates pH values were corrected to the ranges of pH 2-6 (which was desirable range for HPLC) using 7 N potassium hydroxide (KOH), and filtered through 0.22 µm nylon filters prior to analyses. The HPLC system used was consisting of BioRad Aminex HPX-87 column, which was fitted with Cation-H Micro-Guard Cartridge and refractive index detector. The column operating conditions were 0.6 mL/min flow rate, 5 mM H_2SO_4 mobile phase and 65 °C temperature. The concentrations of sugars were determined using the standards calibration curves.

3.2.2.5. Determination of sugars, degraded products and solubilised lignin in liquid fractions

In order to determine monomeric sugars and degraded products in the liquid fractions, the samples were analysed for sugars according to the method highlighted by Brandt et al. (2011). The liquid fractions were then acid hydrolysed using NREL/TP-510-42623 (Sluiter et al., 2008c), but the autoclaving time was reduced to 30 minutes, and analysed for sugars. 60 minutes autoclaving resulted in sugar degradation, so 30 minutes autoclaving was considered (Gomes, 2012). The difference between the sugars in acid hydrolysed and non-hydrolysed liquid samples was used to approximate the effect of fractionation method in producing monomeric and polymeric carbohydrates (Brandt et al., 2011). The solubilised lignin was

determined following NREL/TP-510-42618 method (Sluiter et al., 2012). However, for ionic liquid fractionations, the lignin content was determined using precipitation as per the method by Diedericks et al. (2012b), as ionic liquids interact with the UV-Vis absorption of lignin (Kline et al., 2010).

3.2.2.6. Enzymatic hydrolysis of biomass

The enzymatic hydrolysis of both the treated and untreated biomass was carried out using the NREL/TP-510-42629 method (Selig et al., 2008). The enzyme to biomass (dry weight basis) loading used was 30 FPU/g of glucan (Diedericks et al., 2012b). The glucan content was based on the glucose content of the biomass samples. The cellulase (Optiflow) was further enriched with Novozyme 188 β-glucosidase, in a 1:10 volume ratio with Optiflow. The enzymatic hydrolysis was carried out in 100 mL sealed Erlenmeyer flasks, with the total liquid volume of 50 mL, at 50 °C, at 150 rpm shaking, and for 72 hours. The 50 mL liquid volume consisted of moisture in biomass samples, cellulase, β-glucosidase, sodium azide solution and 0.05 M citrate buffer of pH 4.8. After 72 hours, the samples were prepared for HPLC by centrifuging 2 mL of sample at 14000 rpm for 5 minutes. Following centrifugation, 200 µL of sample, 600 µL of deionised water and 109.8 µL perchloric acid (PCA) of 35 %v/v were mixed and incubated in ice for 10 minutes. 99 µL of 7 N KOH was then added to the mixture, which was mixed and incubated in ice overnight. On the following day, the mixture was centrifuged at 14000 rpm for 2 minutes, and filtered into HPLC vial using 0.22 µm syringe filter. The glucan digestibility was calculated using equation 3.4.

$$Digestibility\ (\%) = \frac{Glucose\ relesed\ in\ enzymatic\ hydrolysis}{Glucose\ added} \times 100 \qquad \text{Equation 3.4}$$

3.2.2.7. Determination of molecular weights of hemicelluloses using size exclusion chromatography

The molecular weights of hemicellulose (commercial and extracted) were determined with size exclusion chromatography, using HPLC. The hemicelluloses were dissolved in water to give a concentration of 1 g/L, and the dissolution was assisted by stirring at 30 °C (Gomes, 2012). The hemicelluloses solutions were filtered through 0.22 µm syringe filters then analysed in HPLC. The HPLC column used consisted of Suprema column configuration of one Suprema 30 column and two Suprema 3000 columns in series, and ELSD detector. 10 µL of hemicellulose sample was injected, and the eluent used was deionised water, at 1 mL/min

rate. The HPLC operation temperature was 30 °C. Pullulan (type of glucan) standards for molecular weight determination were used.

3.2.2.8. Determination of sugar composition of hemicelluloses using Gas chromatography-mass spectroscopy (GC-MS)

The sugar compositions of hemicelluloses (commercial and extracted) were determined using GC-MS. Acidic methanolysis of hemicellulose samples was carried out using methanolysis reagent (MeOH 0.5 M HCl) so as to hydrolyse polysaccharides into neutral and acidic monosaccharides, then methanolysis into their corresponding methyl glycosides (Ayestarán et al., 2004; Pati et al., 2010; Ruiz-Matute et al., 2011). MeOH 0.5 M HCl was prepared by adding 1400 µL acetyl chloride to 5 mL anhydrous methanol (Ayestarán, Guadalupe and León, 2004). Dried hemicellulose samples (10 mg) and 0.025 mg ribitol (internal standard) were treated with 5 mL methanolysis reagent at 80 °C for 18 hours (Ayestarán et al., 2004; Pati et al., 2010; Ruiz-Matute et al., 2011). Following methanolysis, the remaining methanolysis reagent was removed using stream of nitrogen gas (Ayestarán, Guadalupe and León, 2004; Pati et al., 2010). The sugar standards (0.01, 0.02, 0.05, 0.1 and 0.5 mg/mL) were prepared from 1 mg/mL stock solution. The stock solution consisted of 10 mg D(+) glucose, 10 mg D-galactose, 10 mg D-mannose, 10 mg D(+) xylose and D(-) arabinose, all dissolved in 10 mL anhydrous methanol. Non-acid methanolysis of sugar standards with 0.025 mg ribitol (internal standard) was carried out the same conditions as acidic methanolysis. The oximation of glycosides of hemicellulose samples and sugar standards was carried out by adding 100 µL methoxyaminhydrochloride (20 mg/mL in pyridine) to each sample and standards, then heating at 40 °C for 2 hours (Ndimande, 2014). The samples and standards were then derivatised by adding 150 µL of *N,O-bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and heating at 37 °C for 30 minutes (Ndimande, 2014). The derivatised samples and standards then transferred into GC vails with insert. 10 µL of sample was injected onto GC-MS column. The GC consisted of 30 m Rtx®-5Sil MS column (RESTEK) with Integra guard of 0.25 mm inner diameter and 0.25 mm film thickness (Ndimande, 2014). The GC-MS system consisted of an AS 2000 autosampler, trace GC and the quadropole trace MS (ThermoFinnigan) (Ndimande, 2014). The sample flow rate was 1 ml/min. The injection temperature was 230 °C and the ion source temperature was set at 200 °C (Ndimande, 2014). The recording of mass spectra was done at two scans per sec with the scanning range of 500-600 m/z (Ndimande, 2014). The chromatograms and mass

spectra were evaluated using Xcalibar software bundle version 1.2 (Finnigan Corporation 1998-2000) and eluting compounds were identified using NIST library (www.nist.gov) (Ndimande, 2014). The concentrations of different sugars were calculated from the calibration curves prepared from the sugar standards.

3.2.2.9. Structural characterisation of feedstocks and all fractions (cellulose, hemicellulose and lignin) using Fourier-Transformer Infrared (FT-IR) spectroscopy

Lignocellulosic feedstocks, cellulose-rich fractions and hemicelluloses were ground into fine powders and dried in the oven overnight. The samples were structurally characterised on Thermo Nicolet Nexus 870 FT-IR device with the Golden Gate ATR measuring system. The absorbance frequency range of FT-IR considered was 4000-400 cm^{-1}. The spectra were recorded in the Omnic ® 7 software and for each run (background and sample) 64 scans were run. Lignin samples were run in Thermo Nicolet (Nexus 870 FT-IR) Liquid Analysis system with liquid uptake module. Similar absorbance frequency range of FT-IR as with solid state was used and similar number of scans. The bands' assignment for FTIR spectra of lignocellulose components is shown in Table 3.1 below.

Table 3.2: The FTIR bands' assignment

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, xylans 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)
1630-1695	Conjugated C=O stretch of acetyl, ester or carboxylic acid groups
1730	Unconjugated C=O stretch of acetyl, ester or carboxylic acid groups
2900	C-H stretch vibration
3380	O-H stretch vibration

(Adapted from Kline et al., 2010; Ren and Sun, 2010; Hu et al., 2012; Karatzos et al., 2012b; Sun et al., 2012; Wang et al., 2012a; Sun et al., 2014)

3.2.2.10. Determination of lignin's monomer composition (Thioacidolysis and GC-MS)

The determination of lignin's monomer composition was carried out using method developed by Robinson and co-worker, and detailed by Foster and co-workers (Robinson and Mansfield, 2009; Foster et al., 2010). 0.02 g of either extractives free feedstocks or lignin samples was weighed into a screw capped tube for Thioacidolysis. 2000 µL of dioxane solution (which

contained 10 % ethanethiol and 2.5 % boron trifluoride diethyl etherate) was poured into each sample, and headspace purged with nitrogen gas then immediately covered. The sample mixtures were heated at 100 °C for 4 hours, with mixing every hour. The sample mixtures were then cooled in ice bath for 5 minutes, followed by addition of 1500 µL of 0.4 M sodium bicarbonate and vortex. 10 mL of water and 5 mL of ethyl acetate were then added to each sample mixture, vortex and phase separation followed. 150 µL of ethyl acetate layer was transferred into 2 mL glass vial and air evaporated. Two washes with 200 µL of acetone followed. Once dried, derivatisation was done by adding 500 µL ethyl acetate, 20 µL of pyridine and 100µL *N,O-bis*(trimethylsilyl) acetamine to each sample, then incubated for 2 hours at 40 °C. After derivatisation, 100 µL of the sample was into GC-MS vial and 100 µL of acetone added, followed by GC-MS analysis. The GC consisted of 30 m Rtx®-5Sil MS column (RESTEK) with Integra guard of 0.25 mm inner diameter and 0.25 mm film thickness (Ndimande, 2014). The GC-MS system consisted of an AS 2000 autosampler, trace GC and the quadropole trace MS (ThermoFinnigan) (Ndimande, 2014). One microlitre was injected with a splitless injection and the flow rate was 1.1 ml/min with a 30 min solvent delay. The temperature of the program was as follows: initial hold at 130 °C for 3 min, a 3 °C/min ramp to 250 °C and hold for 1 min to allow equilibration to the initial temperature of 130 °C. The peaks were identified by mass spectrum ions of 299 m/z, 269 m/z and 239 m/z for syringyl, guaiacyl and p-hydroxyphenol units respectively.

3.2.3. Fractionation (treatment) of lignocellulosic feedstocks

The quantitative and qualitative methods mentioned in Section 3.2.2 were used to obtain yield, purity, and quality of fractions, as well as in preparation of mass balances. Alkaline extractions and organosolv treatments (which are elaborated in this section) were used to achieve the first and third objectives. Alkaline extractions and ionic liquid treatments were used to achieve the second and third objectives. The objectives were;

i. To study the effects of coupling organosolv fractionation with alkaline extraction on SCB and *E. grandis*, based on yield, purity and quality of fractions.
ii. To study the effects of coupling ionic liquid fractionation with alkaline extraction on SCB and *E. grandis*, based on yield, purity and quality of fractions.
iii. To assess the efficiencies of organosolv and ionic liquid fractionation methods (with or without alkaline extractions) on SCB and *E. grandis*, in terms of mass closure.

Alkaline extractions used lower temperatures, so they were carried out in closed Schott bottles heated in the shaking water baths. However, organosolv and ionic liquid fractionations (treatments) were operated at high temperatures, which also involved pressure build-up, and for those experiments stainless steel tubular reactors were used. The stainless steel reactors consisted of seamless Hastelloy C276 tubes of 11.8 mm inner diameter, 12.7 mm outer diameter and 152.0 mm length (Diedericks et al., 2012b). The tubular reactors were sealed with Swagelok stainless steel end-caps, and the caps were fitted with Teflon plugs to avoid corrosion (Diedericks et al., 2012b). The reactors were heated in fluidised sand baths with temperature control, and the internal temperature of tubular reactors was monitored using temperature probe (thermocouple) (Diedericks et al., 2012b). For the sand baths, the temperature variance from set point was 0.3 °C, and the first sand bath was set 50 °C higher than the second sand bath (Diedericks et al., 2012b). The first sand bath was used to heat reactors to desirable temperature within a short time, while the second sand bath was used as heating medium for entire reaction time.

The lignin-hemicellulose fraction was separated following the alcohol-precipitation method used by Gomes (2012). Prior to precipitation, the pH of the samples was adjusted to near neutral (6-7) using acetic acid. The alcohol precipitation was carried out using 1:3 v/v ratio of lignin-hemicellulose fraction to alcohol mixture (ethanol-methanol-water at 4:5:1 volume ratios), 48 hours precipitation time and 4°C (Gomes, 2012). Precipitated hemicelluloses were filtered and dried, alcohols were recovered using rotary evaporator, and remaining liquor was dried (solid considered as lignin).

3.2.3.1. Alkaline extraction

Alkaline pre-extraction of hemicellulose from the feedstocks was done prior to either organosolv or ionic liquid fractionations. Alkaline pre-extraction of SCB was carried out 1.5 M NaOH solution, with 10 % solids loading, at a reaction temperature of 65 °C and 92 minutes of reaction time (Vena, 2013). The reaction was carried out in closed Schott bottles, in a shaking hot water bath (Vena, 2013). The separation of solids and liquid fractions was done through filtration on 45 µm filter paper under vacuum, and accompanied by four water washes (each wash was equal to the total reaction volume), such that the pH of the solid residues was near neutral (around 7).

For *E. grandis* alkaline pre-extraction, the three level factorial design experiments were carried out in order to get the preferable working conditions, and the experimental design is

shown in Table 3.2. The temperature was fixed at 90 °C, 10 % solids loading was used, sodium hydroxide concentrations of 0.5, 1.0 and 1.5 M were used, reaction times of 4, 5 and 6 hours were used, based on the findings from Vena's work (Vena, 2013). Similar reaction and separation setups as those used in alkaline pre-extraction of SCB were used. Following the factorial design experiments, the pre-extraction of *E. grandis* was carried out at the preferable conditions, which were: 90 °C, 1.5 M NaOH, 10 % solid loading, and 4 hours.

Table 3.3: Factorial design for *E. grandis* alkaline pre-extraction experiments

Run	NaOH conc. (M)	Time (min)
1	0.5	240
2	0.5	300
3	0.5	360
4	1.0	240
5	1.0	300
6	1.0	360
7	1.5	240
8	1.5	300
9	1.5	360

Alkaline post-extraction of hemicellulose was done on the solid residues from both organosolv and ionic liquid treatments of feedstocks (virgin biomass). To 1 g of solid residue from ionic liquid treatments, 30 mL of sodium hydroxide (0.5 M) was added for post-extraction at 80 °C and for 2 hours, the conditions were adapted from several research works (Lan et al., 2011; Sun et al., 2012; da Costa Lopes et al., 2013a; Sun et al., 2013; Xu et al., 2013; Yang et al., 2013). For organosolv treated solids, the alkaline post-extraction of hemicellulose was studied at 0, 0.0625 (SCB only), 0.125 (SCB only), 0.5, 0.75 and 1.0 M NaOH concentrations, all at 80 °C and for 2 hours. In order to compare the effects of coupling organosolv and ionic liquids with alkaline post-extraction of solids, 0.5 M NaOH concentration was used.

The solid residues from both pre-extraction and post-extraction were analysed for moisture content, carbohydrates, lignin, and ash. The wet solid residues were retained (kept at 4 °C), and later enzymatically digested. The liquid fractions were analysed for carbohydrates, lignin and degradation products. Furthermore, the liquid fractions were separated into lignin and

hemicelluloses using alcohol precipitation method. The structural and compositional characterisations of lignin and hemicellulose followed.

3.2.3.2. Organosolv fractionation of virgin and pre-extracted biomass

The organosolv treatment of both virgin and pre-extracted biomass was carried out in stainless steel tubular reactors. The organosolv treatments were carried out using 60 wt.% ethanol solution, 1 g of biomass placed in the reactor such that 10 % solids loading was achieved, 200°C reaction temperature and 60minutes reaction time (Huijgen et al., 2012). To allow for proper liquid transfer into the biomass, the weighed solid was soaked in ethanol solution overnight. Following treatment, filtration accompanied by four water washes was done. The solid residues were analysed for moisture content, carbohydrates, lignin, and ash. The wet solid residues were retained (kept at 4 °C), and later enzymatically digested. The liquid fractions were analysed for carbohydrates, lignin and degradation products. Since lignin and hemicelluloses separation using alcohol precipitation method could not work for organosolv liquid fraction, the stream was not separated further. The solvent was removed and mainly considered as lignin. The structural and compositional characterisations of lignin followed.

3.2.3.3. Ionic liquid fractionation of virgin and pre-extracted biomass

Prior to ionic liquid fractionation of biomass, the screening of ionic liquids was done. The screening was done based on the acidity of aqueous ionic liquid solutions (1 g/L concentration). [BMIM]$MeSO_4$ solutions gave the pH value of 3.78±0.25 and [EMIM]OAc solutions gave the pH value of 6.11±0.15. Since [BMIM]$MeSO_4$ solutions were acidic, only [EMIM]OAc was used in the fractionation experiments.

The ionic liquid fractionation of both virgin and pre-extracted biomass was carried out in stainless steel tubular reactors. 1g of dry biomass was placed into the tubular reactor, followed by addition of ionic liquid solutions such that the solids loading of 10 % was achieved. Prior to capping the reactors, nitrogen gas was purged in. The loaded reactors were then left overnight in upright position in order to allow for diffusion of ionic liquid into the biomass (Diedericks et al., 2012b). The ionic liquid treatment was carried out at the conditions shown in CCD experimental design shown in Table 3.3. Following treatments, the contents of each reactor were transferred into 60mL centrifuge tubes, washed with 30 mL of 50 wt% aqueous acetone mixture (antisolvent) four times accompanied with vortex mixing, centrifuged and filtered (Diedericks et al., 2012b). The acetone was recovered from the liquid

fraction using rotary evaporator, and residual liquid analysed for carbohydrates, lignin and degradation products. The hemicellulose and lignin separation from liquid fraction was done using alcohol precipitation. Lignin remained in ionic liquid solution and was separated using acid precipitation method adapted from previous work in the research group (Diedericks et al., 2012b). The cellulose residue was characterised for moisture content and chemical composition, and the wet solids were taken through enzymatic hydrolysis.

Table 3.4: Central composite design for ionic liquid experiments

Run	Aqueous ionic liquid conc. (%)	Temperature (°C)	Time (min)
1	46.00	100.00	120.00
2	46.00	100.00	300.00
3	46.00	160.00	120.00
4	46.00	160.00	300.00
5	86.00	100.00	120.00
6	86.00	100.00	300.00
7	86.00	160.00	120.00
8	86.00	160.00	300.00
9	32.36	130.00	210.00
10	99.64	130.00	210.00
11	66.00	79.55	210.00
12	66.00	180.45	210.00
13	66.00	130.00	58.64
14	66.00	130.00	361.36
15	66.00	130.00	210.00
16	66.00	130.00	210.00
17	66.00	130.00	210.00
18	66.00	130.00	210.00

The factor variables considered at low and high levels are: temperature (100 ºC and 160 ºC), residence time (120 min and 300 min), and aqueous ionic liquid concentration (46 %w/w and 86 %w/w). 10% solids loading was used. The choice of the levels used was based on literature presented in Section 2.3.3. All central composite designs (CCDs) were carried out using STATISTICA 11. The response variables were solid recovery, glucan (retained and

solubilised), xylan (retained and solubilised), lignin (retained and solubilised), and glucan enzymatic digestibility.

3.2.4. Repeatability and reproducibility

The experimental designs (factorial and central composite design, CCD), and the statistical analysis of the data (ANOVA, Pareto charts, surface response method and desirability) were carried out using STATISTICA 11. The centre points in the CDD were run four times (which means that centre point was replicated three times). The four runs at centre point were used to estimate random error, used in statistical analysis of the CCD experimental results. Three to five runs at centre points are required for CDD experiments (NIST/SEMATECH). The residual errors, pure errors and lack of fit were obtained in the ANOVA analyses, and used foe error analyses. The factorial design experiments, alkaline extractions, organosolv treatments, and ionic liquid desirable conditions were run in triplicates to ensure reliability. Standard deviations were calculated and used. However, the determination of lignin in extracted hemicelluloses (xylan) was carried out in duplicates (due to limited quantity of xylan), so average error was calculated and used.

4. Experimental Results and Discussions

This chapter presents and discuss the results obtained from compositional analysis of the feedstocks, study of alkaline pre- and post-extraction of hemicelluloses, organosolv fractionation, and aqueous ionic liquid optimisation and fractionation experiments. The yields, mass balances, and quality of the fractions, together with operation challenges of the fractionation methods are compared in Chapter 5. Although the two chapters overlap a lot, they have been separated because Chapter 4 is focused on comparing the experimental results obtained from alkaline extractions, organosolv fractionation and ionic liquids to those reported in literature (testing how the extractions and fractionation processes used in this study compares with literature). The results presented in this chapter include preliminary and/or optimisation results, as well testing the fractionation conditions on both SCB and *E. grandis*. Chapter 5 addresses the aim and objectives of this study (comparison of organosolv fractionation and ionic liquid fractionation on both SCB and *E. grandis*), and it is the section, in which the paper will be extracted from, thus leading to separation from Chapter 4.

The method used for determination of carbohydrates detected only monomeric sugars, so xylan was determined as xylose while glucan (cellulose) was determined as glucose. When addressing polymers both xylan and glucan (cellulose) are used.

4.1. Compositional analysis of the feedstocks

The chemical compositions and FTIR analysis results of the feedstocks (SCB and *E. grandis*) are presented in Table 4.1 and Appendix B. The sugar content of both feedstocks was based on the monomeric sugar analysis. The chemical composition of the *E. grandis* sample composition (in Table 4.1) was within the ranges obtained by other researchers. In the literature, cellulose (glucose) content is within the range of 39 to 53 %, xylan is within the range of 11 to 22 %, while lignin is within the range of 21 to 30 % (Baeza et al., 1991; Cotterill and Macrae, 1997; Emmel et al., 2003; Alves et al., 2010; Gomes, 2012; Postma, 2012; Vena, 2013; Joubert, 2015).

The chemical composition of the SCB sample composition (presented in Table 4.1) was within the ranges obtained by other researchers. In the literature, cellulose (glucose) content is within the range of 35 to 45 %, xylan is within the range of 17 to 28 %, while lignin is within the range of 19 to 23 % (da Cunha et al., 2009; Alves et al., 2010; Carrasco et al., 2010; Krishnan et al., 2010; Mesa et al., 2011; Rezende et al., 2011; Diedericks et al., 2012a;

Diedericks et al., 2012b; Gomes, 2012; Zhang et al., 2012; Vena, 2013; Khuong et al., 2014). Both the first wash approach (washing of SCB samples prior to milling) and the second wash approach (washing of SCB after milling) significantly reduced the ash content (sand particles) in the SCB. The ash content after washing (1.55 to 0.98 %) was significantly lower than values of 4 and 4.8 %, measured previously for SCB samples used in the research group (Diedericks et al., 2012a; 2012b; Gomes; 2012).

Table 4.1: Chemical composition analysis results for lignocellulosic feedstocks

Feedstock	Glucose (g/100g)	Xylose (g/100g)	Arabinose (g/100g)	Lignin (g/100g)	Ash (g/100g)	Extractives (g/100g)
SCB (W1)	42.62 ± 1.73	22.79 ± 0.34	2.00 ± 0.16	22.42 ± 0.43	0.98 ± 0.45	5.16 ± 0.30
SCB (W2)	40.70 ± 0.69	20.26 ± 0.39	1.24 ± 0.07	21.17 ± 0.02	1.55 ± 0.07	5.08 ± 0.02
E. grandis	48.18 ± 1.87	13.63 ± 0.66	0.08 ± 0.02	27.48 ± 0.32	0.07 ± 0.02	4.00 ± 0.32

The higher ash in SCB as compared to *E. grandis* was attributed to high silica content in SCBe, and to soil contamination of SCB during harvesting and storage (normally SCB is thrown into piles to dry after sugar extraction) (Sun et al., 2002b). Even after washing, the extractives content in SCB was higher than in *E. grandis* (Table 4.1). The higher extractives might be due to residual sugars (sucrose, which are water soluble) after sugar extraction in sugar mills which are water soluble (Li et al., 2011b). Hemicellulose content in SCB is higher than in *E. grandis*. The higher hemicellulose content in SCB is a characteristic of grasses or herbaceous plants as compared to woody material (Sun et al., 2002b; Fengel and Wegener, 1989; Ren and Sun, 2010; Vena, 2013).

The lignin content is higher in *E. grandis* (27 %) than in SCB (22 %), and this trend is characteristic of grasses or herbaceous plants (Fengel and Wegener, 1989). The lower lignin content in SCB is attractive for easier delignification at milder processing conditions (Vena, 2013). However, low lignin content alone is not sufficient to achieve easier delignification as other factors like monolignols content, lignin's DP, and lignin-carbohydrate interactions are worth considering (Steward et al., 2006; Vena, 2013). Furthermore, the monolignols content in both feedstocks were determined, and the syringyl to guaiacyl ratios (S/G) were found to be 1.68 and 4.35 for SCB and *E. grandis* respectively. The S/G ratio of 1.68 in SCB is

comparable to the value of 1.79 for SCB (diffent sample from the one used in this study), which was obtained using the same protocol (Ndimande, 2014). For *E. grandis*, the S/G ratio of 4.35 was in the higher end of the range of 1 to 4 for hardwood lignins (Lapierre, 2008; Sannigrahi et al., 2010; Wen et al., 2013a). The S/G ratio of lignin in lignocellulosic biomass presents an essential factor for estimating required processing or pre-treatment conditions, in order to achieve desired delignification (Lupoi et al., 2014). Although the correlation between lignin S/G ratio and enzymatic hydrolysis of polysaccharides has not been established, the observation during pre-treatment of lignocellulosic materials which have high S/G ratios has been release of larger amounts of monomeric sugars (Lupoi et al., 2014).

4.2. Alkaline pre-extraction of hemicelluloses from feedstocks (SCB and *E. grandis*)

4.2.1. Alkaline pre-extraction of hemicelluloses from SCB

The chemical compositions of the fractions obtained from alkaline pre-extraction of SCB (W2) are shown in Table 4.2. The solid recovery following alkaline pre-extraction (59.3 %) is within the range of 50 to 83 % obtained by other researchers as shown in Section 2.3.1. The solubilised lignin (in Table 4.2) corresponds to 65.72 % of lignin in SCB. The 65.72 % lignin dissolution falls within the range of 24 to 89 % shown in Section 2.3.1. The solubilised xylan (in Table 4.2) corresponds to 71.17 % of xylan in SCB. The 71.17 % xylan dissolution is within the range of 16 to 96 % shown in Section 2.3.1. Furthermore, the 71.17 % xylan dissolution is in agreement with 69.1 % obtained at the similar operating conditions by Vena (2013).

Table 4.2: The chemical compositions of liquid and solid fractions obtained from alkaline pre-extraction of hemicelluloses from SCB

	Alkaline pre-extraction hydrolysate	**Solid residue**	**Mass balance (%)**
Solid recovery (%)		59.31 ± 0.86	
Glucose (g/100g)	0.38 ± 0.02	38.47 ± 1.60	95.5 ± 3.9
Xylose (g/100g)	14.42 ± 1.19	6.27 ± 0.19	100.9 ± 6.5
Lignin (g/100g)	13.92 ± 0.00	8.28 ± 0.30	104.8 ±1.4

As shown in Section 2.3.1, most researchers did not analyse the liquid fraction for solubilised lignin and xylan, so calculated extraction yields based only on the differences in chemical composition between the raw biomass and residual solids. In this study, chemical characterisation of both streams (liquid and solid fractions) allowed for closing the mass balances of the alkaline pre-extraction process, and assessment of possible sugar degradation under extraction conditions. The mass balances of glucan (based on glucose), xylan (based on xylose) and lignin were found to be 95.5±3.9, 100±6.5 and 104.8±1.4 % respectively. The mass balance values obtained in this study corresponded to 94.6, 90.9 and 97.3 % of glucan, xylan and lignin respectively, which were reported by Vena (2013).

The dissolved xylan was tested for degradation as stated in Section 3.2.2.5. There were no monomeric sugars (xylose) detected in the liquid fraction before autoclaving (acid hydrolysis). The zero xylose in liquid fraction indicated that the extracted hemicelluloses (xylan) were mainly in oligomeric and polymeric form. Characterisation of extracted hemicelluloses for molecular weight, sugar composition, and structure (FTIR) were carried out. The average molecular weight of the extracted hemicelluloses was found to be 34298 Da. The molecular weight obtained in this study was similar to those obtained in alkaline extraction of hemicelluloses from SCB (32793, 35200, 35450, 35600, 35820, 36760, and 37430 Da), barley straw and husks (34100, 34220, 35100, 39000 and 40400 Da), and (35540 Da) from rice straw (Sun et al., 2000; Sun, and Sun, 2002; Höije et al., 2005; Xu et al., 2006; Gomes, 2012; Vena, 2013).

The neutral sugar composition of the extracted hemicellulose (in relative percentages), was calculated based on the total detected neutral sugars shown in Appendix C. The neutral sugar composition is shown in Figure 4.1. Xylose was the dominant neutral sugar (75 % of neutral sugars), followed by arabinose (21 %), glucose and galactose (each being 2 %). The trend of high xylose content in the alkaline extracted hemicelluloses followed by arabinose is common for hemicelluloses from herbaceous biomass and agricultural residues, whereby xylose ranging from 50 to 97 % and arabinose ranging from 3 to 17 % have been observed (Sun et al., 2002a; Höije et al., 2005; Xu et al., 2006b; Peng et al., 2009; Chimphango, 2010; Peng et al., 2010a; Gomes, 2012). The low content of residual glucose is within the range of 0.02 to 27 %, which have been obtained in alkaline extracted hemicelluloses from herbaceous and agricultural residues (Sun et al., 2002a; Höije et al., 2005; Xu et al., 2006b; Peng et al., 2009; Chimphango, 2010; Peng et al., 2010a; Gomes, 2012). The acid insoluble lignin

content of SCB xylan (NaOH pre-extracted) was determined gravimetrically (masses shown in Appendix C), and was found to be 12.03±0.02 %. The lignin content obtained is within the range of 1.4 to 16 %, which were found in alkaline extracted xylan from herbaceous and agricultural residues (Sun et al., 2002a; Höije et al., 2005; Xu et al., 2006b; Peng et al., 2009; Chimphango, 2010; Peng et al., 2010a; Gomes, 2012; Vena, 2013).

The neutral sugar composition of commercial Beechwood xylan is shown in Figure 4.1. The xylose (88 %) and glucose (4 %) content (obtained in this study) were found to be similar to 89 % and 1.6 % for Beechwood xylan, which were measured previously in the research group (Gomes, 2012). The Beechwood xylan was reported to have 7.72% uronic acid and 3.57% lignin (Gomes, 2012). The SCB xylan (NaOH pre-extracted) has higher arabinose content than Beechwood xylan. This trend (high arabinose in SCB xylan) is related to the type of biomass, and has been observed in literature (Sun et al., 2002a; Höije et al., 2005; Xu et al., 2006b; Peng et al., 2009; Chimphango, 2010; Peng et al., 2010a; Gomes, 2012).

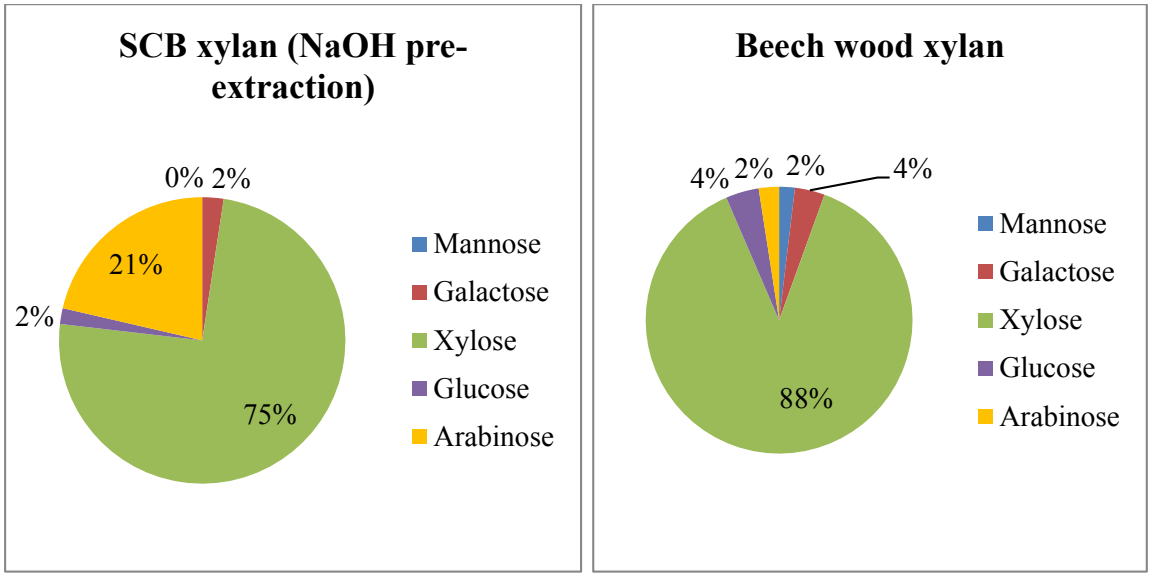

Figure 4.1: Neutral sugar compositions of SCB xylan (NaOH pre-extraction) and Beechwood xylan (commercial)

Both Beechwood xylan and SCB xylan (NaOH pre-extraction) have pyranose ring stretch vibrations (as shown in Appendix B), which indicates the presence of hemicelluloses, mainly xylans. The xylans also had β-glycosidic linkages between sugar monomers in the hemicellulose, as well as lignin, and the observation from FTIR is in agreement with compositional analyses of xylans.

As shown in Section 3.2.3, the soluble lignin fraction in the liquid product was obtained as a residue from alcohol precipitation of hemicelluloses, and was considered to be crude lignin. The S/G ratio of the lignin was found to be 1.81. There is slight increase in the ratio when compared to the feedstock (S/G ratio of 1.68). The slight increase in S/G ratio of the extracted lignin implies that lignin in SCB was reactive to degradation/cleavage reactions taking place during extraction (Wang et al., 2012b).

The FTIR analysis SCB lignin (NaOH pre-extracted), is shown in Appendix B. The recovered lignin was oxidised (conjugated carboxylic acids or ester groups), but had negligible carbohydrates contamination. Low carbohydrates contamination confirms that alcohol precipitation of hemicelluloses was effective (almost all hemicelluloses were precipitated). The intensity of aromatic ring vibrations indicates that the alkaline pre-extraction enhanced lignin separation from other components of the lignocellulosic biomass.

NaOH pre-extraction of SCB produced solid residues which had 94.83 % glucan recovery (in relation to glucan in the virgin SCB). This glucan recovery is within the range of 70 to 98% mentioned in Section 2.3.1, and is in agreement with the one reported by Vena (2013) of 94.6% (which was obtained at the similar conditions). Alkaline pre-extraction is therefore attractive; as it does not solubilise significant amounts of cellulose (minimum cellulose loses).

Following wet chemistry, the solid residue was further structurally characterised using FTIR and its enzymatic digestibility was also determined. The glucan digestibility of the solid residue was found to be 80.14 %. Glucan digestibilities ranging from 64 to 90 % have been reported for solid residues after alkaline treatments of herbaceous lignocellulose or agricultural residues (Li et al., 2012; Yang et al., 2012; Jin et al., 2013; Eliana et al., 2014; Kim et al., 2014a; Kim et al., 2014b; Liu et al., 2014; Toquero and Bolado, 2014). The glucan digestibility of 80.14% measured here is reasonable for biofuels and/or high value chemicals production, as compared to ≥80% which is considered as high cellulose digestibility (Yang and Wyman, 2008; Alvira et al., 2010; da Silva et al., 2013). Therefore, alkaline pre-extraction can be considered as a good fractionation method for SCB and/or other agricultural residues.

The FTIR analysis of alkaline pre-extracted solid residue (shown in Appendix B) indicates that SCB was deacetylated and delignified during alkaline pre-extraction of hemicelluloses.

Significant hemicellulose dissolution was also observed, and cellulose peaks were more intense (indicating that the material was cellulose rich).

Alkaline pre-extraction of SCB solubilised significant amount of polymeric hemicellulose (71% xylan solubilisation). Minimum xylan degradation (6% of xylan in virgin SCB) was observed. High extraction and preservation of hemicelluloses (xylan) indicate that alkaline pre-extraction is effective for herbaceous/agricultural residues (SCB). Furthermore, high cellulose digestibility (80%) and high delignification (66%) coupled with hemicellulose extraction, improve the effectiveness of alkaline pre-extration method. Therefore, alkaline pre-extraction can be intergrated in biorefineries.

4.2.2. Alkaline pre-extraction of hemicelluloses from *E. grandis*

4.2.2.1. Factorial experimental design and analysis for alkaline pre-extraction of hemicelluloses from *E. grandis*

In literature, alkaline pre-extractions of hemicellulose from *E. grandis* have been limited because the pulps were to be feedstocks to paper production (Vena, 2012). NaOH concentration was found to be most significant factor on xylan solubilisation, followed by reasidence time, while reaction temperature was the last factor (Vena, 2013). In this study, the alkaline pre-extraction of *E. grandis* was studied further using 3^2 factorial experimental design, and results are shown in Table 4.3. Temperature was fixed at 90°C, while the levels of NaOH concentration (0.5, 1.0 and 1.5M) and reaction time (240, 300 and 360min) were used, and conditions were adapted from (Vena, 2013). Low NaOH concentractions were considered in order to ensure that the extractions are mild (to minimise sugar degradation and loses), while residence times were chosen such that longer times (8-24 hours) are avoided and shorter times (<4 hours, Vena's best residence time) are avoided as at the used conditions xylan extraction was very low.

Table 4.3: The 3^2 factorial experimental design's results for alkaline pre-extraction of *E. grandis*

Pre-extraction conditions		Stream 1 (alkaline pre-extraction hydrolysate)			Stream 2 (solid residue)			Sum of the streams			Overall recovery			Solid recovery
NaOH conc. (M)	Time (min)	Glucose (g/100g)	Xylose (g/100g)	Lignin (g/100g)	Glucose (g/100g)	Xylose (g/100g)	Lignin (g/100g)	Glucose (g/100g)	Xylose (g/100g)	Lignin (g/100g)	Glucose (g/100g)	Xylose (g/100g)	Lignin (g/100g)	(%)
0.5	240	0.13	2.11	6.47	46.61	10.87	22.54	46.75	12.98	29.01	97.03	95.27	105.58	78.69
0.5	240	0.00	2.17	6.55	45.56	10.74	21.92	45.56	12.91	28.47	94.57	94.76	103.60	78.79
0.5	240	0.00	2.23	6.62	45.97	10.93	23.11	45.97	13.16	29.73	95.42	96.61	108.18	78.04
1.0	240	0.00	4.56	6.95	45.87	8.62	22.69	45.87	13.19	29.64	95.20	96.77	107.86	74.73
1.0	240	0.00	4.73	6.93	46.16	8.48	21.44	46.16	13.21	28.37	95.81	96.96	103.22	74.24
1.0	240	0.00	4.89	6.79	46.50	8.48	22.70	46.50	13.37	29.50	96.52	98.14	107.34	74.77
1.5	240	0.00	5.19	5.60	46.07	7.19	23.02	46.07	12.38	28.62	95.63	90.84	104.14	73.86
1.5	240	0.00	3.72	6.98	46.11	7.28	22.89	46.11	11.00	29.88	95.70	80.74	108.72	73.96
1.5	240	0.00	6.87	7.12	46.93	7.18	22.66	46.93	14.05	29.78	97.41	103.14	108.37	73.85
0.5	300	0.00	2.12	5.90	45.10	10.49	23.31	45.10	12.61	29.21	93.61	92.55	106.29	78.25
0.5	300	0.00	2.14	6.44	46.54	10.65	23.03	46.54	12.78	29.47	96.59	93.81	107.26	78.07
0.5	300	0.00	2.16	6.59	45.57	10.28	23.60	45.57	12.44	30.19	94.59	91.29	109.87	78.41
1.0	300	0.00	3.43	4.97	45.14	8.19	22.76	45.14	11.62	27.73	93.70	85.27	100.91	74.34
1.0	300	0.00	4.77	6.63	45.60	8.34	22.99	45.60	13.11	29.61	94.65	96.24	107.76	74.64
1.0	300	0.00	4.51	6.59	32.92	6.19	21.87	32.92	10.70	28.46	68.32	78.53	103.58	74.62
1.5	360	0.00	6.37	6.54	45.51	7.19	22.64	45.51	13.55	29.18	94.45	99.47	106.19	73.26
1.5	360	0.16	6.72	6.85	46.21	7.40	22.42	46.37	14.11	29.26	96.25	103.59	106.49	73.07
1.5	360	0.26	6.81	6.50	44.48	6.94	22.32	44.75	13.75	28.82	92.88	100.92	104.88	73.63
0.5	360	0.00	2.28	6.69	44.42	10.57	21.84	44.42	12.85	28.52	92.19	94.34	103.80	78.12
0.5	360	0.00	2.31	6.43	44.37	10.73	22.37	44.37	13.04	28.79	92.10	95.72	104.78	78.07
0.5	360	0.16	2.19	6.41	38.00	8.88	23.03	38.16	11.06	29.44	79.21	81.20	107.13	78.51
1.0	360	0.00	4.58	6.76	40.78	7.50	21.14	40.78	12.08	27.90	84.65	88.68	101.52	74.37
1.0	360	0.87	5.00	7.29	42.78	7.59	17.37	43.66	12.58	24.66	90.62	92.36	89.74	74.24
1.0	360	0.78	3.61	5.07	32.97	5.89	20.93	33.75	9.50	26.00	70.06	69.75	94.62	74.18
1.5	360	1.16	6.91	6.81	43.54	6.71	21.70	44.69	13.62	28.52	92.76	99.95	103.77	72.88
1.5	360	0.90	6.51	6.78	43.33	6.63	19.99	44.23	13.14	26.78	91.80	96.44	97.44	73.61
1.5	360	0.90	4.72	5.01	42.94	6.81	20.02	43.84	11.53	25.03	91.00	84.62	91.09	73.36

The experimental results obtained were statistically analysed at 95 % confidence level. The major responses which were statistically analysed are solid recovery, extracted xylan (reported as xylose), extracted lignin and glucan in solids (reported as glucose). The glucan in the solid residue was considered important for marking the impact of severity on glucan losses (which are undesirable). The regression coefficients and ANOVA of response surfaces obtained in alkaline pre-extraction of hemicelluloses from *E. grandis* are shown in Table 4.4. NaOH concentration (M) is the first factor (1) while reaction time (min) is second factor (2). The second-order polynomial regression model for alkaline pre-extraction of hemicelluloses from *E. grandis* is shown Equation 4.1 Where Y is the response (solid recovery, xylan solubilised, lignin solubilised and glucan retained in the solids), β_0 is constant coefficient, β_i is linear coefficient and β_{ii} is quadratic coefficient.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 \qquad \text{Equation 4.1}$$

Table 4.4: Regression coefficients and ANOVA of the response surfaces obtained in alkaline pre-extraction of hemicelluloses from *E. grandis*

Coefficient	**Solid recovery**	**Xylan dissolution**	**Lignin dissolution**	**Glucan retention in solids**
β_0	74.45	4.17	6.42	44.17
β_1	-4.77	3.66	-0.081^{NS}	1.07^{NS}
β_2	-0.41	0.36^{NS}	0.23^{NS}	-4.15
β_{11}	-1.49	0.43^{NS}	0.028^{NS}	-3.14
β_{22}	-0.01^{NS}	-0.20^{NS}	0.39^{NS}	0.14^{NS}
R^2	0.989	0.843	0.089	0.368
R^2 adjusted	0.986	0.814	0	0.253
Lack of fit	0.164	1.93	0.315	32.57

^{NS} – not significant at 95% confidence level

From statistical analysis, the significance of NaOH concentration and reaction time on solid recovery is presented in Figure 4.2. On the Pareto chart, p is the probability (at 95% confidence interval, probability is 0.05), L represents linear/first order terms while Q represents quadratic/second order terms. In the alkaline pre-extraction of hemicelluloses from *E. grandis*, the most significant factor is NaOH concentration (in first and second orders), while reaction time (first order is slightly significant while second order is insignificant) has

minimal impact on solid recovery. With increasing NaOH concentration, more components of cell wall (lignocellulose) are dissolved, and that results in reduction in solid recovery. The trend of reduced solid recovery as concentration of alkali increases has also been observed, whereby percentage wood loss increased as alkali (NaOH) concentration was increased (Lehto and Alén, 2013; Joubert, 2015). The solid recoveries ranging from 73% (for high NaOH concentration, enhanced dissolution of wood components) to 78% (for low NaOH concentration) obtained are in accordance with some of the solid recoveries presented in Section 2.3.1.

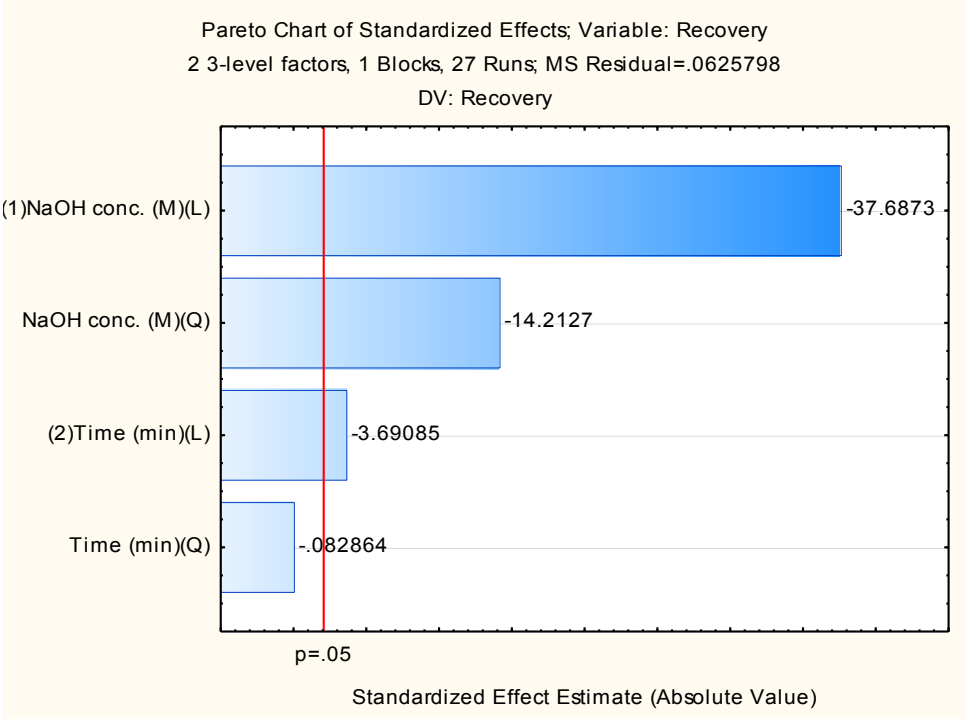

Figure 4.2: Pareto chart showing the standardised effects of NaOH concentration and reaction time on solid recovery

From statistical analysis, the significance of NaOH concentration and reaction time on xylan dissolution is presented in Figure 4.3. In alkaline pre-extraction of hemicelluloses from *E. grandis*, the most significant factor is NaOH concentration (only first order significant) while reaction time (both first and second orders) has minimal impact on xylan dissolution. The trend of increasing xylan dissolution as alkali (NaOH) concentration increases has been observed in alkaline hemicelluloses pre-extractions (Júnior et al., 2013; Lehto and Alén, 2013; Vena, 2013; Joubert, 2015). The xylan dissolution values obtained, which range from 2.2 g/100g of biomass (16 % of xylan in original biomass) to 6.7 g/100g of biomass (49 % of xylan in original biomass), are in agreement with those presented in Section 2.3.1.

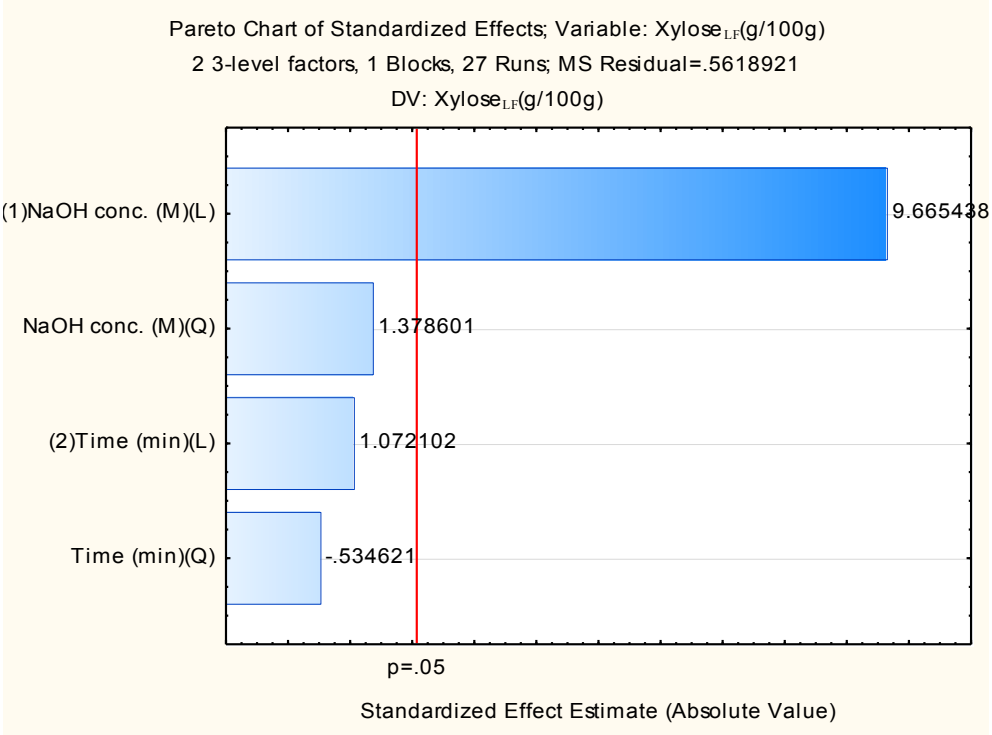

Figure 4.3: Pareto chart showing the standardised effects of NaOH concentration and reaction time on xylan dissolution

From statistical analysis, the significance of NaOH concentration and reaction time on lignin dissolution is presented in Figure 4.4. In alkaline pre-extraction of hemicelluloses from *E. grandis*, all the factors had minimal significance on lignin dissolution, at the conditions used. The minimal significance of both NaOH concentration and reaction time are observed mainly because the reaction temperature was held constant and it was fairly low. For delignification, even in mild alkaline conditions, an increase in temperature will improve the rate and degree of delignification (McIntosh and Vancov, 2010).

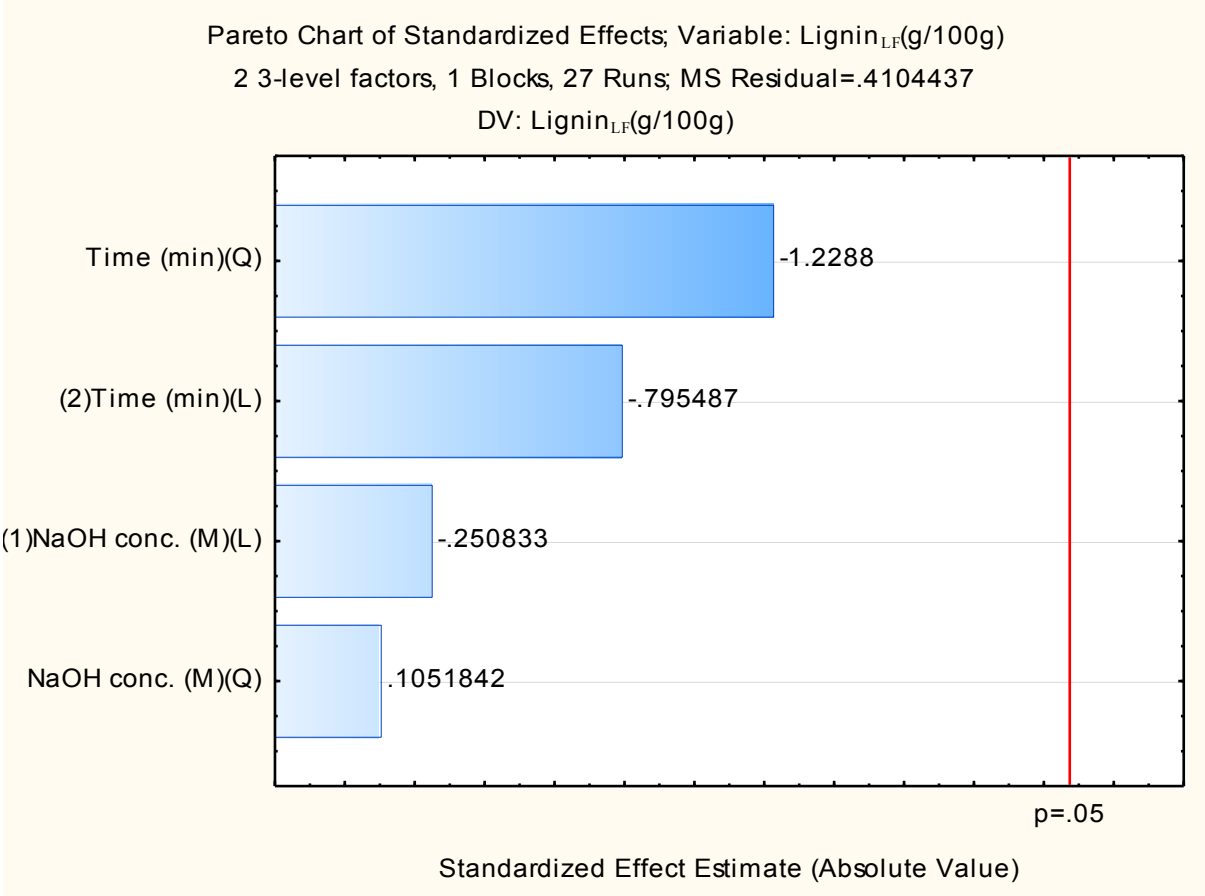

Figure 4.4: Pareto chart showing the standardised effects of NaOH concentration and reaction time on lignin dissolution

From statistical analysis, the significance of NaOH concentration and reaction time on glucan remaining in solid residue is presented in Figures 4.5. In alkaline pre-extraction of hemicelluloses from *E. grandis*, reaction time is the most significant factor (only first order), followed by NaOH concentration (only second order is significant) on glucan retention in the solids. At high severities (long reaction times and high NaOH concentrations), significant amounts of glucan are dissolved, which implies glucan loss in the fractionation scheme. It is worth noting that at reaction times beyond 240 min, significant glucan losses are observed, irrespective of the alkali concentration. It has been mentioned earlier that one of the parameters used to measure the efficiency of pre-treatment or fractionation methods is degree of glucan losses, that is, the minimum glucan dissolution (loss) is desirable for best pre-treatment or fractionation methods (Mosier et al., 2005; Yang and Wyman, 2008; Alvira et al., 2010; Vena, 2013). Although there is increasing xylan dissolution with increasing severity, the compromise has to be made in order to minimise glucan dissolution (Vena, 2013).

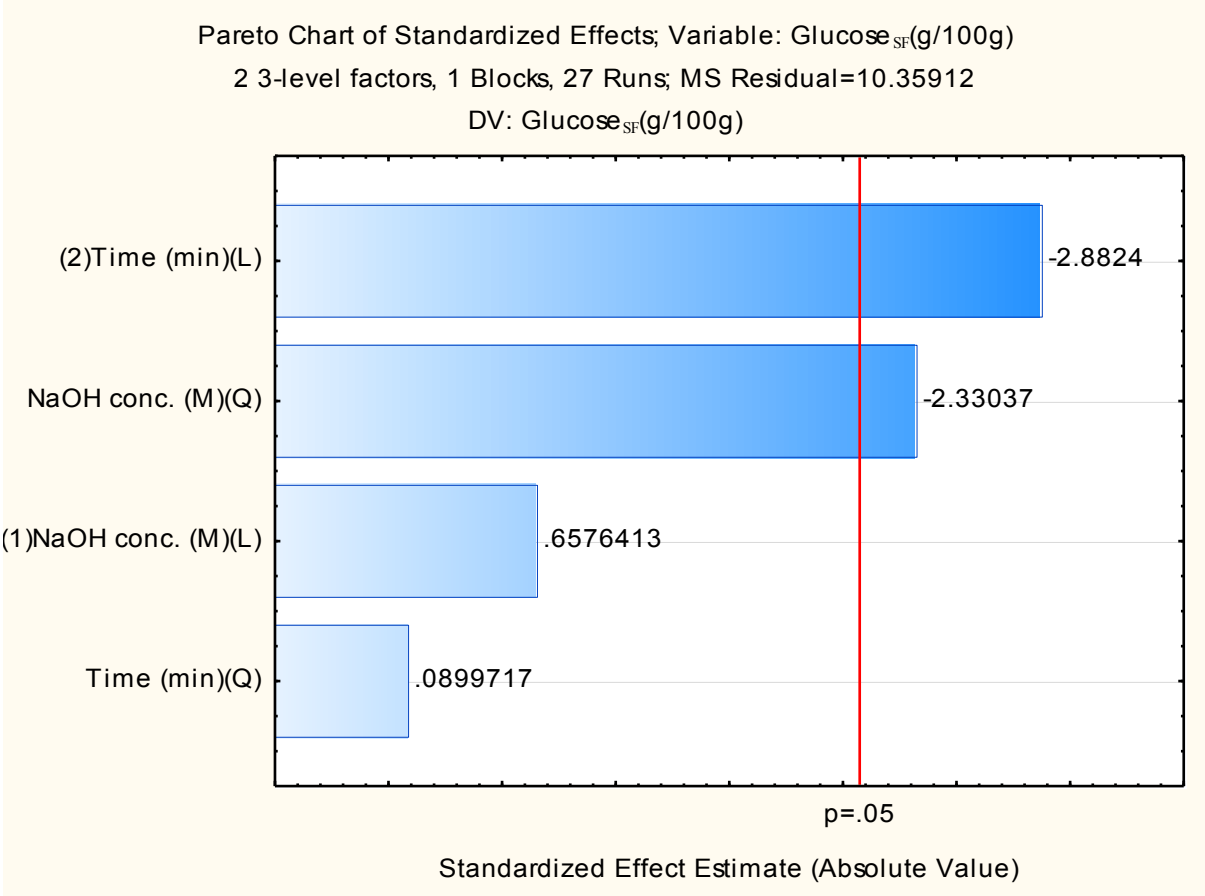

Figure 4.5: Pareto chart showing the standardised effects of NaOH concentration and reaction time on glucan retention in solid residue

Following statistical analysis of the factors' effects on the responses, the closer comparison of xylan dissolution and glucan retention was considered. The plots of xylan dissolution into liquid fraction and glucan retention in solid residues are shown in Figures 4.6 and 4.7 respectively. The focus reaction time for comparison was chosen to be 240 min because beyond 240 min there was significant glucan loss. At 240 min, increasing NaOH concentration led to increasing xylan dissolution but no significant dissolution (loss) of glucan. From that observation, the pre-extraction conditions of 240 min and 1.5 M NaOH were considered as the better option for compromise, and were later used in alkaline pre-extraction of hemicelluloses from *E. grandis*. The residual solids from alkaline pre-extraction of hemicelluloses were used as feed for organosolv and ionic liquids studies.

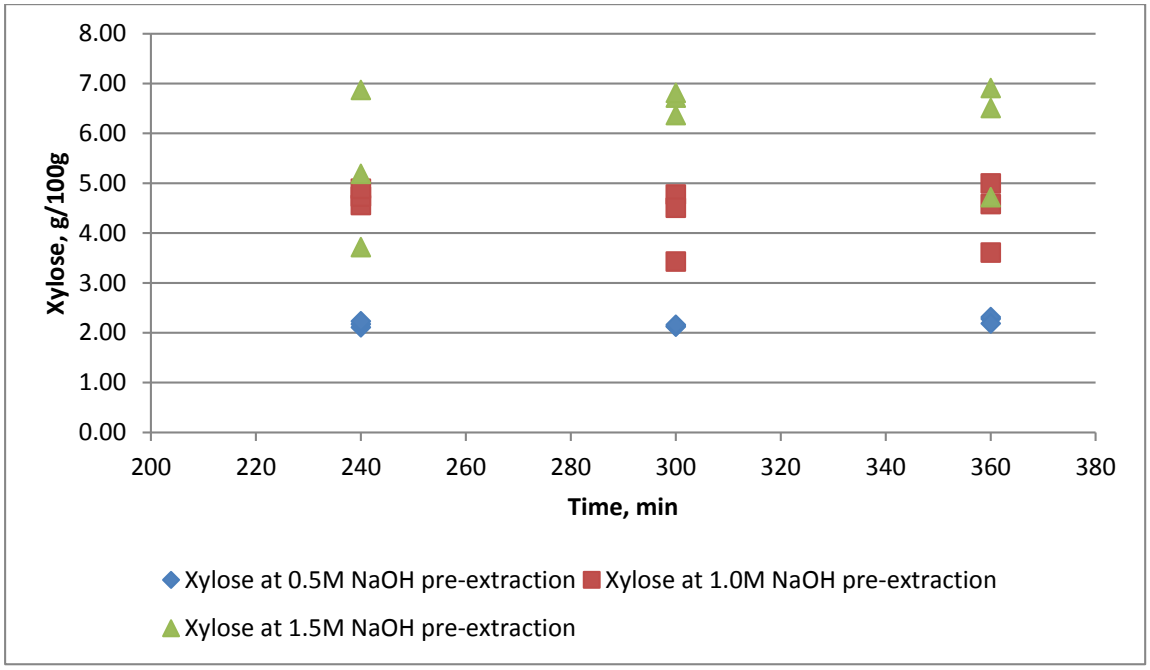

Figure 4.6: Xylan dissolution into liquid at different pre-extraction times and NaOH concentrations

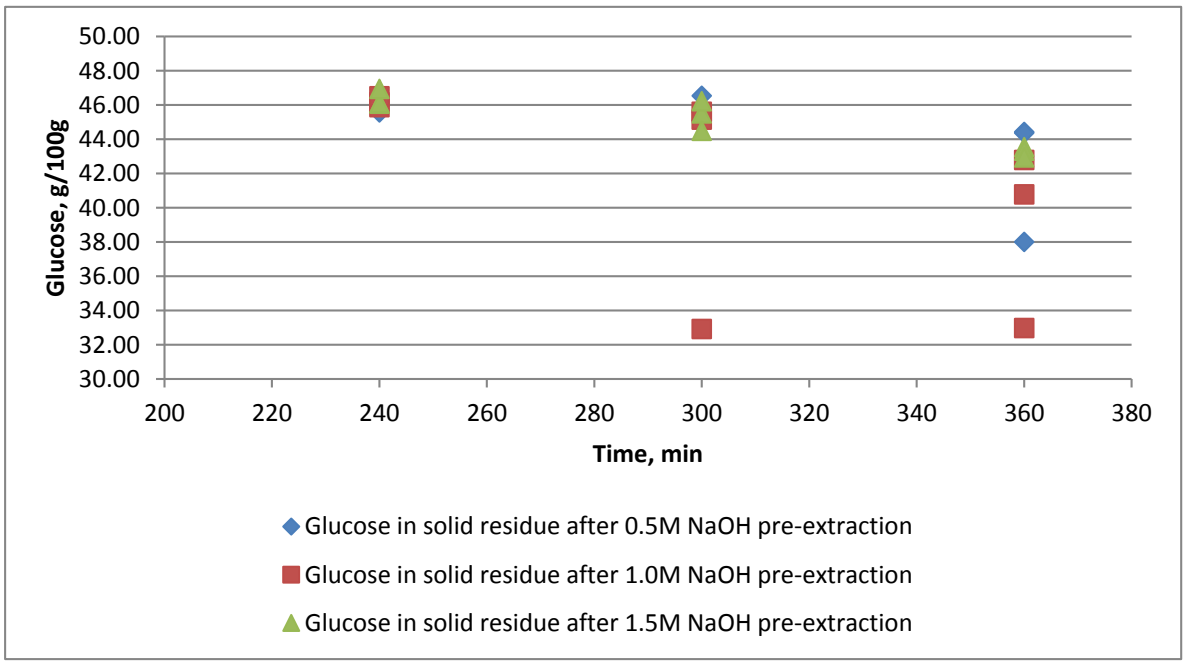

Figure 4.7: Glucan retention in solid residues at different pre-extraction times and NaOH concentrations

From this experimental design, NaOH concentration was found to be the most significant factor affecting xylan dissolution. The effect of residence time on glucan dissolution resulted in the compromise of pre-extraction conditions mentioned in the preceding paragraph.

4.2.2.2. Alkaline pre-extraction of hemicelluloses from *E. grandis* (at desirable conditions)

Following alkaline pre-extraction of hemicelluloses from *E. grandis*, the chemical compositions of the solid residues and liquid fraction were determined, and are shown in Table 4.5. After alkaline pre-extraction of hemicelluloses from *E. grandis*, solid recovery was found to be 78.1 %, and this recovery is within the range of 63 to 93 % obtained by other researchers as shown in Section 2.3.1. The solubilised lignin was 26.16 % of lignin in virgin *E. grandis*. The 26.16 % lignin dissolution obtained is above the range of 3 to 22 % shown in Section 2.3.1. Since pre-extraction factors were found to have minimal impact on lignin dissolution, the increase in lignin dissolution in comparison to the one obtained by Vena (2013) might be related not only to changes in pre-extraction conditions but also to origin of the *E. grandis*.

The solubilised xylan corresponds to 55.39 % of xylan in virgin *E. grandis*. The 55.39 % xylan dissolution obtained is above the range of 8 to 43 % shown in Section 2.3.1. The increase in xylan dissolution can be attributed to changes in severity in this as compared to the limited pre-extractions (mainly because the pulps were to be feed for papermaking) performed by Vena (2013).

As mentioned earlier, determining chemical composition of both liquid and solid fraction enables one to carry out mass balances. The mass balance of glucan, xylan and lignin were found to be 90.1±4.2 %, 104±5.3 % and 103.5±3.1 % respectively. The mass balance values obtained by Vena (2013) were 94.4, 94.1 and 95.3 % of glucan, xylan and lignin respectively, and when comparing with the mass balances in this study, both xylan and lignin balances are slightly higher while glucan balance is slightly lower. However, the difference in the mass balances is less than 10 % for all constituents, so the results can be said to be comparable.

Table 4.5: The chemical compositions of liquid and solid fractions obtained from alkaline pre-extraction of hemicelluloses from *E. grandis*

	Alkaline pre-extraction hydrolysate	**Solid residue**	**Mass balance (%)**
Solid recovery (%)		78.06 ± 2.67	
Glucose (g/100g)	0.99± 0.02	42.44 ± 0.73	90.1 ± 4.2
Xylose (g/100g)	7.55 ± 0.27	6.35 ± 0.12	104.5 ± 5.3
Lignin (g/100g)	7.19 ± 0.21	21.24 ± 0.78	103.5 ± 3.1

The degradation of dissolved xylan was tested as stated in Section 3.2.2.5. There were no monomeric sugars (xylose) detected in the liquid fraction prior to autoclaving (acid hydrolysis). The zero xylose in liquid fraction indicated that alkaline pre-extraction solubilised polymeric and oligomeric hemicelluloses.

The average molecular weight of the extracted hemicelluloses was found to be 55991 Da. The molecular weight obtained in this study is in relation with those obtained in alkaline extraction of hemicelluloses from *E. grandis* and other hardwoods (14500, 16000, 40021, 41000, 45000, 51589, 53400, 63809, 79420, 91530 and 99960 Da) (Gomes, 2012; Postma, 2012; Júnior et al., 2013; Vena, 2013; Wei et al., 2013). It is import to note that the lower molecular weights (14500 and 16000 Da) obtained by Júnior et al. (2013), resulted from severe (12.5 M) NaOH extractions while higher molecular weights (79420, 91530 and 99960 Da) obtained by Wei et al. (2013) resulted from very mild (1 M NaOH, 70 °C and 180 min) extractions. As a result, it can be expected to have decrease in molecular weights as severity of alkaline extractions increases (mainly because most bonds will be cleaved).

Xylan was found to be the major component of neutral sugars in the extracted hemicelluloses, as calculated in Appendix C. The neutral sugar composition is shown in Figure 4.8. Xylose was the dominant neutral sugar (87 % of neutral sugars), followed by galactose (6 %), glucose (4 %), arabinose (2 %) and mannose (1 %). The trend of high xylose content in the alkaline extracted hemicelluloses followed by either glucose or galactose is common for hardwoods' hemicelluloses, whereby xylose ranging from 41 to 85 % and glucose ranging from 1 to 51 %, and galactose ranging from 1 to 5 % have been reported by other researchers (Chimphango, 2010; Gomes, 2012; Postma, 2012; Júnior et al., 2013; Wei et al., 2013).As the

severity increases, the amount of xylose, in relation to glucose detected, increases because more side chains are cleaved (Júnior et al., 2013).

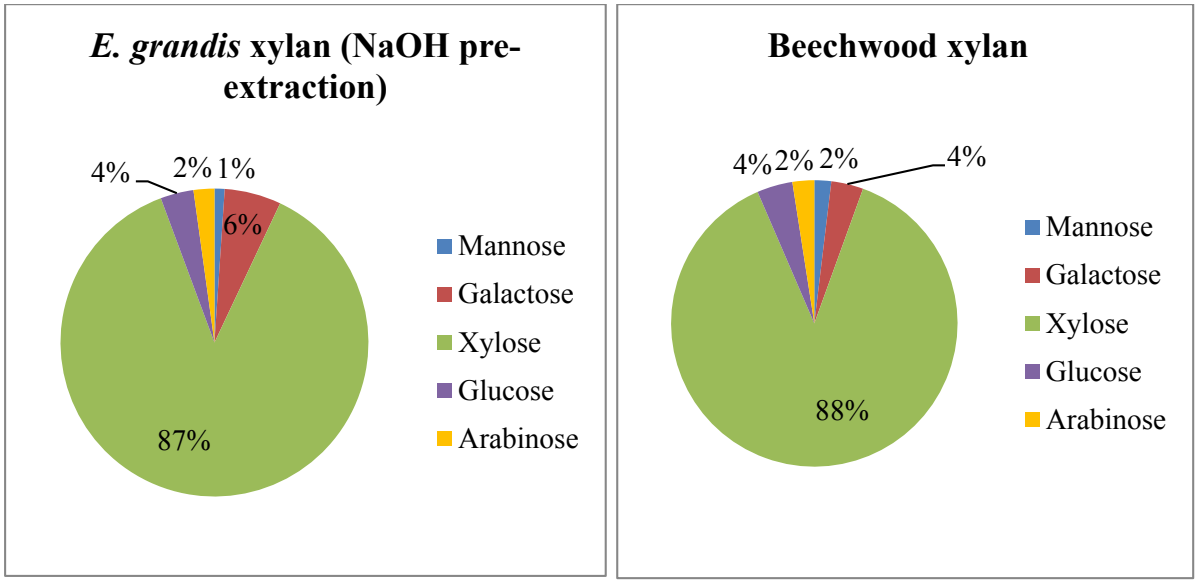

Figure 4.8: Neutral sugar compositions of *E. grandis* xylan (NaOH pre-extraction) and Beechwood xylan (commercial)

The acid insoluble lignin content of *E. grandis* xylan (NaOH pre-extracted) was determined gravimetrically (masses shown in Appendix C), and was found to be 13.92±0.04 %. The lignin content obtained is within the range of 3 to 38 %, which was found in alkaline extracted xylan from Eucalypts and other hardwoods (Chimphango, 2010; Gomes, 2012; Postma, 2012; Júnior et al., 2013; Vena, 2013).

The neutral sugar compositions of *E. grandis* xylan (NaOH pre-extracted) and Beechwood xylan are comparable (as shown in Figure 4.8). The major difference is the lignin content, *E. grandis* xylan (NaOH pre-extracted) has higher lignin content of 13.92 % than Beechwood xylan of 3.57 % (Gomes, 2012). Therefore, it can be said that alkaline pre-extraction and alcohol precipitation of hemicelluloses were effective in producing good quality and purity xylan.

FTIR analysis of *E. grandis* xylan (NaOH pre-extraction), provided in Appendix B, indicates that confirmed that the obtained fraction was hemicellulose (mainly xylan), as it had strong pyranose ring stretch vibrations Extracted xylan had β-glycosidic linkages between sugar monomers and arabinosyl side chains. The extracted hemicelluloses were completely

deacetylated. Lignin contamination was also present, although the intensities of lignin peaks were low, and that corresponded with lignin determined using wet chemical method.

The S/G ratio of the lignin was found to be 4.96. There is slight increase in the ratio when compared to the feedstock (S/G ratio of 4.35). The slight increase in S/G ratio of the extracted lignin resulted from increased dissolution of syringyl-type lignin structures during alkaline extraction (Fengel and Wegener, 1989; Bose et al., 2009).

FTIR analysis of *E. grandis* lignin (NaOH pre-extracted), shown in Appendix B, indicates that the recovered lignin was oxidised (high intensity of conjugated carbonyl groups) (Bai et al., 2013; Zhang et al., 2013b; Sun et al., 2014). Carbohydrates contamination was very low, so the separation of hemicellulose from lignin (using alcohol precipitation) was effective.

The glucan recovered in the solid residue, following NaOH pre-extraction of *E. grandis*, was 88.09 % of the glucan in virgin material. This recovery is within the range of 74 to 100 % mentioned in Section 2.3.1. Following wet chemistry, the solid residue was further structurally characterised using FTIR and its enzymatic digestibility was also determined.

The glucan digestibility of the solid residue was found to be 66.09 %. For alkaline treatments of *Eucalypts* and other hardwoods, the glucan digestibilities ranging from 5 to 100 % have been reported (Um and Walsum, 2010; Park and Kim, 2012; Castro et al., 2013; Lima et al., 2013). The high digestibility (100 %) was obtained from hybrid *E. grandis x urophylla* (Lima et al., 2013). Although the type of hardwood (hybrid type, which has different lignin migration, coalescence on cell wall surface and higher S/G ratio) could affect digestibility of pre-treated material, high temperatures and pressures (autoclaving), and mechanical stirring in alkaline treatments also tend to enhance digestibility significantly (Castro et al., 2013; Lima et al., 2013). The glucan digestibility of 66.09 % is not good enough for biofuels and/or high value chemicals production, as compared to ≥80 % which is considered as high cellulose digestibility (Yang and Wyman, 2008; Alvira et al., 2010; da Silva et al., 2013). Therefore, mild alkaline pre-extraction of hemicelluloses from hardwoods can be succeeded by other treatments to enhance increase digestibility further or the solid residues can be used as feed for other cellulose applications beside biofuels (like pulp and paper, biomaterials, solvents, etc.).

The FTIR analysis of alkaline pre-extracted solid residue, shown in Appendix B, indicates that the material was completely deacetylated during alkaline pre-extraction of

hemicelluloses. Furthermore, there is an indication that significant delignification occurred, which resulted cellulose-rich solid residues.

Alkaline pre-extraction of *E. grandis* resulted in solubilisation of polymeric xylan (55% of xylan in virgin material), and the process was accompanied by very low xylan degradation (about 5% of xylan in virgin material). Alkaline pre-extraction is attractive not only for extracting and preserving hemicelluloses (xylan) in polymeric form, but also in extracting good quality lignin and resulting in digestible solid residues. Alkaline pre-extraction of hemicellulose is therefore effective on hardwoods, so it can be included in biorefineries (both integrated forest biorefineries and fuel-chemical-material based biorefineries).

4.3. Organosolv Fractionation

4.3.1. Organosolv fractionation of SCB

4.3.1.1. Organosolv fractionation of virgin SCB

The chemical compositions of the fractions obtained from organosolv fractionation of SCB are shown in Table 4.6. The solid recovery following organosolv fractionation of SCB was found to be 66.74 %, and this recovery is within the range of 43 to 90 % obtained by other researchers, as shown in Section 2.3.2. The lowest solid recovery (43 %) was obtained in the acid catalysed organosolv, due to enhanced delignification and hemicelluloses' hydrolysis (Pan et al., 2006a; Brosse et al., 2009; Zhao et al., 2009; Hallac et al., 2010b; Ingram et al., 2011; Koo et al., 2011a; 2011b; Huijgen et al., 2012; Abeywickrama et al., 2013; Wildschut et al., 2013). The high solid recovery (90 %) was obtained in an alkaline catalysed organosolv, which was carried out at lower temperature and high solid loading of 17 % (Mesa et al., 2010). Increasing temperature leads to lower solid recovery, as it enhances delignification and hemicelluloses' hydrolysis (Pan et al., 2006a; Brosse et al., 2009; Zhao et al., 2009; Hallac et al., 2010b; Ingram et al., 2011; Koo et al., 2011a; 2011b; Huijgen et al., 2012; Abeywickrama et al., 2013; Wildschut et al., 2013). Solid recovery is not only affected by catalyst, temperature and solid loading, but also by solvent concentration. Solvent concentration alters solid recovery in organosolv treatments as follows: high ethanol (alcohol) concentration gives high solid recovery because of decreased delignification and hemicelluloses' hydrolysis (Jiménez et al., 1997; Wildschut et al., 2013). It is also important to note that solvent concentration does not only affect solid recoveries, but enzymatic digestibility of recovered solids (at high alcohol concentration and high solid recovery, the

enzymatic digestibility tends to decrease) (Jiménez et al., 1997; Wildschut et al., 2013). The solid recovery (66.74 %) obtained in this study is comparable to those obtained by Huijgen etal. (2012) of 57.9 %, and by Wildschut et al. (2013) of 63.2 %, both on wheat straw and carried out at similar conditions as this study.

Table 4.6: The chemical compositions of liquid and solid fractions obtained from organosolv fractionation of virgin SCB

	Organosolv hydrolysate	**Solid residue**	**Mass balance (%)**
Solid recovery (%)		66.74 ± 0.21	
Glucose (g/100g)	0.66 ± 0.04	39.25 ± 0.71	93.6 ± 4.4
Xylose (g/100g)	8.05 ± 0.04	7.79 ± 0.21	69.5 ± 2.0
Lignin (g/100g)	9.55 ± 0.25	14.79 ± 0.48	108.6 ± 2.9

42.60% of lignin in virgin SCB was solubilised during organosolv fractionation, and obtained delignification is within the range of 9 to 93 % shown in Section 2.3.2. The 9 % delignification corresponded to 90% solid recovery, while 93% delignification corresponded to 43% solid recovery (Mesa et al., 2010; Ingram et al., 2011; Wildschut et al., 2013). As mentioned earlier, higher process severities (high temperatures, high acid catalyst concentrations and lower alcohol (50-60 %) concentrations) tend to enhance delignification, resulting in high amounts of solubilised lignin (Jiménez et al., 1997; Pan et al., 2006a; Brosse et al., 2009; Zhao et al., 2009; Hallac et al., 2010b; Ingram et al., 2011; Koo et al., 2011a; 2011b; Huijgen et al., 2012; Abeywickrama et al., 2013; Wildschut et al., 2013). When compared with the solubilised lignin obtained from organosolv treatment of wheat straw (at similar conditions to those used in this study) 42.60 % is found to be slightly lower than 58.9 and 67.27 % obtained by other researchers (Huijgen et al., 2012; Wildschut et al., 2013), and the difference might be attributed to different feedstocks.

Xylan detected in organosolv liquor was 35% of xylan in virgin SCB. The 35 % xylan dissolution is within the range of 0.5 to 93% shown in Section 2.3.2. 0.5 % detected xylan in organosolv liquor was obtained at high temperatures (≥200 °C) while 93 % detected xylan in organosolv liquor was obtained at moderate temperatures (167 °C) (Huijgen et al., 2010; Ingram et al., 2011; Huijgen et al., 2012). At high temperatures (≥200 °C), xylan dissolution occurs, but the degradation reactions are enhanced by that high temperature leading to low

amounts of xylan being detected in the organosolv liquor (Jiménez et al., 1997; Brosse et al., 2009; Zhao et al., 2009; Ingram et al., 2011; Huijgen et al., 2012; Abeywickrama et al., 2013; Wildschut et al., 2013). Although 51 % and 67 % xylans were dissolved in organosolv treatment of wheat straw, only 0.5 % and 7.8 % xylans were detected in the liquid products from those treatments, as both monomeric and oligomeric xylans (Huijgen et al., 2012; Wildschut et al., 2013). Furthermore, in those treatments concentrations of degradation products (furfural and HMF) were significant, thus confirming degradation of sugars (Huijgen et al., 2012; Wildschut et al., 2013). Similarly, in this study, xylan (xylose) degradation had been observed as only 35 % xylan was detected but mass closure indicated 66 % xylan dissolution.

Mass balances of the organosolv process were prepared after compositional analyses of solid and liquid fractions. The mass balance of glucan, xylan and lignin were found to be 93.6±4.4 %, 69.5±2.0 % and 108.6±2.9 % respectively. The mass balance values obtained in this study corresponds to 97.9, 75.7 and 109.8 % of glucan, xylan and lignin respectively, which were obtained in wheat straw organosolv treatment (Wildschut et al., 2013). Organosolv treatment appears to be good for enriching and conserving glucan in herbaceous and agricultural residues, but the dissolved xylans are susceptible to degradation (Jiménez et al., 1997; Brosse et al., 2009; Zhao et al., 2009; Goh et al., 2011; Ingram et al., 2011; Huijgen et al., 2012; Abeywickrama et al., 2013; Wildschut et al., 2013). Huijgen et al. (2010; 2012) also found that xylan recoveries (using xylan and its derivatives) were low due to some unidentified xylan products like condensates with lignin.

Following the organosolv treatment of virgin SCB and chemical compositional analyses of solid and liquid fractions, the fractions were further characterised for structural properties, monomer composition, and enzymatic digestibility in order to have an idea regarding downstream applications. The liquid fraction (which comprised of dissolved hemicelluloses, dissolved lignin and degradation products) was dried and considered as crude lignin. The hemicelluloses were not separated out as alcohol precipitation (used in this study) did not work, and time limitations did not allow for exploring other separation techniques like membrane technology, size exclusion chromatography and sub/supercritical antisolvent precipitation.

Analysis of lignin for monolignols resulted in S/G ratio of 2.11which was slightly higher than S/G ratio (1.68) of virgin SCB. As mentioned earlier, increase in S/G ratio corresponds to

reactivity of lignin in virgin material. Some sugar (glucose) was also detected during thioacidolysis analysis of lignin. The presence of sugar was expected as the lignin was crude and obtained from herbaceous and agricultural residues which have been observed to have high residual sugars (Hu et al., 2012; Sammons et al., 2013). Furthermore, the organosolv lignin was sensitive to moisture (became sticky when put outside oven or desiccator), and the moisture sensitivity could have been attributed to hemicelluloses in lignin as they have high affinity for water (Gomes, 2012; Postma, 2012).

FTIR analysis of SCB lignin (organosolv), shown in Appendix B, had strong intensity of aromatic ring (confirming that the recovered fraction is lignin) and was oxidised. Carbohydrates contamination was confirmed by the presence of pyranose stretch vibration (Zhang et al., 2013b). High intensity of lignin bands in extracted lignin than in feedstock showed that the organosolv treatment enhanced lignin separation from other components of the lignocellulosic biomass.

Recovered glucan in the solid residue, following organosolv treatment of SCB, was 92.09 % of glucan in virgin SCB. This recovery is within the range of 71 to 100 %, mentioned in Section 2.3.2. Obtained glucan recovery corresponds to 95.6 and 96.8 % obtained from wheat straw at similar organosolv conditions (Huijgen et al., 2012; Wildschut et al., 2013). Following wet chemistry, the solid residue was further structurally characterised using FTIR and its enzymatic digestibility was also determined.

The glucan digestibility of the solid residue was found to be 63.02%. The 63.02% enzymatic digestibility obtained in this study is within the range of 17 to 98% obtained for organosolv treatments of herbaceous biomass or agricultural residues, as shown in Section 2.3.2. The enzymatic digestibility obtained in this study (63.02 %) is slightly higher than those obtained on organosolv treated wheat straw (at similar organosolv conditions) of 44.4 and 51 % (Huijgen et al., 2012; Wildschut et al., 2013). The slight increase in enzymatic digestibility is alleged to higher enzyme loading (30FPU/g of glucan) used in this study, as compared to 20FPU/g glucan used on wheat straw (Huijgen et al., 2012; Wildschut et al., 2013). The glucan digestibility of 63.02 % is low for biofuels and/or high value chemicals production, as compared to ≥80 % which is considered as high cellulose digestibility (Yang and Wyman, 2008; Alvira et al., 2010; da Silva et al., 2013). Therefore, auto-catalysed organosolv is not good enough for treating virgin SCB and/or other agricultural residues for biofuels and/or high value chemicals (ethanol) production.

FTIR analysis of organosolv treated solid residue, shown in Appendix B, indicates that delignification and partial deacetylation of SCB occurred during organosolv fractionation.

Low delignification, high hemicellulose (xylan) degradation, and low cellulose digestibility indicate that auto-catalysed organosolv is not effective in fractionating virgin SCB. In order to improve hemicellulose recovery and preservation in polymeric form, delignification as well as cellulose digestibility, alkaline conditions can be included.

4.3.1.2. NaOH post-extraction of organosolv treated SCB

The study of coupling organosolv fractionation plus NaOH post-extraction was carried out in order to enable fair comparison with ionic liquid fractionation plus NaOH post-extraction. NaOH post-extraction of organosolv treated solids was carried out at 80°C for 120min at various NaOH concentrations. Both xylan and lignin dissolutions, which are shown in Figure 4.9, increased with increasing NaOH concentration. The increasing xylan dissolution and delignification with increasing NaOH concentration has been observed in alkaline extraction of hemicelluloses (McIntosh and Vancov, 2010; Júnior et al., 2013; Lehto and Alén, 2013; Vena, 2013; Joubert, 2015).

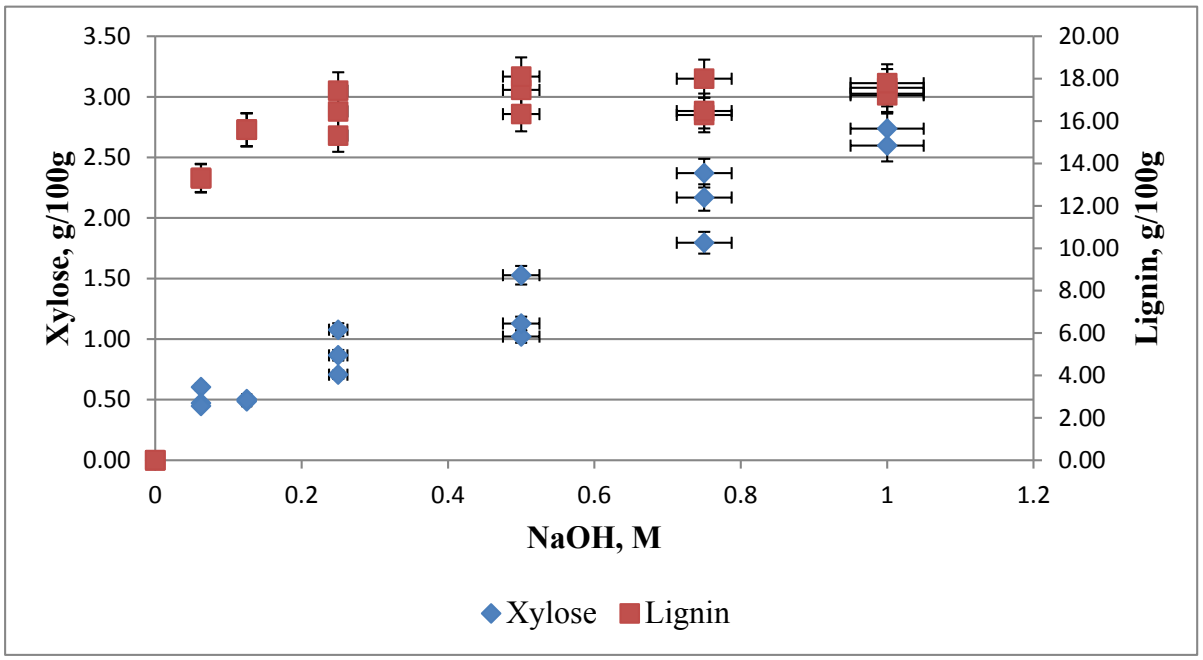

Figure 4.9: Xylan and lignin dissolution from organosolv treated SCB solids at different NaOH concentrations

Figure 4.10 shows the decrease in glucan retention in solid residues as NaOH concentrations increased. The decreasing glucan retention in solid residues, as NaOH concentration increases, is attributed to enhanced glucan dissolution.

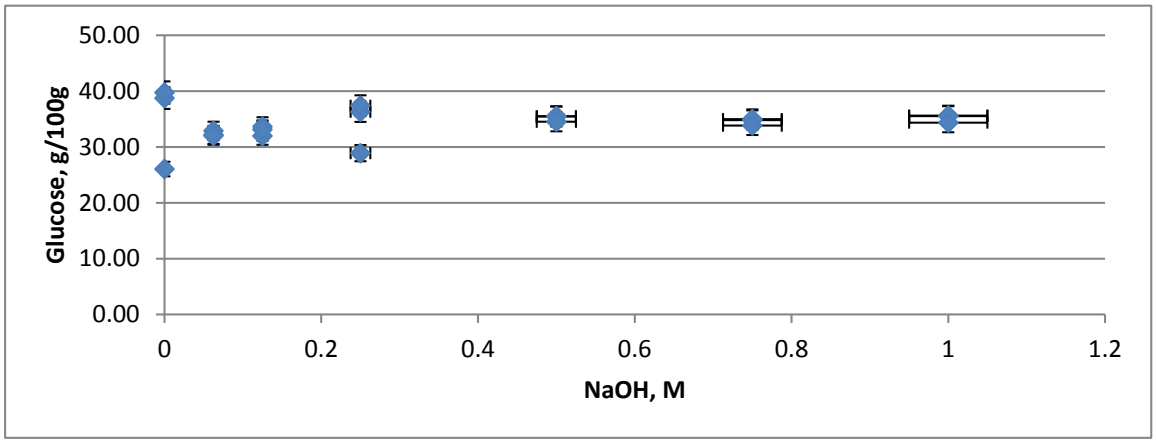

Figure 4.10: Glucan retention in SCB solids treated with organosolv method and NaOH post-extraction at different NaOH concentrations

The relation of enzymatic digestion and NaOH concentration is shown in Figure 4.11. Addition of NaOH improved cellulose digestibility up to 100 %. However, the effect of increasing NaOH concentration on enzymatic digestibility of solids obtained from organosolv treatment and NaOH post-extraction was not significant, as NaOH concentrations as low as 0.0625 M NaOH resulted in 98 % cellulose digestibility. As the NaOH concentration was increased, enzymatic digestibilities reached 100 %. The fractionation method which combines organosolv and NaOH post-extraction yields highly digestible solids of treated herbaceous and agricultural residues.

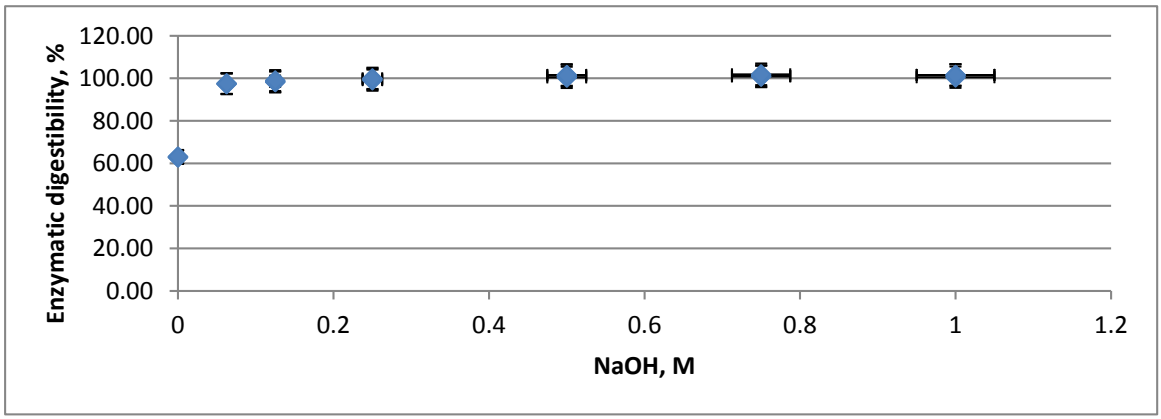

Figure 4.11: Enzymatic digestibility of organosolv treated and NaOH post-extracted SCB solids at different NaOH concentrations

Although very low NaOH concentrations enhanced cellulose digestibility, xylan dissolution increased with increasing NaOH concentration. The compromise which was reached for alakaline post-extraction (based on xylan extraction, delignification and cellulose digestibility) was 80 °C, 0.5 M NaOH and 120 min of SCB.

Table 4.7 presents the results obtained when NaOH post-extraction of organosolv treated SCB was carried out. The solid recovery following the treatment of SCB was found to be 43.4 %. The obtained solid recovery is lower than the one obtained in organosolv treatment alone (66.7 %). The decrease in solid recovery results from solubilisation of hemicelluloses and lignin in the alkaline post-extraction.

Table 4.7: The chemical compositions of liquid and solid fractions obtained from NaOH post-extraction of organosolv treated SCB

	Organosolv hydrolysate	**Solid residue**	**Mass balance (%)**	
			Based on virgin material	Based on organosolv treated material
Solid recovery (%)		43.38 ± 1.71		
Glucose (g/100g)	0.42± 0.05	35.17 ± 0.56	83.5 ± 3.6	90.7 ± 2.2
Xylose (g/100g)	0.84 ± 0.94	5.98 ± 0.09	65.2 ± 6.5	87.6 ± 12.4
Lignin (g/100g)	17.63 ± 1.58	1.47 ± 0.09	85.2 ± 7.2	129.1 ± 11.5

Lignin detected in the liquid fractions (alkaline post-extraction) was 17.63 (g/100g) based on dry raw biomass, which corresponded to 77.6 % of lignin in virgin SCB, and 119.2 % of lignin in organosolv treated SCB. The 119 % total lignin detected in the NaOH post-extraction liquor implies that near complete delignification was achieved. The 19 % beyond 100 % can be related to formation of pseudo-lignin (Tan et al., 2009; Huijgen et al., 2010; Huijgen et al., 2012).

The solubilised xylan was 0.84 (g/100g) based on dry raw biomass, which corresponds to 3.7 % of xylan in virgin SCB, and 10.8 % of xylan in organosolv treated SCB. The low xylan detection has already been related to degradation and other side reactions of xylan in organosolv treatment.

Chemical characterisation of both streams (liquid and solid fractions) allow for closing the mass balances of the organosolv plus NaOH post-extraction process. The mass balance of glucan, xylan and lignin were found to be 83.5±3.6 %, 65.2±6.5 % and 85.2±7.2 % based on virgin SCB. On the basis of organosolv treated material, the recoveries were 90.7±2.2, 87.6±12.4 and 129.1±11.5 %for glucan, xylan and lignin. The glucan balance following this treatment combination has decreased by 12.8 % from the one obtained in organosolv treatment alone. The decrease is attributed to glucose dissolution in alkaline (NaOH) treatment and degradation of dissolved glucan (Vena, 2013). The increase in lignin is attributed to pseudo-lignin (lignin-xylan/xylan derivatives condensation products) formation (Huijgen et al., 2010; Huijgen et al., 2012).

The hemicelluloses and lignin (NaOH post-extraction) were obtained from the liquid fraction of NaOH post-extraction. Hemicelluloses were obtained using alcohol precipitation, and crude lignin from drying the residual liquor. The characterisations of extracted hemicelluloses for molecular weight, sugar composition, and structure (FTIR) were carried out.

The molecular weight of the extracted hemicelluloses was found to be 8148 Da. The molecular weight obtained in this treatment is very low when compared to those obtained in alkaline extraction of hemicelluloses from SCB (32793, 35200, 35450, 35600, 35820, 36760, and 37430 Da), barley straw and husks (34100, 34220, 35100, 39000 and 40400 Da), and (35540 Da) from rice straw (Sun et al., 2000; Sun, and Sun, 2002; Höije et al., 2005; Xu et al., 2006c; Gomes, 2012; Vena, 2013). The significant decrease in size of extracted hemicelluloses is attributed to cleavage of lignin-carbohydrates bonds and depolymerisation of hemicellulose during organosolv treatment (Xu et al., 2006c).

Extracted hemicelluloses were analysed for neutral sugar composition (in relative percentages), and calculations are presented in Appendix C. The neutral sugar composition is shown in Figure 4.12. Xylose was the dominant neutral sugar (79 % of neutral sugars), followed by arabinose (11 %), and glucose (10 %). The trend of high xylose content in the alkaline extracted hemicelluloses followed by arabinose is common for hemicelluloses from herbaceous biomass and agricultural residues, whereby xylose ranging from 50 to 97 % and arabinose ranging from 3 to 17 % have been observed (Sun et al., 2002a; Höije et al., 2005; Xu et al., 2006c; Peng et al., 2009; Chimphango, 2010; Peng et al., 2010a; Gomes, 2012). The residual glucose obtained is within the range of 0.02 to 27 %, which have been obtained in alkaline extracted hemicelluloses from herbaceous and agricultural residues (Sun et al.,

2002a; Höije et al., 2005; Xu et al., 2006c; Peng et al., 2009; Chimphango, 2010; Peng et al., 2010a; Gomes, 2012).

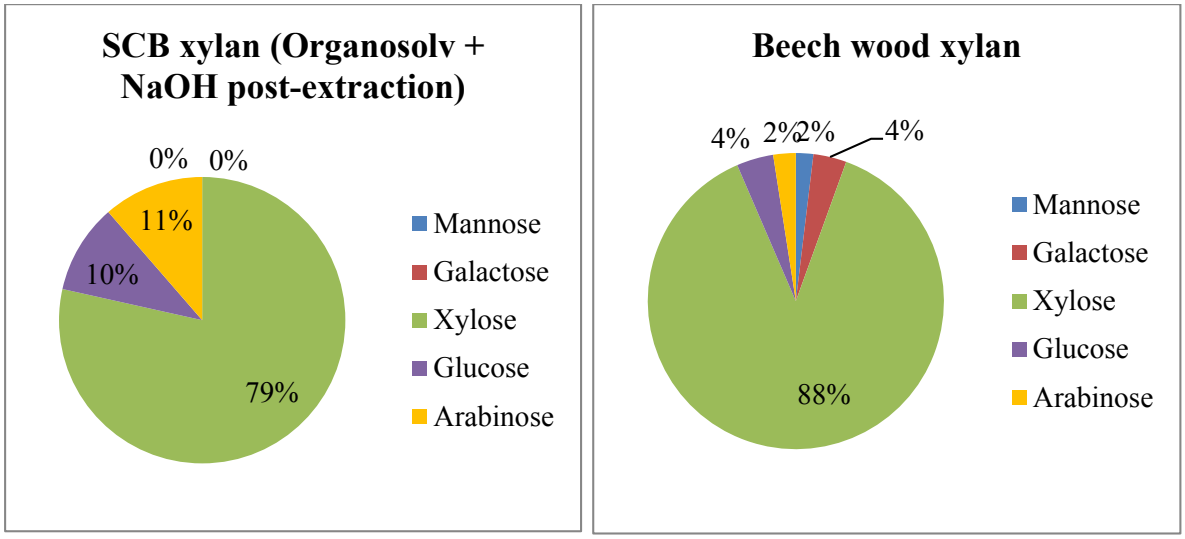

Figure 4.12: Neutral sugar compositions of SCB xylan (organosolv plus NaOH post-extraction) and Beechwood xylan (commercial)

The acid insoluble lignin content of SCB xylan (organosolv plus NaOH post-extraction) was found to be 26.86±0.04 %. The lignin content obtained is very high when compared to the range of 1.4 to 16 %, which were found in alkaline extracted xylan from herbaceous and agricultural residues (Sun et al., 2002a; Höije et al., 2005; Xu et al., 2006c; Peng et al., 2009; Chimphango, 2010; Peng et al., 2010a; Gomes, 2012; Vena, 2013). The increase in lignin content (by 2.23 times of the one obtained in alkaline pre-extraction) in extracted hemicelluloses has been observed in this study. The increase of lignin content in post-extraction might be influenced by organosolv step which solubilised significant amount of lignin and hemicellulose through cleavage of LCCs (Xu et al., 2006c; Xu et al., 2008). So the remaining LCCs in solids could not be significantly cleaved by alkaline post-extraction, which used lower NaOH concentration (0.5M) as compared 1.5M NaOH used in pre-extraction (which enhanced cleavage of LCCs). When compared with Beechwood xylan, SCB xylan (organosolv plus NaOH post-extraction) has higher arabinose and glucose.

FTIR analysis of SCB xylan (organosolv plus NaOH post-extraction), is presented in Appendix B. SCB xylan (organosolv plus NaOH post-extraction) has strong hemicellulose peaks and lignin peaks. High intensity of lignin peaks is in agreement with high lignin content obtained from wet chemical method.

The obtained lignin had S/G ratio of 3.99. The increase in the ratio when compared to the feedstock (S/G ratio of 1.68), indicates that syringyl units are more reactive and easily hydrolysed than guaiacyl units. Some sugar (glucose) was also detected during thioacidolysis analysis of lignin. The presence of sugar was expected as the lignin was crude and obtained from herbaceous and agricultural residues which have been observed to have high residual sugars (Sammons et al., 2013).

FTIR analysis of SCB lignin (organosolv plus NaOH post-extraction), presented in Appendix B, had strong intensity of lignin bands. The obtained lignin was oxidised, and had carbohydrates contamination.

Glucan recovered in the solid residue, following organosolv and NaOH post-extraction of SCB, was 82.52 %. This recovery is lower than 92.09 % obtained organosolv only. This low glucan recovery shows that 9.57 % of glucan was solubilised in NaOH post-extraction. The solid residues were further structurally characterised and tested for enzymatic digestibility.

The glucan digestibility of the solid residue was found to be 101.10 %. The 101.10% enzymatic digestibility obtained in this treatment is higher than the one obtained with organosolv alone (63.02 %). NaOH post-extraction increased enzymatic digestibility of glucan by 38.08 %. The glucan digestibility of 101.10 % is good for application of cellulose in fermentation-based biorefinery.

Low lignin content, complete deacetylation, and high cellulose content (confirmed by FTIR analysis) of organosolv plus NaOH post-extracted solid residue indicate that this treatment combination is attractive for fermentation-based biorefineries.

The combination of organosolv with alkaline post-extraction yields highly digestible solid residues. This combination is accompanied by high delignification. Both high digestibility and delignification makes this fractionation combination attractive. However, its limitation is high degradation of hemicelluloses. Since this study desires fractionation methods which favour hemicellulose preservation (oligomers and polymers), this fractionation combination is concluded be harsh on SCB.

4.3.1.3. Organosolv fractionation of NaOH pre-treated SCB

The organosolv treatment was also carried out on alkaline pre-extracted SCB. The chemical compositions of the fractions obtained from organosolv fractionation of SCB are shown in

Table 4.8. The solid recovery following organosolv fractionation of SCB was found to be 41.08 %, and this recovery is lower than 66.74 % (obtained in organosolv treatment of virgin SCB). The similar trend of decreasing solid recovery when pre-extraction step is included in organosolv has been observed by Huijgen and co-workers (Huijgen et al., 2012). The solid recovery (41.08 %) obtained in this treatment is slightly lower than the one obtained in organosolv plus NaOH post-extraction (43.38 %). The decrease in solid recovery from this treatment when compared to organosolv plus NaOH post-extraction is attributed to different severities in alkaline treatments (1.5 M NaOH in pre-extraction as compared to 0.5 M NaOH in post-extraction). Higher alkaline concentration enhanced delignification and hemicellulose dissolution, thus yielding lower solid recovery.

Table 4.8: The chemical compositions of liquid and solid fractions obtained from organosolv fractionation of NaOH pre-extracted SCB

	Organosolv hydrolysate	Solid residue	Mass balance (%)	
			Based on virgin material	Based on pre-extracted material
Solid recovery (%)		41.08 ± 0.60		
Glucose (g/100g)	0.04 ± 0.00	27.56 ± 0.46	67.8 ± 2.8	71.73 ± 3.21
Xylose (g/100g)	0.24 ± 0.01	5.89 ±0.07	30.3 ± 2.2	97.77 ± 3.17
Lignin (g/100g)	2.72 ± 0.07	5.25 ± 0.03	37.7 ± 1.0	96.26 ± 3.61

The solubilised lignin was 2.72 (g/100g) based on dry raw biomass, which corresponds to 12.85 % of lignin in virgin SCB. But when comparing lignin solubilisation obtained to the one in alkaline pre-extracted solid, solubilised lignin is 32.85 %. The 32.85 % delignification is lower than 42.60 % lignin dissolution obtained with organosolv treatment of virgin SCB. The decrease in delignification could have been caused by lack of acid catalyst (absence of acetic acid from alkaline pre-extracted biomass and neutralising effect of any residual NaOH).

The solubilised xylan (detected) was 0.24 (g/100g) based on dry raw biomass, which corresponds to 1.18 % of xylan in SCB. But when comparing xylan solubilisation obtained to the one in alkaline pre-extracted solid, solubilised xylan is 3.83 %. The 3.83 % xylan dissolution is lower than 35 % xylan dissolution obtained with organosolv treatment of virgin

SCB. Decrease in xylan dissolution in organosolv following alkaline pre-extraction might be attributed to lack of acetic acid in pre-extracted material and together with lower hydroxyl ions (due to neutralisation effect of any residual NaOH), which limit catalysis of xylan dissolution and delignification.

The mass balances of the organosolv process for glucan, xylan and lignin were found to be 67.8±2.8 %, 30.3±2.2 % and 37.7±1.0 % respectively. The mass balances for organosolv fractionation of pre-extracted SCB were determined by relating the amount of components after organosolv to the virgin biomass. But when comparing with the NaOH pre-extracted material the balances would be 71.73 %, 97.79 % and 96.39 % for glucose, xylose and lignin respectively. The mass balance values obtained in this treatment are different from the values obtained in organosolv treatment of virgin SCB, which are 93.64, 69.50 and 108.56% of glucan, xylan and lignin respectively. In this treatment, significant amount of glucan (21.87 %) is degraded, while xylan and lignin are reserved due to limited solubilisation. With this observation, one can conclude that coupling organosolv fractionation with alkaline post-extraction is not the good approach.

The hemicelluloses (in organosolv liquor) were not separated out as alcohol precipitation (used in this study) did not work, and time limitations did not allow for exploring other separation techniques like membrane technology, size exclusion chromatography and sub/supercritical antisolvent precipitation.

The obtained lignin had S/G ratio of 2.37. The slight increase in the ratio when compared to the feedstock (S/G ratio of 1.68), indicate that the treatment favoured solubilisation of syringyl. Some sugar (glucose) contamination was observed from during thioacidolysis analysis. FTIR analysis of SCB lignin (NaOH pre-extraction plus organosolv) confirmed that the obtained fraction is lignin, and it was oxidised. Presence of peaks related to carbohydrates confirmed sugar contamination, which was observed in thioacidolysis analysis.

Glucan recovered in the solid residue, following NaOH pre-extraction plus organosolv treatment of SCB, was 67.81 % based on virgin SCB, but 71.74 % based on pre-extracted SCB. Although this enrichment (71.74 %) is in within the range of 71 to 100 % mentioned in Section 2.3.2 for organosolv treatment, it is relatively lower than the one obtained through organosolv only (92.09 %). The decrease in glucan enrichment by 20.35 % results from combined effect of pre-extraction and organosolv. In NaOH pre-extraction, 5.48 % glucan is

dissolved, and 28.36 % glucan is dissolved in organosolv. The glucan dissolution of pre-extracted material is higher than of virgin SCB in organosolv. The enhanced glucan dissolution might be attributed to increased surface area, reduced lignin and xylan.

The glucan digestibility of the solid residue was found to be 100.41 %, and has been significantly improved (by 37.39 %) when compared to only organosolv treated solids (63.02 %). The glucan digestibility (100.41 %) obtained in this treatment corresponds to the one obtained in organosolv plus NaOH post-extracted solids (101.10 %). FTIR analysis of NaOH pre-extraction plus organosolv treated solid residue showed complete deacetylation, and near complete delignification.

Combining alkaline pre-extraction with organosolv fractionation yielded high lignin and xylan balances. High xylan balance is attractive for this study, as it confirms that degradation reactions were minimal (on solubilised oligomers and monomers). This fractionation combination also improved cellulose digestibility significantly. Since this fractionation approach yields highly digestible solid residues, has minimal sugar degradation, and achieves high delignification, it can be considered as best fractionation approach for SCB.

4.3.2. Organosolv fractionation of *E. grandis*

4.3.2.1. Organosolv fractionation of virgin *E. grandis*

The chemical compositions of the fractions obtained from organosolv fractionation of *E. grandis* are shown in Table 4.9. The solid recovery following organosolv fractionation of *E. grandis* was found to be 60.62 %, and this recovery is within the range of 47 to 75 % obtained by other researchers as shown in Section 2.3.2. As discussed in Section 4.3.1.1, solid recovery decreases with increasing severity of fractionation method.

Table 4.9: The chemical compositions of liquid and solid fractions obtained from organosolv fractionation of virgin *E. grandis*

	Organosolv hydrolysate	**Solid residue**	**Mass balance (%)**
Solid recovery (%)		60.62 ± 0.56	
Glucose (g/100g)	0.14 ± 0.03	38.61 ± 0.93	80.4 ± 4.6
Xylose (g/100g)	3.85 ± 0.03	4.81 ± 0.33	63.5 ± 3.9
Lignin (g/100g)	10.18 ± 0.14	14.93 ± 0.54	91.4 ± 2.5

The solubilised lignin was 10.18 (g/100g) based on dry raw biomass, which corresponds to 37.05% of lignin in *E. grandis*. The 37.05 % lignin dissolution falls within the range of 24 to 75 % shown in Section 2.3.2. The lower delignification corresponds to mild organosolv treatment, which resulted in higher solid recovery, while higher delignification corresponds to severe organosolv treatment, which resulted in lower solid recovery (Pan et al., 2006a; Hallac et al., 2010b; Koo et al., 2011a; 2011b; Romani et al., 2011). As mentioned earlier, high severity tends to enhance delignification.

The solubilised xylan was 3.85 (g/100g) based on dry raw biomass, which corresponds to 28.25 % of xylan in *E. grandis*. The 28.25 % xylan dissolution is within the range of 21 to 81 % shown in Section 2.3.2. The lower detected xylan in organosolv liquor was obtained at alkaline catalysed organosolv treatment, which was carried at lower temperatures and resulted in minimal xylan dissolution (Koo et al., 2011a; 2011b). Higher solubilised xylan in severe organosolv treatment liquor, but degradation of extracted xylan (~29 %) was also observed in severe organosolv treatment and led to lower xylan being detected (Pan et al., 2006a; Hallac et al., 2010b; Koo et al., 2011a). Also in autocatalysed organosolv, significant xylan was solubilised (Romani et al., 2011). The 28.25% xylan detected in organosolv liquor when compared to 64.71% which appears to be dissolved (in relation to xylan mass closure) is found to be lower, and this implies that degradation of xylans occurred.

The mass balance of glucan, xylan and lignin were found to be 80.4±4.6 %, 63.5±6.2 % and 91.4±2.5 % respectively. The mass balance values obtained in this treatment are slightly lower than 89.3, 71.3 and 115.8 % of glucan, xylan and lignin respectively, which were obtained in acid catalysed organosolv treatment of hybrid poplar (Pan et al., 2006a). In this treatment significant amount of carbohydrates (cellulose and hemicellulose) were solubilised, and solubilised sugars were degraded (leading to losses).

The obtained lignin had S/G ratio of 3.24, which is slight lower than S/G ratio of virgin *E. grandis* (4.35). The slight reduction in S/G ratio in the organosolv lignin (*E. grandis*) suggests that both guaiacyl and syringyl units, in *E. grandis*, were reactive towards condensation (Hallac et al., 2010a). FTIR analysis of *E. grandis* lignin (organosolv) had strong lignin peaks, as well as strong band of conjugated carbonyl groups (indicating oxidation). Carbohydrates contamination was very low.

Glucan recovery in the solid residue, following organosolv treatment of *E. grandis*, was 80.14 %. This enrichment is closer to the range of 83 to 109 % mentioned in Section 2.3.2. The lower glucan enrichment corresponds to lower solid recovery and results from severe organosolv treatment, while higher glucan enrichment corresponds to higher solid recovery and results from moderate organosolv (Pan et al., 2006a; Hallac et al., 2010b; Koo et al., 2011a; 2011b; Romani et al., 2011). Some glucan was solubilised in severe organosolv, and some of dissolved glucan was degraded which results in lower glucan enrichment in treated solids (Pan et al., 2006a; Koo et al., 2011a). Therefore, there was significant glucan dissolution in this study.

The glucan digestibility of the solid residue was found to be 45.70 %. The 45.70 % enzymatic digestibility obtained in this treatment is within the range of 39 to 97 % obtained for organosolv treatments of hardwoods, as shown in Section 2.3.2. The lower enzymatic digestibility was obtained in autocatalysed organosolv treatment (at 175°C) while higher enzymatic digestibility was obtained in severe organosolv treatment (Pan et al., 2006a; Hallac et al., 2010b; Koo et al., 2011a; 2011b; Romani et al., 2011). The glucan digestibility of 45.70 % is low for fermentation applications. FTIR analysis of organosolv treated solid residue (presented in Appendix B) shows partial deacetylation and delignification.

Auto-catalysed organosolv treatment of *E. grandis* resulted in low carbohydrates recoveries (for both cellulose and hemicellulose), due to degradation of solubilised sugars. The digestibility of recovered solids was also low. Based on recoveries and quality of fractions, auto-catalysed organosolv is not good enough for treating *E. grandis* and/or other hardwoods for biofuels and/or high value chemicals production. To enhance its performance, auto-catalysed organosolv can be coupled with alkaline extractions.

4.3.2.2. NaOH post-extraction of organosolv treated E. grandis

Similar conditions, as those used in Section 4.3.1 (80°C for 120 min at various NaOH concentrations), were explored. Both xylan and lignin dissolutions, which are shown in Figure 4.13, were observed to increase with increasing NaOH concentration (especially when NaOH concentration exceeds 8 M).

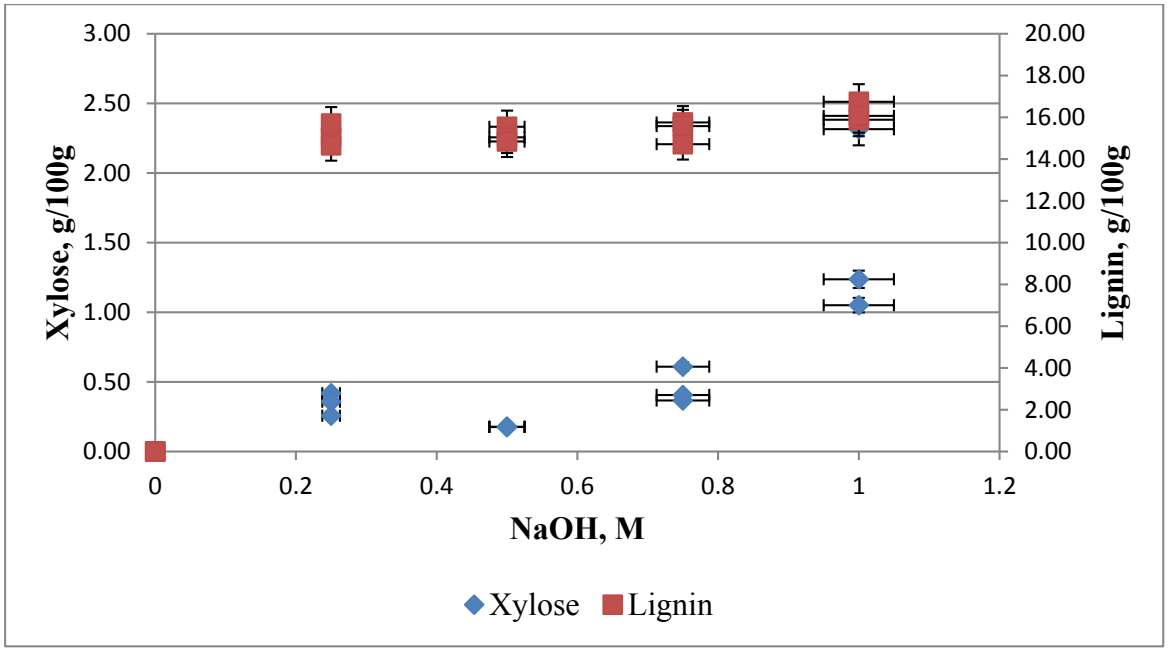

Figure 4.13: Xylan and lignin dissolution from organosolv treated *E. grandis* solids at different NaOH concentrations

Figure 4.14 shows the glucan retention in solid residues as NaOH concentrations increase. Glucan recovery was observed to decrease as NaOH concentrations increase, but the decrease was to very significant at those NaOH. However, to allow for clear comparison with SCB, similar conditions were used (0.5 M NaOH, 80°C for 120 min).

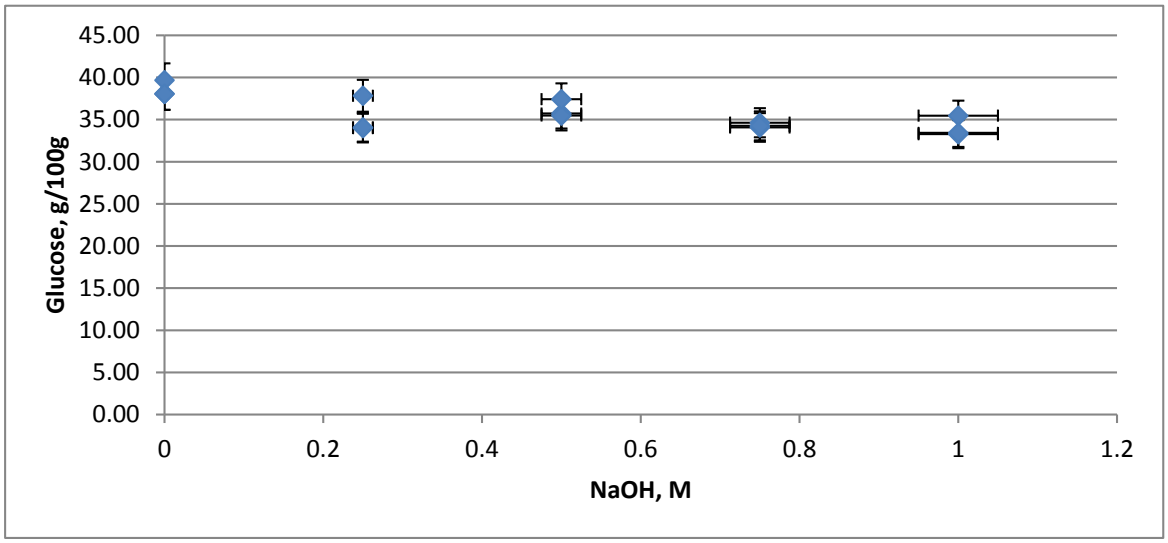

Figure 4.14: Glucan retention in *E. grandis* solids treated with organosolv method and NaOH post-extraction at different NaOH concentrations

The relation of enzymatic digestion and NaOH concentration is shown in Figure 4.15. The enzymatic digestibility of solids obtained from organosolv treatment and NaOH post-extraction of *E. grandis* increased with increasing NaOH concentration. The increase on enzymatic digestibility from 45.70 % (organosolv only) by 26 % at 0.25 M NaOH and by 48 % at 1.0 M NaOH was observed. As the NaOH concentration was increased (to 1.0 M), enzymatic digestibilities reached 93 %. This treatment yields highly digestible solids, which are good for biofuels and/or high value chemicals production.

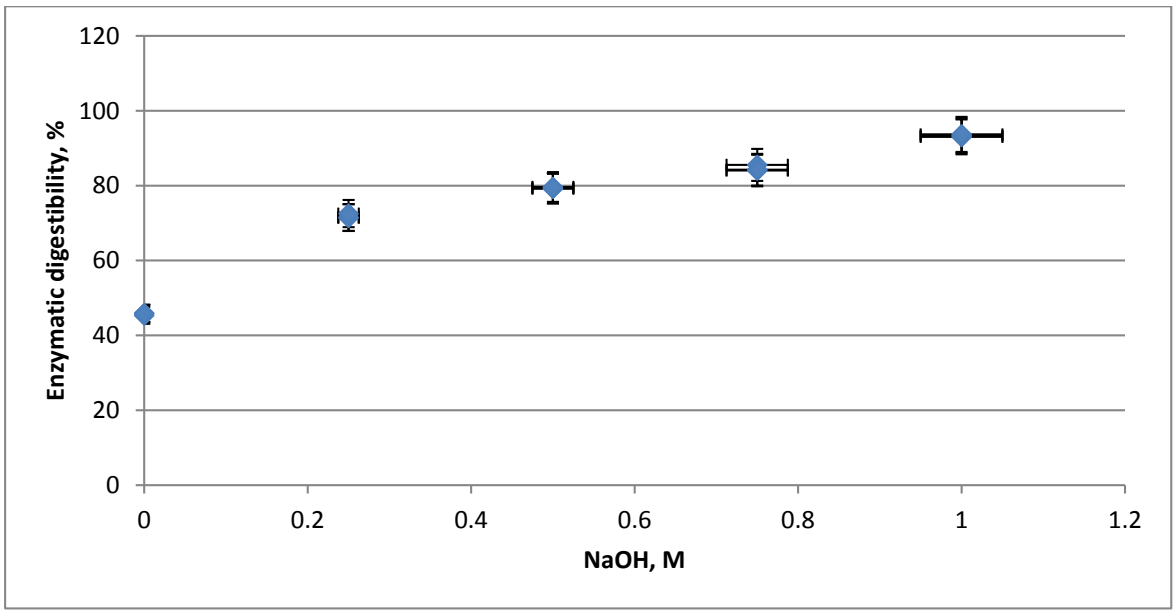

Figure 4.15: Enzymatic digestibility of organosolv treated and NaOH post-extracted *E. grandis* solids at different NaOH concentrations

The chemical compositions of the fractions obtained from organosolv fractionation plus NaOH post-extraction (at 80 °C, 0.5 M NaOH and 120 min) of *E. grandis* are shown in Table 4.10. The solid recovery following the treatment of *E. grandis* was found to be 40.3 %. The obtained solid recovery is lower than the one obtained in organosolv treatment alone (60.6 %). The decrease in solid recovery results from solubilisation of hemicelluloses and lignin in the alkaline post-extraction.

Table 4.10: The chemical compositions of liquid and solid fractions obtained from NaOH post-extraction of organosolv treated *E. grandis*

	Organosolv hydrolysate	**Solid residue**	**Mass balance (%)**	
			Based on virgin material	Based on organosolv treated material
Solid recovery (%)		40.27 ± 1.37		
Glucose (g/100g)	0.37 ± 0.04	36.21 ± 1.06	75.9 ± 3.7	94.7 ± 3.6
Xylose (g/100g)	0.08 ± 0.04	4.25 ± 0.23	31.8 ± 2.3	90.0 ± 7.9
Lignin (g/100g)	15.48 ± 0.88	1.91 ± 0.19	63.3 ± 1.3	116.5 ± 7.2

The solubilised lignin was 15.48 (g/100g) based on dry raw biomass, which corresponds to 56.3 % of lignin in virgin *E. grandis*, and 103.6 % of lignin based on organosolv treated material. The 103.6 % total lignin detected in the NaOH post-extraction liquor implies that near complete delignification was achieved.

The solubilised xylan was 0.08 (g/100g) based on dry raw biomass, which corresponds to 0.6 % of xylan in virgin *E. grandis*, and 1.7 % of xylan in organosolv treated *E. grandis*. The detected xylan in solution when NaOH post-extraction v. The low xylan detection indicates that alkaline post-extraction does not improve xylan recovery from organosolv treated materials.

The mass balance of glucan, xylan and lignin are shown in Table 4.10, for both virgin and organosolv treated materials' bases. The glucan balance following this treatment combination has decreased by 4.2% from the one obtained in organosolv treatment alone. The decrease is attributed to glucose dissolution in alkaline (NaOH) treatment and degradation of dissolved glucan (Vena, 2013). The xylan balance following this treatment is much lower than the one obtained using organosolv alone. The decrease is attributed to degradation of xylan. The lignin balance following this treatment combination has increased to 116.5 %. The increase in lignin is attributed to pseudo-lignin formation (Huijgen et al., 2010; Huijgen et al., 2012).

The molecular weight of the extracted hemicelluloses was found to be 8970 Da. The molecular weight obtained in this treatment is very low when compared to those obtained in alkaline extraction of hemicelluloses from *E. grandis* in this study (55991 Da). The significant decrease in size of extracted hemicelluloses is attributed to cleavage of lignin-

carbohydrates bonds and depolymerisation of hemicellulose during organosolv treatment (Xu et al., 2006c).

Extracted hemicellulose comprised mainly xylan. Xylose content (89% of neutral sugars) is the highest, followed by arabinose (11%), as shown in Figure 4.16. The high arabinose content might be related to the organosolv treatment that occurred prior to hemicelluloses extraction, which might have favoured retention of arabinosyl groups in organosolv treated *E. grandis*. The acid insoluble lignin content of *E. grandis* xylan (organosolv plus NaOH post-extraction) was found to be 28.41±0.38 %. When compared with Beechwood xylan, *E. grandis* xylan (organosolv plus NaOH post-extraction) has higher arabinose content, but xylose content is comparable.

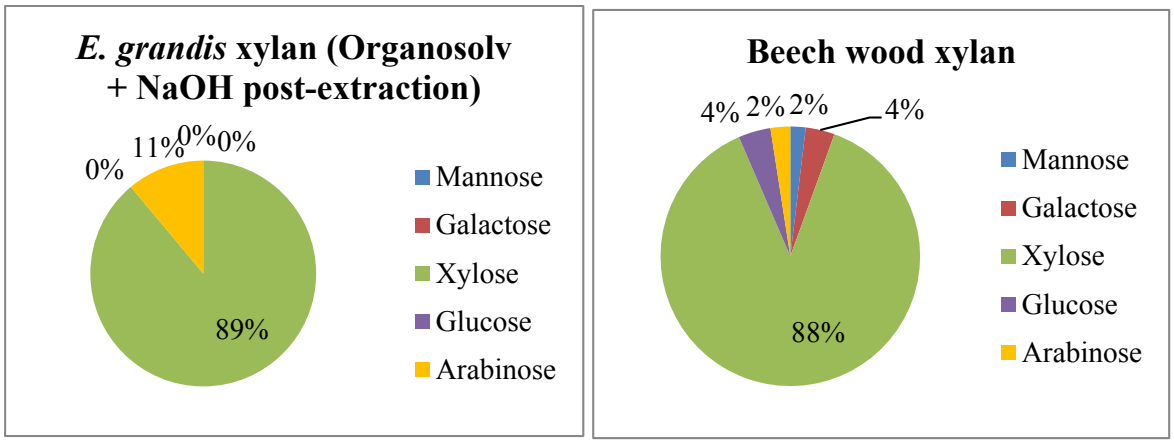

Figure 4.16: Neutral sugar compositions of *E. grandis* xylan (organosolv plus NaOH post-extraction) and Beechwood xylan (commercial)

The lignin content obtained is within the range of 3 to 38 %, which was found in alkaline extracted xylan from Eucalypts and other hardwoods (Chimphango, 2010; Gomes, 2012; Postma, 2012; Júnior et al., 2013; Vena, 2013). FTIR analysis of *E. grandis* xylan (organosolv plus NaOH post-extraction) confirms that the recovered material is hemicellulose. The strong intensity of lignin bands confirmed lignin contamination obtained when wet chemical method was used.

The obtained lignin had S/G ratio of 6.13, which is higher than S/G ratio of virgin *E. grandis* (4.35). An increase in S/G ratio indicates that dissolution of syringyl units was enhanced during this treatment. FTIR analysis of *E. grandis* lignin (organosolv plus NaOH post-extraction) confirmed that recovered material was lignin (aromatic ring vibrations). The recovered lignin was oxidised, and had some carbohydrates contamination.

Glucan recovered in the solid residue, following organosolv and NaOH post-extraction of *E. grandis*, was 75.16 %. This glucan recovery is lower than of 80.14 % (obtained organosolv only). This low glucan enrichment shows that 4.98 % of glucan was solubilised in NaOH post-extraction. Glucan digestibility of the solid residue was found to be 79.37 %. The 79.37 % enzymatic digestibility obtained in this treatment is higher than the one obtained with organosolv alone (45.70 %). NaOH post-extraction increased enzymatic digestibility of glucan by 33.67 %. The glucan digestibility of 79.37 % is good for biofuels and/or high value chemicals production. FTIR analysis of organosolv plus NaOH post-extracted solid residue shows complete deacetylation, near complete delignification, and pronounced cellulose peaks.

Coupling organosolv with alkaline post-extraction resulted in high delignification of virgin *E. grandis*. The recovered solid residues were highly digestible. High delignification and digestibility render this process attractive for fermentation-based biorefineries. However, the focus of this study is utilising hemicellulose biopolymers (oligomers and polymers), in biorefinery approach. So, this fractionation method does not meet the requirements, and is considered inadequate in this study.

4.3.2.3. Organosolv fractionation of NaOH pre-extracted E. *grandis*

Chemical compositions of the fractions obtained from organosolv fractionation of *E. grandis* are shown in Table 4.11. The solid recovery following organosolv fractionation of pre-extracted *E. grandis* was found to be 58.77 %, and this recovery is lower than 60.62 % (obtained in organosolv treatment of virgin *E. grandis*). The similar trend of decreasing solid recovery when pre-extraction step is included in organosolv has been observed by Huijgen et al. (2012). The solid recovery (58.77 %) obtained in this treatment is higher than the one obtained in organosolv plus NaOH post-extraction (40.27 %). The increase in solid recovery from this treatment when compared to organosolv plus NaOH post-extraction does not show direct impact of different severities in alkaline treatments (1.5 M NaOH in pre-extraction as compared to 0.5 M NaOH in post-extraction). Higher alkaline concentration enhanced delignification and hemicellulose dissolution, and is expected to yield lower solid recovery. So the higher solid recovery might be caused by neutralising effect of any residual NaOH (from pre-extraction) on hydronium ions (which enhance dissolution).

Table 4.11: The chemical compositions of liquid and solid fractions obtained from organosolv fractionation of NaOH pre-extracted *E. grandis*

	Organosolv hydrolysate	Solid residue	Mass balance (%)	
			Based on virgin material	Based on pre-extracted material
Solid recovery (%)		58.77 ± 2.01		
Glucose (g/100g)	0.28 ± 0.02	37.82 ± 0.04	79.08 ± 3.88	89.77 ± 1.55
Xylose (g/100g)	0.42 ± 0.04	5.09 ± 0.05	41.89 ± 4.95	86.77 ± 1.93
Lignin (g/100g)	7.23 ± 0.03	13.34 ± 0.04	74.85 ± 1.19	96.85 ± 3.56

The solubilised lignin was 7.23 (g/100g) based on dry raw biomass, which corresponds to 26.31 % of lignin in virgin *E. grandis*. But when comparing lignin solubilisation obtained to the one in alkaline pre-extracted solid, solubilised lignin is 34.04 %. The 34.04 % delignification is lower than 37.05 % lignin dissolution obtained with organosolv treatment of virgin *E. grandis*. The decrease in delignification could have been caused by lack of acid catalyst (absence of acetic acid from alkaline pre-extracted biomass and neutralising effect of any residual NaOH).

The solubilised xylan (detected) was 0.42 (g/100g) based on dry raw biomass, which corresponds to 3.08 % of xylan in *E. grandis*. But when comparing xylan solubilisation obtained to the one in alkaline pre-extracted solid, solubilised xylan is 6.61 %. The 6.61 % xylan dissolution is lower than 28.25% xylan dissolution obtained with organosolv treatment of virgin *E. grandis*. Decrease in xylan dissolution in organosolv following alkaline pre-extraction might be attributed to lack of acetic acid in pre-extracted material, which limits catalysis of xylan dissolution and delignification.

The mass balances of glucan, xylan and lignin were found to be 79.1±3.9 %, 41.9±5.0 % and 74.9±1.2 % respectively. The mass balances for organosolv fractionation of pre-extracted *E. grandis* were determined by relating the amount of components after organosolv to the virgin biomass. But when comparing with the NaOH pre-extracted material the balances would be 89.78, 89.92 and 96.84 % for glucose, xylose and lignin respectively. The mass balance values obtained in this treatment are different from the values obtained in organosolv treatment of virgin *E. grandis*, which are 80.4±4.6 %, 63.5±6.2 % and 91.4±2.5 % of glucan,

xylan and lignin respectively. In this treatment, minimal glucan and xylan degradations are observed, and lignin is reserved due to limited solubilisation.

The obtained lignin had S/G ratio of 4.75, which was slightly higher than ratio S/G ratio of virgin *E. grandis* (4.35). The slight increase in S/G ratio of the extracted lignin relates to high syringyl units being solubilised during fractionation. FTIR analysis of *E. grandis* lignin (NaOH pre-extraction plus organosolv) shows that recovered material was lignin (aromatic ring vibrations). The lignin sample was oxidised, and had some carbohydrates contamination.

Glucan recovery in the solid residue, following NaOH pre-extraction plus organosolv treatment of *E. grandis*, was 78.50 % based on virgin *E. grandis*, but 89.11 % based on pre-extracted *E. grandis*. Glucan recovery in solid residue of 89.11 % is higher than the one obtained through organosolv only (80.14 %). In NaOH pre-extraction, 11.91 % glucan is dissolved, and 10.89 % glucan is dissolved in organosolv. The glucan dissolution of pre-extracted material is lower than of *E. grandis* (19.86 % dissolved glucan) in organosolv. The decreased glucan dissolution might be attributed to removal of acetyl and carboxylic acid groups during alkaline pre-extraction, which limited autocatalysis to hydroxyl ions from water molecules only.

Glucan digestibility of the solid residue was found to be 100.82 %. The glucan digestibility, following this treatment, has been significantly improved (by 55.12 %) when compared to only organosolv treated solids (45.70 %). The glucan digestibility (100.82 %) obtained in this treatment is higher than the one obtained in organosolv and NaOH post-extracted solids (79.37 %). The difference in glucan digestibility might be attributed to different alkaline extraction severities (high severity in pre-extraction (1.5 M NaOH, 90 °C and 240 min) as compared to post-extraction (0.5 M NaOH, 80 °C and 120 min). The similar trend of increasing glucan digestibility of organosolv treated *E. globulus* as pre-extraction (auto-hydrolysis) severity increased has been observed (Romani et al., 2011). FTIR analysis of NaOH pre-extraction plus organosolv treated solid residue had complete deacetylation and near complete delignification.

Coupling organosolv with alkaline pre-extraction of hemicellulose, on treatment of E. grandis, enhances delignification and digestibility of solid residues. Alkaline pre-extraction also preserves hemicellulose in polymeric form, so when coupled with organosolv, it improves hemicellulose balance. Highly digestible solid residues, high delignification, and

improved hemicellulose balance allow this fractionation combination to be attractive in biopolymers (xylan) based biorefineries.

4.4. Ionic liquid fractionation

4.4.1. Ionic liquid fractionation of SCB

It was observed that when ionic liquids solutions of ≥66 % were used there was gel formation. The gel formation is attributed to either high viscosity of biomass to ionic liquid mixture, high solid loading (>5 %), or the type of antisolvent used (Dibble et al., 2011; Li et al., 2011a; Li et al., 2013). To break the gels, the method by Li and co-workers who used blender or homogenizer was employed (Li et al., 2013). It is also worth noting that xylose detection in liquid fraction was challenging. In ionic liquid liquor before autoclaving (acid hydrolysis), no xylose was detected in most treatments combinations. After acid hydrolysis of ionic liquid liquor, xylose was also not detected. When taking the pH values of acid hydrolysis of the liquor, the pH read ≥5. The near neutral pH of the liquor (which shows buffering effect of acetate ion in the ionic liquid) did not allow for hydrolysis of dissolved xylan, and that led to non-detection of xylose (Brandt et al., 2011). Since wet chemistry method did not work for xylose detection, other detection methods which do not require acid hydrolysis step like FTIR spectroscopy and capillary electrophoresis can be considered (Hyvärinen et al., 2014). The obtained solid fractions were further treated with cellulase and β-glucosidase, in order to evaluate their glucan digestibilities.

4.4.1.1. Optimisation of ionic liquid fractionation of virgin SCB

The experimental results for the CCD are shown in Table 4.12 for ionic liquid treatment of virgin SCB. The aqueous ionic liquid treatment of virgin SCB gave the solid recoveries ranging from 61 to 89%. The lowest solid recovery was obtained at the most severe treatment (at the highest temperature of 180.5°C). The highest temperature (180.5°C) is above the glass transition of lignin, so it does not only enhance carbohydrates dissolution and xylan degradation, but also gives high delignification (Li et al., 2011b). The higher solid recoveries corresponded to lower glucan digestibility, while lower solid recoveries corresponded to higher glucan digestibility. The similar trend for solid recoveries was observed in aqueous ionic liquid treatment of pre-extracted SCB. As for glucan digestibility of solid residues following aqueous ionic liquid treatment of pre-extracted SCB, there were few values which deviated (were lower than digestibility of pre-extracted SCB).

The obtained experimental results were analysed statistically in order to assess the impact of the factor variables on the response variables. The ANOVA table is presented in Table 4.13.

Table 4.12: The experimental results for ionic liquid treatment of virgin SCB

Run	IL conc. (%)	Temp (°C)	Time (min)	Solid fraction			Liquid fraction			Solid recovery (%)	Glucan enzymatic digestibility (%)
				Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)	Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)		
1	46.00	100.00	120.00	39.16	12.64	22.53	0.076	0.018	1.93	87.65	44.25
2	46.00	100.00	300.00	32.11	17.24	13.40	0.098	0.020	1.65	77.76	48.05
3	46.00	160.00	120.00	39.28	19.31	10.05	0.064	0.000	7.90	76.47	84.25
4	46.00	160.00	300.00	39.17	12.70	8.90	0.087	0.031	11.43	72.41	90.58
5	86.00	100.00	120.00	40.48	19.62	20.21	0.065	0.020	2.76	84.57	21.19
6	86.00	100.00	300.00	38.34	18.99	18.50	0.061	0.022	2.75	83.79	22.68
7	86.00	160.00	120.00	43.28	15.18	16.50	0.090	0.022	8.24	77.14	69.86
8	86.00	160.00	300.00	31.56	15.36	9.03	0.091	0.022	6.81	76.61	76.10
9	32.36	130.00	210.00	29.60	14.78	16.09	0.048	0.000	2.05	89.09	24.85
10	99.64	130.00	210.00	35.80	15.30	10.10	0.091	0.009	4.53	62.44	91.84
11	66.00	79.55	210.00	38.27	12.55	25.94	0.019	0.000	1.47	89.81	43.75
12	66.00	180.45	210.00	35.45	7.62	5.14	0.132	0.018	11.99	60.90	100.15
13	66.00	130.00	58.64	39.68	14.05	22.37	0.084	0.010	3.69	75.37	42.52
14	66.00	130.00	361.36	39.61	12.80	15.90	0.117	0.009	8.62	73.75	72.32
15	66.00	130.00	210.00	26.46	12.73	14.05	0.056	0.000	4.03	81.72	79.92
16	66.00	130.00	210.00	26.39	12.72	14.34	0.061	0.000	4.02	81.33	80.22
17	66.00	130.00	210.00	24.23	11.80	14.51	0.062	0.000	4.28	81.20	84.04
18	66.00	130.00	210.00	17.93	8.66	14.73	0.057	0.000	5.73	81.85	81.89

Table 4.13: The regression coefficients and ANOVA of response surfaces for ionic liquid treatment of virgin SCB

Coefficient	Solid recovery	Glucan (SF)	Xylan (SF)	Lignin (SF)	Glucan (LF)	Xylan (LF)	Lignin (LF)	Glucan EH
Mean/Interc.	81.2954	23.71514	11.31072	14.46046	0.059221	-0.000541	4.51529	81.7252
(1)Ionic liquid conc. (%)(L)	-5.4193	2.10216	1.19052	-0.10279	0.008225	0.004861	0.27047	5.1812
Ionic liquid conc. (%)(Q)	-2.0354	6.65421	4.01055	-1.41446	0.005563	0.007921	-0.87177	-18.2441
(2)Temp (°C)(L)	-11.6796	-0.22531	-2.08572	-9.54308	0.032428	0.003735	6.29669	40.9300
Temp (°C)(Q)	-2.3245	9.59424	0.51004	0.31515	0.009820	0.011153	1.56327	-8.6305
(3)Time (min)(L)	-2.6363	-3.09795	-0.66825	-4.44521	0.014131	0.004875	1.48187	9.9536
Time (min)(Q)	-2.8856	11.56412	2.86865	2.85553	0.027701	0.011661	1.15449	-18.9039
1L by 2L	0.4825	-2.78847	-2.55122	0.95227	0.019859	0.002150	-1.55317	4.8912
1L by 3L	3.1578	-1.67634	0.38994	0.27381	-0.011893	-0.007765	-1.17158	-0.5995
2L by 3L	1.5167	-0.65931	-2.60066	0.55614	0.001607	0.006903	0.59892	1.8198
Lack of fit	416.977	69.5883	59.5459	76.6094	0.003755	0.000689	12.0252	549.193
R^2	0.611	0.855	0.617	0.845	0.681	0.629	0.923	0.712
Std. Error	0.095	16.117	3.722	0.082	0.000	0.000	0.667	3.575

The ANOVA table was obtained by fitting the data into the second-order polynomial regression model at 95 % confidence interval; the lack of fit was significant for solid recovery, lignin retention, glucan dissolution, and glucan digestibility. The lack of fit was not significant for glucan retention, xylan retention, xylan dissolution, and lignin dissolution. However, the regression coefficients for xylan retention and xylan dissolution were low. Low regression coefficient for xylan dissolution is related to lack of quantification of dissolved xylan (other techniques needed). As for retained xylan, the low regression coefficient was accompanied by high standard error. The lack of fit for recovered solids, retained lignin, and solubilised glucan corresponds to very small standard error (pure error), and the similar trend of lack of fit for small pure error has been observed in aqueous ionic liquid treated of wheat straw (Fu and Mazza, 2011b). Significant lack of fit occurs when either the model poorly fits the data or when model fits the data sufficiently but measurements are very precise so that the pure error becomes very small (Fu and Mazza, 2011b). As for lack of fit of enzymatic hydrolysis was accompanied by large pure error.

The Pareto charts and response surfaces were used to study the effects of all factors involved in ionic liquid treatment. The effects of ionic liquid treatment factors (ionic liquid concentration, temperature and time) on the responses (glucan, xylan, lignin, solid recovery and enzymatic digestibility) are shown in Appendix D for treatment of virgin SCB. For glucan retention, both quadratic terms of time and temperature are statistically significant. This implies that glucan retention decreases as longer times and higher temperatures are used. The effect of time and temperature on glucan retention is also supported by linear and quadratic terms of both time and temperature being statistically significant on glucan dissolution. Xylan retention was mainly affected by ionic liquid concentration (quadratic term), followed by time (quadratic term), temperature (linear term), interaction of temperature with time, and interaction of ionic liquid concentration with time. Xylan retention was favoured by higher ionic liquid concentration. The higher ionic liquid content favoured xylan retention because when there is low water content, minimum hydroxyl ions from water molecules are produced, thus limiting/reducing the acidity of the reaction medium, and allowing for enhanced buffering effect of acetate anion in the [EMIM]OAc used (Brandt et al., 2011; Zhang et al., 2013a). The aqueous ionic liquid (with lower ionic liquid content) requires elevated temperatures (>150 °C) or longer reaction times to enhance the treatment, which turn to solubilise and degrade significant amount of xylan (Brandt et al., 2011; Fu and Mazza, 2011a; 2011b; Zhang et al., 2013a). Although none of the factors are

statistically significant at 95% confidence level, the quadratic terms of time, temperature and ionic liquid tend to affect xylan dissolution to some extent. For lignin retention and dissolution, temperature was the most statistically significant factor. As temperature increases, the delignification rate increases, thus leading to increase in concentration of lignin in the liquid fraction. Longer reaction times and higher ionic liquid concentration also enhanced delignification, which resulted in lower lignin content in solid fraction. The similar trend of delignification has been observed on aqueous ionic liquid treatment of wheat straw (Fu and Mazza, 2011b).

The Pareto charts and response surface plots (shown in Appendix D) were used to obtain the extent at which the factors were significant. The effect of temperature was the most significant factor in both solid recovery and glucan digestibility of solids. As temperature increased, delignification and xylan dissolution increased, leading to lower solid recoveries and highly digestible solids. The effects of ionic liquid concentrations and longer reaction times also enhanced delignification and xylan dissolution, thus reducing solid recovery and improving glucan digestibility.

The ionic liquid fractionation of virgin SCB into cellulose, hemicellulose and lignin was desirable. The antisolvent (acetone/water mixture) used was supposed to be selective for separation of solubilised lignin from carbohydrates, through precipitation of most carbohydrates into the solid residue (Lan et al., 2011; Diedericks et al., 2012b). However, following ionic liquid CCD experiments, it was observed that significant portion of carbohydrates (particularly xylan) was not recovered in solid fraction. As a result, the desirability plots of the CCD experimental results had to be performed in order to get the desirable treatment conditions (considered as a compromise) for all the components. The method for the determination of desirabilities shown in Table 4.14 was used in the analysis of CCD experimental results, which was carried out in STATISTICA 11.

Table 4.14: The approach used for determining desirabilities in aqueous ionic liquid fractionation of virgin SCB (Adapted from (Diedericks et al., 2012b))

Stream	Component	Goal	Min. conc.	Max. conc.
Liquid	Glucose	Min	40.70	0.0
	Xylose	Min	20.26	0.0
	Lignin	Max	0.0	21.17
Solid	Glucose	Max	0.0	40.70
	Xylose	Max	0.0	20.26
	Lignin	Min	21.17	0.0
	Desirability	Max	0.0%	100%

The desirability plot shown in Figure 4.26 was obtained from STATISTICA 11 analysis, and it provided the desirable treatment conditions of 32 % ionic liquid concentration, 180.45 °C and 361.36 min. The low ionic liquid concentration is attractive as it limits the viscosity challenges affiliated to high ionic liquid concentrations. The temperature of 180.45 °C is very high, it is higher than glass transition temperature of lignin, so it is expected to enhance delignification, xylan dissolution, as well as xylan degradation to some extent (Li et al., 2011b). The desirable conditions obtained are different from the optimum conditions obtained on aqueous treatment of wheat straw, which were 49.5 % ionic liquid concentration, 158° C and 216 min (Fu and Mazza, 2011b). The difference is attributed to different factor levels used, consideration of liquid fraction in this study which was not considered on wheat straw treatment, and desirability which was based on mass balance in this study while optimum conditions were based on fermentable sugars recovery in treatment of wheat straw (Fu and Mazza, 2011b).

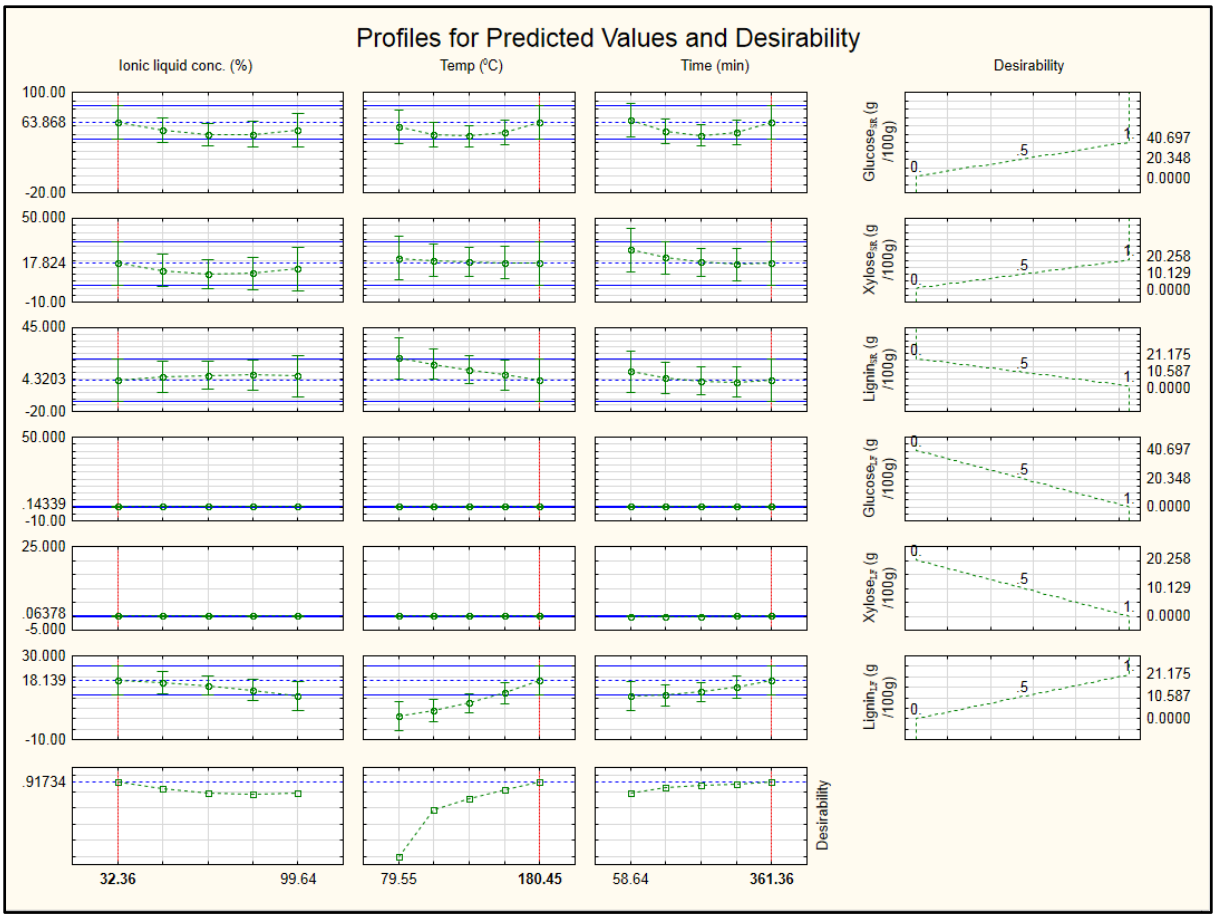

Figure 4.17: The desirability plot for aqueous ionic liquid treatment of virgin SCB

It was observed that treatment temperature significantly affected the ionic liquid fractionation of virgin SCB. High temperatures enhanced cellulose (glucan), hemicellulose (xylan) and lignin dissolutions. High ionic liquid concentrations limited glucan and xylan dissolutions. Therefore, it can be said that the most significant factors in ionic liquid fractionation of virgin SCB are ionic liquid concentration and temperature, while residence time is the least. The obtained desirable conditions were later tested and compared with other approaches.

4.4.1.2. Optimisation of ionic liquid fractionation of NaOH pre-extracted SCB

The experimental results for the CCD are shown Table 4.15 for ionic liquid treatment of pre-extracted SCB. The solid recovery for ionic liquid treatment of pre-extracted SCB is abbreviated as P-SCB and as V-SCB in relation to virgin SCB (in Table 4.15). The ANOVA table is presented in Table 4.16 for statistical analysis of data.

Table 4.15: The experimental results for ionic liquid treatment of pre-extracted SCB

Run	IL conc. (%)	Temp (°C)	Time (min)	Solid fraction			Liquid fraction			Solid recovery (%)		Glucan enzymatic digestibility (%)
				Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)	Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)	P-SCB	V-SCB	
1	46.00	100.00	120.00	35.90	1.40	2.85	0.011	0.037	1.83	75.81	44.96	87.30
2	46.00	100.00	300.00	38.17	2.01	2.43	0.041	0.015	2.24	73.26	43.45	91.39
3	46.00	160.00	120.00	36.90	1.78	1.50	0.030	0.000	6.65	71.75	42.55	98.17
4	46.00	160.00	300.00	35.09	1.29	2.39	0.040	0.015	11.96	63.91	37.90	100.87
5	86.00	100.00	120.00	34.13	1.73	2.54	0.022	0.016	3.44	74.46	44.16	83.23
6	86.00	100.00	300.00	37.03	1.84	2.46	0.018	0.014	2.74	74.02	43.90	79.19
7	86.00	160.00	120.00	34.85	1.61	1.48	0.023	0.017	9.54	73.08	43.34	95.28
8	86.00	160.00	300.00	33.07	1.60	1.04	0.312	0.042	4.87	71.66	42.50	97.72
9	32.36	130.00	210.00	31.23	1.50	2.28	0.034	0.000	2.46	92.31	54.75	69.31
10	99.64	130.00	210.00	32.09	1.20	2.33	0.037	0.072	3.07	62.00	36.77	100.88
11	66.00	79.55	210.00	35.91	1.60	1.85	0.006	0.014	1.44	94.58	56.10	45.65
12	66.00	180.45	210.00	33.25	1.07	0.92	0.029	0.007	11.51	61.15	36.27	101.85
13	66.00	130.00	58.64	32.50	1.27	2.21	0.010	0.016	3.57	66.50	39.44	89.11
14	66.00	130.00	361.36	31.48	1.28	1.77	0.024	0.005	9.13	64.64	38.34	93.21
15	66.00	130.00	210.00	31.27	1.44	2.12	0.031	0.000	3.85	72.31	42.89	89.70
16	66.00	130.00	210.00	29.95	1.38	2.20	0.036	0.000	3.67	72.75	43.14	90.32
17	66.00	130.00	210.00	29.10	1.35	2.22	0.049	0.000	3.82	72.39	42.93	89.62
18	66.00	130.00	210.00	28.89	1.35	2.13	0.049	0.000	3.67	71.88	42.63	89.23

Table 4.16: The regression coefficients and ANOVA of response surfaces for ionic liquid treatment of pre-extracted SCB

Coefficient	Solid recovery	Glucan (SF)	Xylan (SF)	Lignin (SF)	Glucan (LF)	Xylan (LF)	Lignin (LF)	Glucan EH
Mean/Interc.	72.3997	29.67122	1.361483	2.157874	0.022162	0.000007	0.868854	89.29568
(1)Ionic liquid conc. (%)(L)	-6.2215	-0.80973	-0.028214	-0.229607	0.021198	0.003841	-0.036043	4.50885
Ionic liquid conc. (%)(Q)	2.8213	2.48836	0.128354	0.187890	0.007326	0.004592	-0.149158	0.49708
(2)Temp (°C)(L)	-10.7443	-1.43448	-0.231393	-0.796352	0.028692	-0.000548	1.348408	21.29986
Temp (°C)(Q)	3.3237	4.55712	0.117397	-0.460676	0.000271	0.001349	0.458270	-7.52388
(3)Time (min)(L)	-2.2514	-0.02060	0.034802	-0.115932	0.028587	-0.000047	0.328865	1.76804
Time (min)(Q)	-5.3743	2.72804	0.073732	-0.035590	-0.000018	0.001341	0.437977	4.78863
1L by 2L	2.4176	-0.28981	-0.004967	-0.272106	0.038755	0.002998	-0.365626	2.55984
1L by 3L	2.1316	0.16586	-0.011511	-0.246059	0.034358	0.001450	-0.642375	-2.09802
2L by 3L	-1.5659	-2.18816	-0.306875	0.237472	0.038292	0.002978	0.053360	1.27261
Lack of fit	549.193	28.7430	0.561504	0.840310	0.008895	0.000042	1.02957	0.606
R^2	0.510	0.757	0.448	0.813	0.635	0.789	0.898	0.643
Sdt. Error	0.125	4.029	0.002	0.002	0.000	0.000	0.129	0.202

For ionic liquid treatment of pre-extracted SCB, the CCD statistical analysis (shown in Table 4.16) was obtained by fitting the data into the second-order polynomial regression model. At 95% confidence interval, the lack of fit was significant for solid recovery, xylan retention, lignin retention, glucan dissolution, lignin dissolution and glucan digestibility, and lack of fit was insignificant for glucan retention and xylan dissolution. When compared with the lack of fit in ionic liquid treatment of virgin SCB, the lack of fit is significant for xylan retention and lignin dissolution in the treatment of pre-extracted SCB while non-significant in treatment of virgin SCB. The R^2 values in fitting the experimental data obtained in treatment of pre-extracted SCB into second-order regression model showed a decrease from a range of 0.61–0.92 to the range of 0.45–0.90. A change in the range might be related to poor fitting of retained xylan after treatment of pre-extracted SCB. In the alkaline pre-extraction of SCB, 71 % of the original xylan and all acetyl groups were removed, and this removal together with residual alkali might have masked the effect of ionic liquid treatment on pre-extracted SCB.

In ionic liquid fractionation of pre-extracted SCB, glucan retention was mainly affected by reaction temperature. At higher temperatures, glucan dissolution was enhanced, resulting in lower glucan retained in the solid fraction. Time and ionic liquid concentration affected glucan retention, as well. As for dissolved glucan, higher temperatures, longer reaction times and high ionic liquid concentration lead to increased glucan concentration in liquid fraction.

Xylan retention was mostly affected by ionic liquid concentration. With increasing ionic liquid concentration, xylan retention increased. Xylan retention was favoured by higher ionic liquid concentration. This trend is similar to the one observed in ionic liquid treatment of virgin SCB. For xylan dissolution, none of the treatment factors was statistically significant. However, the quadratic terms of time, temperature and ionic liquid concentration tend to affect xylan dissolution to some extent.

Lignin retention was affected by linear terms of temperature and time, quadratic terms of time and ionic liquid concentration, as well as interaction of ionic liquid concentration with temperature were statistically significant. As temperature increases, delignification increased, which yielded lower lignin retention. As ionic liquid concentration increased delignification increased, while very longer reaction times did not have a large impact on delignification increase. Temperature strongly influenced degree of delignification. Near complete delignification was achieved at higher temperatures. The delignification trend in this treatment corresponded to the one observed in ionic liquid treatment of virgin SCB.

Temperature was the most significant factor in solid recovery and glucan digestibility of the solids. The treatment at higher temperatures yielded low solid recoveries, but highly digestible solids. Both ionic liquid concentration and time needed to be coupled with higher temperatures (as shown in response surfaces) in order to enhance delignification and xylan dissolution, which yielded lower solid recoveries but highly digestible solids.

The desirability test was performed, following statistical analysis in order to obtain desirable fractionation conditions. The method for the determination of desirabilities shown in Table 4.17 was used in the analysis of CCD experimental results, which was carried out in STATISTICA 11.

Table 4.17: The approach used for determining desirabilities in aqueous ionic liquid fractionation of pre-extracted SCB (Adapted from (Diedericks et al., 2012b))

Stream	Component	Goal	Min. conc.	Max. conc.
Liquid	Glucose	Min	38.47	0.0
	Xylose	Min	6.27	0.0
	Lignin	Max	0.0	8.28
Solid	Glucose	Max	0.0	38.47
	Xylose	Max	0.0	6.27
	Lignin	Min	8.27	0.0
	Desirability	Max	0.0%	100%

The desirability plot shown in Figure 4.27 was obtained from STATISTICA 11 analysis, and it provided the desirable treatment conditions of 99.64 % ionic liquid concentration, 180.45 °C of temperature and 58.64 min of time. Although high ionic liquid concentration is obtained as desirable concentration in this treatment, high ionic liquid concentration has viscosity challenges, as well as gel formation challenges. The temperature of 180.45°C is very high, it is higher than glass transition temperature of lignin, so it is expected to enhance delignification, xylan dissolution, as well as xylan degradation to some extent (Li et al., 2011b). The shorter treatment time of close to an hour is attractive as it can minimise degradation of dissolved carbohydrates. The desirable conditions obtained differ from those obtained in ionic liquid treatment of virgin SCB. The difference is attributed to significant delignification and xylan dissolution which occurred in alkaline pre-extraction step.

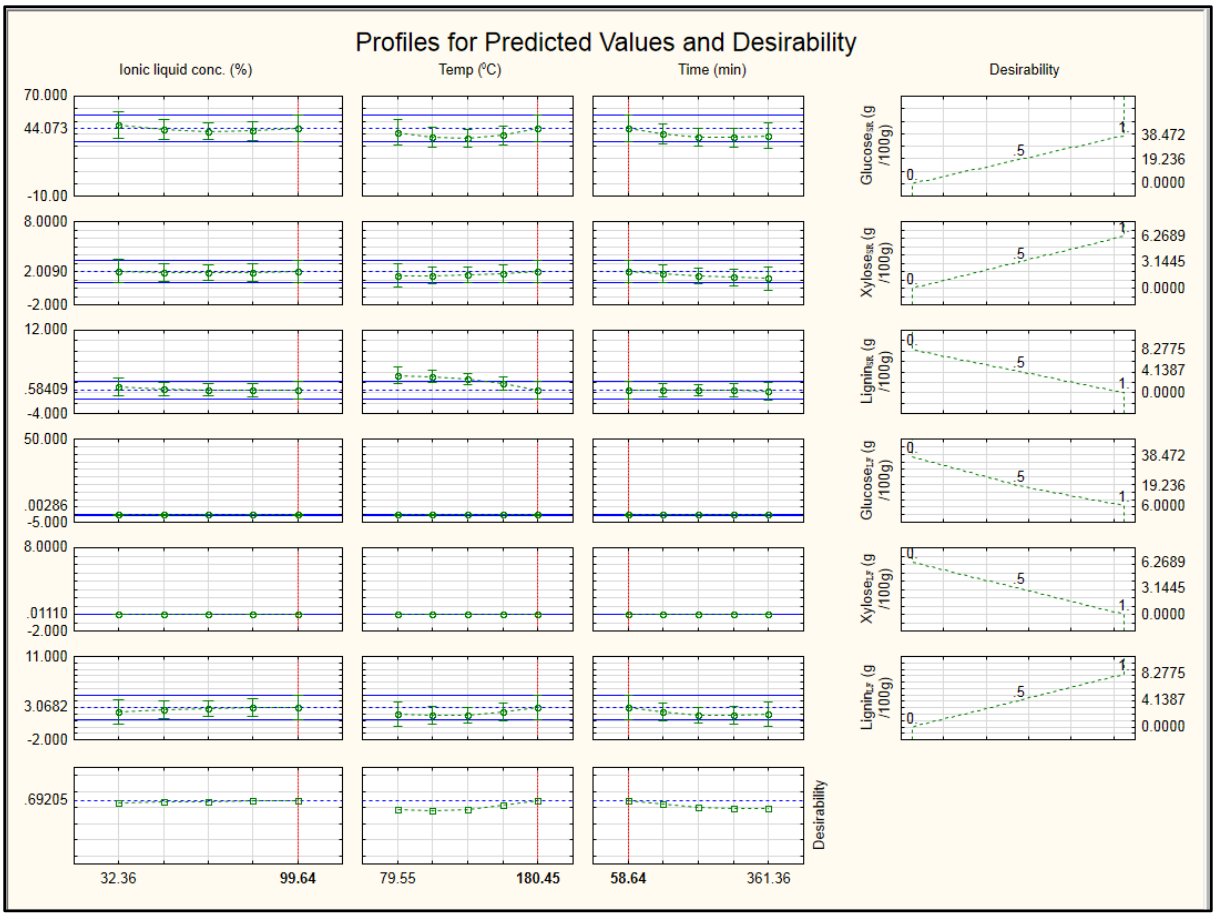

Figure 4.18: The desirability plot for aqueous ionic liquid treatment of pre-extracted SCB

Following ionic liquid fractionation of both virgin and pre-extracted SCB, it is clear that temperature enhances delignification and glucan digestibility of recovered solid residues. At high temperature and low ionic liquid concentration, xylan was susceptible to degradation. Testing desirable conditions on both virgin (32 %w/w ionic liquid, 180.5 ºC and 361 min) and pre-extracted (99.6 %w/w ionic liquid, 180.5 ºC and 59 min) SCB will allow for clear understanding of impacts of ionic liquid fractionation on lignocellulose components, particularly xylan and lignin (glucan digestibility has been already been explored during optimisation experiments).

4.4.1.3. Ionic liquid treatments of SCB at the desirable conditions

The desirable conditions for aqueous ionic liquid treatment of virgin SCB were found to be 32 % ionic liquid concentration, 180.5 °C, and 361.64 min. The reaction time obtained was not only longer than the one obtained on wheat straw treatment (216min) by Fu and Mazza (2011b), but also longer than the one obtained on *E. grandis* treatment (210 min) from this study. Considering the type of biomass, SCB treatment is supposed to use less severe

conditions when compared to *E. grandis*/hard woods treatment. Hemicellulose and lignin in herbaceous materials are extremely reactive to pre-treatment as compared those in hardwoods and softwoods (Ren and Sun, 2010; Vena, 2013). As a result, 210 min was used for treatment of virgin SCB at desirable conditions instead of 361.64 min.

The chemical compositions of the fractions obtained from ionic liquid fractionation of virgin SCB are shown in Table 4.18. The solid recovery following ionic liquid fractionation of SCB was found to be 45.43 %, and this recovery is slightly the range of 47.7 to 94.4 % obtained by other researchers as shown in Section 2.3.3. The lowest solid recovery (47.7 %) was obtained when cholinium ionic liquid was used (An et al., 2015). The lowest solid recovery was accompanied by significant solubilisation of xylan (63.2 %) and lignin (80.6 %), 90 % of original glucan was retained in the solids, and the solids obtained had 74.6 % glucan digestibility, which is considered as fair digestibility (An et al., 2015). The highest solid recovery (94.4 %) was obtained when imidazolium ([BMIM]OAc) ionic liquid was used (Audu et al., 2012). The highest solid recovery was accompanied by significant glucan retention (99.6 %), lower delignification (11.8 %) and low xylan dissolution (11.2%), and the solids obtained had 84.6 % glucan digestibility, which is considered as highly digestible (Audu et al., 2012).

Table 4.18: The chemical compositions of liquid and solid fractions obtained from ionic liquid fractionation of virgin SCB

	Ionic liquid hydrolysate	**Solid residue**	**Mass balance (%)**
Solid recovery (%)		45.43 ± 1.60	
Glucose (g/100g)	0.40 ± 0.01	36.53 ± 0.80	90.7 ± 1.9
Xylose (g/100g)	3.77 ± 0.12	5.50 ± 0.15	45.8 ± 2.8
Lignin (g/100g)	10.53 ± 0.01	5.31 ± 0.25	74.8 ± 1.6

The determined lignin in liquid fraction was 10.53 (g/100g) based on dry raw biomass, which corresponds to 49.74 % of lignin in original SCB. However, 74.92 % of lignin in original SCB was solubilised, so 25.18 % remained in liquid fraction following acid precipitation as both acid soluble lignin and degraded lignin products. The 74.92 % lignin dissolution falls within the range of 3.9 to 82.8 % shown in Section 2.3.3. The 4 % detected lignin in liquid fraction was obtained when phosphate containing ionic liquid was used (Socha et al., 2014).

The 4 % precipitated lignin content from ionic liquid fraction (16.4 % delignification) was obtained in moderate ionic liquid treatment (Diedericks et al., 2012b). Diedericks and co-workers (Diedericks et al., 2012b) were not able to precipitate 74 % of solubilised lignin, and that was related to acid soluble lignin and lignin degradation products. The incomplete precipitation of lignin matches the observation in this study (although only 34 % of solubilised lignin was not precipitated in this study).

The xylan detected in liquid fraction was 3.77 (g/100g) based on dry raw biomass, which corresponds to 18.61 % of xylan in original SCB. However, 72.85 % of xylan in original SCB was solubilised, so 54.24 % of xylan was degraded during this treatment. The 72.85 % xylan dissolution is within the range of 2.1 to 89.4 % shown in Section 2.3.3. 2.1 % detected xylan in liquid fraction corresponded to 9.5 % xylan dissolution, and was obtained when combination of high ionic liquid concentration (as received) with low temperature (100 °C) and short reaction time (60 min) was used (Diedericks et al., 2012b). 89.4 % xylan dissolution was obtained when a combination of high ionic liquid concentration (as received) with high temperature (160 °C) and long reaction time (180 min) was used (Li et al., 2011a). When comparing the xylan dissolution ranges with the xylan dissolution obtained in this treatment, it can be seen that high temperatures and long reaction times lead to high xylan solubilisation. As for xylan degradation, it is evident that it occurs even at moderate conditions (lower temperatures and shorter reaction times). Diedericks and co-workers obtained 78 % degradation of solubilised xylan at moderate conditions (Diedericks et al., 2012b).

The mass balance of glucan, xylan and lignin were found to be 90.74±1.88, 45.76±2.81 and 74.82±1.58 % respectively. In aqueous ionic liquid treatments (with ≤ 50% ionic liquid content), the researchers focused mainly on solid residue and extracted lignin, which led to not preparing the mass balances (Fu and Mazza, 2011a; 2011b; Hou et al., 2013). When considering the non-aqueous ionic liquid treatment of biomass, it is evident that pre-treatment to obtain digestible solids for biofuels and/or high value chemicals production was key objective of most researchers as compared to fractionation for materials and chemicals production (see Section 2.3.3). The mass balances from the ionic liquid fractionation of herbaceous and agricultural residues include glucan balances of 94.8, 95.0 and 95.4 % (Diedericks et al., 2012b; Karatzos et al., 2012b; Li et al., 2013). The xylan mass balances reported include 77.1, 92.53 and 94.84 %, while lignin mass balances include 49.18 and

87.83 % (Diedericks et al., 2012b; Karatzos et al., 2012b; Li et al., 2013). The lignin determination in ionic liquid fraction was based on acid precipitation method, and that did not account for acid soluble lignin and lignin degradation products, leading to low mass balances of lignin (Diedericks et al., 2012b; Karatzos et al., 2012b). When comparing the reported mass balances, the low xylan balance observed in this study is attributed to degradation of dissolved xylan at the temperature and ionic liquid concentrations used. Therefore, these fractionation conditions are not favourable for xylan as 54.24 % xylan is degraded.

The obtained lignin had S/G ratio of 2.13. There is slight increase in the ratio when compared to the feedstock (S/G ratio of 1.68), and this implies that syringyl units were highly solubilised during ionic liquid fractionation. This S/G ratio (2.13) is slightly higher than the one obtained from ionic liquid treatment of bamboo (1.18), and the difference might be attributed to different feedstocks and reaction conditions (Yang et al., 2013). FTIR analysis of SCB lignin (ionic liquid treatment) showed oxidation of lignin sample.

Glucan recovery in the solid residue, following ionic liquid treatment of SCB, was 89.75 %. This enrichment is within the range of 78.2 to 100 % for both aqueous and non-aqueous ionic liquid treatment of herbaceous and agricultural residues, as mentioned in Section 2.3.3. The glucan digestibility of the solid residue was found to be 101.21 %. The 101.21 % enzymatic digestibility obtained in this study is within the range of 25.1 to 100 % obtained for ionic liquid treatments of herbaceous biomass or agricultural residues, as shown in Section 2.3.3. The high digestibility obtained in this study was attributed to significant xylan dissolution and delignification (75 %) at high treatment temperature. FTIR analysis of ionic liquid treated solid residue (the centre point solids used) indicates that ionic liquid fractionation of virgin SCB resulted in partial deacetylation and significant delignification.

Following ionic liquid fractionation of virgin SCB, the effect of using alkaline post-extraction was explored, and are presented below.

The chemical compositions of the fractions obtained from ionic liquid fractionation plus NaOH post-extraction (at 80 °C, 0.5 M NaOH and 120 min) of SCB are shown in Table 4.19. The solid recovery following the treatment of SCB was found to be 45.04 %. The obtained solid recovery is slightly lower than the one obtained in ionic liquid treatment alone (45.43 %). The decrease in solid recovery results from solubilisation of cellulose, hemicelluloses and lignin in the alkaline post-extraction.

Table 4.19: The chemical compositions of liquid and solid fractions obtained from NaOH post-extraction of ionic liquid treated SCB

	Ionic liquid hydrolysate	**Solid residue**	**Mass balance (%)**	
			Based on virgin material	Based on ionic liquid treated material
Solid recovery (%)		45.04 ± 1.50		
Glucose (g/100g)	0.79 ± 0.10	32.90 ± 0.59	82. 8 ± 2.0	92.2 ± 2.6
Xylose (g/100g)		4.54 ± 0.27	22.4 ± 1.3	82.5 ± 5.4
Lignin (g/100g)	3.63 ± 0.00	4.26 ± 0.63	37.3 ± 3.0	148.6 ± 13.8

The determined lignin in all liquid fractions (ionic liquid and alkaline) was 3.63 (g/100g), based on dry raw biomass, which corresponds to 17.9 % of lignin in virgin SCB, and 66 % in ionic liquid treated SCB. The high mass balance of lignin (148.6 %), indicates near-complete delignification and formation of pseudo-lignin (Tan et al., 2009). No xylan was detected in NaOH post-extraction liquor. The non-detection of solubilised xylan indicates that some degradation and side reactions (particularly formation of pseudo-lignin) occurred (Tan et al., 2009). The mass balance of glucan shown in Table 4.19 indicates that glucan dissolved in alkaline post-extraction was degraded.

The molecular weight of the extracted hemicelluloses was found to be 11040 Da. This molecular weight is as good as the one obtained in ionic liquid treatment of bamboo, which is 10020 Da (Yang et al., 2013). However, when compared with the molecular weights obtained in alkaline extraction of hemicelluloses from herbaceous and agricultural residues (shown in Section 4.2.1), this molecular weight is very low. The significant decrease in size of extracted hemicelluloses is attributed to cleavage of lignin-carbohydrates bonds and depolymerisation of hemicellulose during ionic liquid treatment plus NaOH post-extraction (Yang et al., 2013).

The neutral sugar composition of the extracted hemicellulose, mainly xylan (in relative percentages) was calculated the total detected neutral sugars shown in Appendix C. The neutral sugar composition is shown in Figure 4.28. Xylose was the dominant neutral sugar (81.4 % of neutral sugars), followed by arabinose (9.1 %), glucose (4.4 %), galactose (3.4 %), and mannose (1.7 %). The trend of high xylose content in the hemicelluloses obtained from

alkaline post-extraction of ionic liquid treated solids, followed by arabinose was observed in hemicelluloses from ionic liquid treatment of corncob, whereby xylose ranged from 70.79 to 71.77 % and arabinose ranged from 19.13 to 18.66% have been observed (Sun et al., 2012). Although xylose was the dominant neutral sugar (52.97 and 73.25 %) in ionic liquid fractionation of bamboo and SCB, glucose (42.75 and 16.58 %) was second instead of arabinose (Lan et al., 2011; Yang et al., 2013).

The acid insoluble lignin content of SCB xylan (ionic liquid treatment plus NaOH post-extraction) was determined gravimetrically (masses shown in Appendix B), and was found to be 3.99±0.10 %. The lignin content obtained is comparable to the 3.54 % obtained in hemicellulose fraction from ionic liquid fractionation of SCB (Lan et al., 2011). When compared with Beechwood xylan, SCB xylan (ionic liquid treatment plus NaOH post-extraction) has higher arabinose, but glucose, arabinose and mannose are comparable.

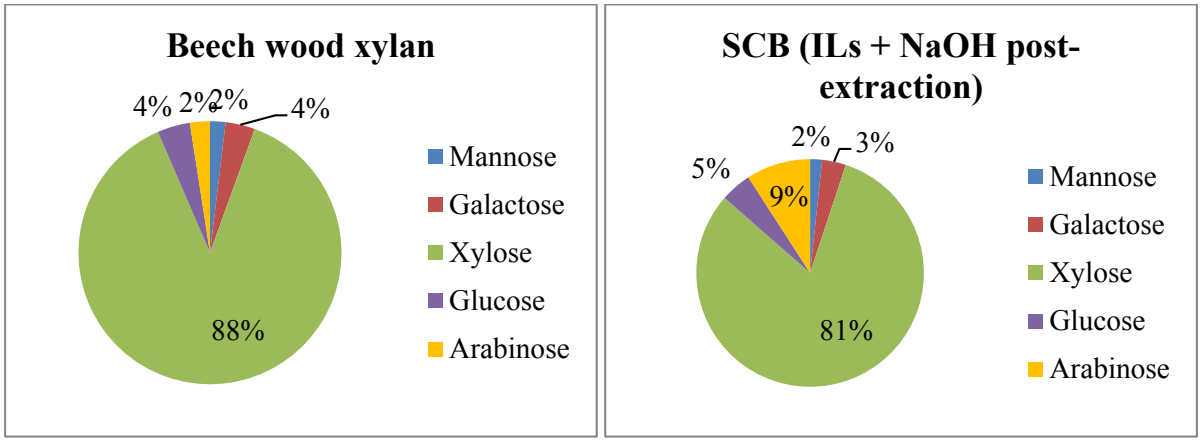

Figure 4.19: Neutral sugar compositions of SCB xylan (ionic liquid treatment plus NaOH post-extraction) and Beechwood xylan (commercial)

The acid insoluble lignin content of SCB xylan (ionic liquid treatment plus NaOH post-extraction) was determined gravimetrically (masses shown in Appendix B), and was found to be 3.99±0.10 %. The lignin content obtained is comparable to the 3.54 % obtained in hemicellulose fraction from ionic liquid fractionation of SCB (Lan et al., 2011). When compared with Beechwood xylan, SCB xylan (ionic liquid treatment plus NaOH post-extraction) has higher arabinose, but glucose, arabinose and mannose are comparable. FTIR spectra of SCB xylan (ionic liquid treatment plus NaOH post-extraction) confirmed that obtained xylan had low lignin content.

The recovered lignin was oxidised, and clean of sugar contaminants, as provided by FTIR analysis.

Glucan recovery in the solid residue, following ionic liquid treatment and NaOH post-extraction of SCB, was 80.84 %. This enrichment is within the range of 78.2 to 100 % mentioned in Section 2.3.3. When compared to glucan enrichment of 89.75 % (obtained ionic liquid treatment only), this glucan enrichment is low. This low glucan enrichment shows that 8.91 % of glucan was solubilised in NaOH post-extraction. The glucan digestibility of the solid residue was found to be 100.94 %. The 100.94 % enzymatic digestibility obtained in this treatment is comparable to the one obtained with ionic liquid treatment alone (101.21 %). The similarity of enzymatic digestibility shows that ionic liquid treatment gave highly digestible solids, and effect of NaOH post-extraction could not be accounted for. The glucan digestibility of 100.94% is good for biofuels and/or high value chemicals production. However, this high digestibility has been achieved at the expense of glucan recovery, and high glucan losses are undesirable (Kim et al., 2014a). Solid residue obtained from ionic liquid treatment and NaOH post-extracted was completely deacetylated and highly delignified.

Ionic liquid treatment was also carried out on alkaline pre-extracted SCB, and chemical compositions of the fractions obtained are shown in Table 4.20. The solid recovery following ionic liquid fractionation of pre-extracted SCB was found to be 46.09 %, and this recovery is slightly higher than 45.43 % (obtained in ionic liquid treatment of virgin SCB). The solid recovery (46.09 %) obtained in this treatment is slightly higher than the one obtained in ionic liquid treatment plus NaOH post-extraction (45.04 %). The increase in solid recovery from this treatment when compared to ionic liquid treatment only and to ionic liquid plus NaOH post-extraction is attributed to different severities in ionic liquid treatments (high ionic liquid concentration plus short reaction time as compared to low ionic liquid concentration plus longer reaction time in treatment of virgin SCB). Higher ionic liquid concentration protected hemicelluloses and lignin from excessive dissolution and degradation, thus yielding higher solid recovery compared to low ionic liquid concentration treatments.

Table 4.20: The chemical compositions of liquid and solid fractions obtained from ionic liquid fractionation of pre-extracted SCB

	Ionic liquid hydrolysate	**Solid residue**	**Mass balance (%)**	
			Based on virgin material	Based on pre-extracted material
Solid recovery (%)		46.09 ± 1.70		
Glucose (g/100g)	1.14 ± 0.07	28.52 ± 1.29	72.87 ± 4.41	77.10 ± 4.64
Xylose (g/100g)	0.78 ± 0.05	4.31 ± 0..47	25.12 ± 9.43	81.18 ± 7.93
Lignin (g/100g)	4.99 ± 0.05	4.59 ± 0.40	45.25 ± 4.18	115.70 ± 6.42

The solubilised lignin (determined) was 4.99 (g/100g) based on dry raw biomass, which corresponds to 23.57 % of lignin in virgin SCB. But when comparing lignin solubilisation obtained to the one in alkaline pre-extracted solid, solubilised lignin is 60.27 %. The 60.27 % delignification is higher than 49.74 % lignin dissolution obtained with ionic liquid treatment of virgin SCB.

The solubilised xylan (detected) was 0.78 (g/100g) based on dry raw biomass, which corresponds to 3.85 % of xylan in SCB. However, when comparing xylan solubilisation obtained to the one in alkaline pre-extracted solid, solubilised xylan is 12.44 %. The 12.44 % xylan dissolution is lower than 18.61 % xylan dissolution obtained with ionic liquid treatment of virgin SCB. Decrease in xylan dissolution in ionic liquid treatment following alkaline pre-extraction might be attributed to by lack of acid catalyst (absence of acetic acid from alkaline pre-extracted biomass, neutralising effect of any residual NaOH and buffering effect of ionic liquid used), which limits catalysis of xylan dissolution.

Chemical characterisation of both streams (liquid and solid fractions) allow for preparing the mass balances of the ionic liquid fractionation of pre-extracted SCB. The mass balance of glucan, xylan and lignin were found to be 72.87±4.41, 25.12±9.43 and 45.25±4.18 % respectively. The mass balances for ionic liquid fractionation of pre-extracted SCB were determined by relating the amount of components after ionic liquid to the virgin biomass. But when comparing with the NaOH pre-extracted material the balances would be 77.10, 81.18 and 115.70 % for glucose, xylose and lignin respectively. The mass balance values obtained in this treatment are different from the values obtained in ionic liquid treatment of virgin

SCB, which are 90.74, 45.76 and 74.82 % of glucan, xylan and lignin respectively. In this treatment, significant amount of glucan (13.64 %) is degraded, while xylan and lignin are reserved due to limited solubilisation.

The S/G ratio of the obtained lignin was found to be 0.96. There is slight decrease in the ratio when compared to the feedstock (S/G ratio of 1.68). The slight decrease in S/G ratio of the extracted lignin might contributed to impact of high delignification during NaOH pre-extraction. The recovered lignin was oxidised (based on FTIR analysis).

Glucan recovery in the solid residue, following NaOH pre-extraction plus ionic liquid treatment of SCB, was 70.07 % based on virgin SCB, but 74.02 % based on pre-extracted SCB. This glucan recovery is relatively lower than the one obtained through ionic liquid treatment only (89.75 %). The decrease in glucan recovery results from combined effect of pre-extraction and ionic liquid treatment. In NaOH pre-extraction, 5.48 % glucan is dissolved, and 25.86 % glucan is dissolved in ionic liquid treatment. The glucan dissolution of pre-extracted material is higher than of virgin SCB in ionic liquid treatment. The enhanced glucan dissolution might be attributed to increased surface area, reduced lignin and xylan.

The glucan digestibility of the solid residue was found to be 100.73 %. The glucan digestibility following this treatment is comparable to only ionic liquid treated solids (101.21 %), and to that of ionic liquid treatment plus NaOH post-extracted solids (100.94 %).

It was observed that all ionic liquid fractionation approaches yielded highly digestible cellulose-rich solid residues. Ionic liquid proved to be appropriate in producing high quality cellulosic materials, which can be applied in production of fermentation-based fuels and chemicals. High cellulose digestibility was accompanied by high delignification and xylan dissolution. Since high severity (high temperature coupled with low solvent concentration) was used, degradation of solubilised xylan was significant. High xylan degradation is undesirable for high value application of hemicelluloses, and renders ionic liquid fractionation of virgin SCB undesirable for biopolymer-based biorefineries. Coupling ionic liquid fractionation with alkaline conditions, particularly pre-extraction, showed improved hemicellulose (xylan) preservation. Improved xylan preservation, rendered combination of alkaline pre-extraction and ionic liquid fractionation to be the best ionic liquid fractionation approach for SCB.

4.4.2. Ionic liquid fractionation of *E. grandis*

The optimisations of ionic liquid fractionation of *E. grandis* were carried out following CCD experimental design. The levels of temperature (100 and 160 ºC), residence time (120 min and 300 min), and aqueous ionic liquid concentration (46 %w/w and 86 %w/w), which were motivated for in Section 4.4, were used. Similarly, the response variables were: solid recovery, glucan (retained and solubilised), xylan (retained and solubilised), lignin (retained and solubilised), and glucan enzymatic digestibility.

The challenges of gel formation when ≥66 % ionic liquid concentrations were used, was also observed during fractionation of *E. grandis*. The gels were broken down using blender.

4.4.2.1. Optimisation of ionic liquid fractionation of virgin *E. grandis*

The experimental results of the CCD for ionic liquid fractionation of virgin *E. grandis* are shown in Table 4.21. The ANOVA table is presented in Table 4.22 for statistical analysis of data.

Table 4.21: The experimental results for ionic liquid treatment of virgin *E. grandis*

Run	IL conc. (%)	Temp (°C)	Time (min)	Solid fraction			Liquid fraction			Solid recovery (%)	Glucan enzymatic digestibility (%)
				Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)	Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)		
1	46.00	100.00	120.00	37.71	8.53	25.63	0.01	0.01	1.45	86.12	5.10
2	46.00	100.00	300.00	38.20	7.92	25.11	0.01	0.01	1.98	83.40	5.66
3	46.00	160.00	120.00	40.75	11.16	15.98	0.03	0.00	5.06	69.58	62.07
4	46.00	160.00	300.00	39.03	6.62	14.07	0.01	0.05	11.85	67.35	85.49
5	86.00	100.00	120.00	43.65	13.42	25.33	0.02	0.00	1.27	72.65	5.42
6	86.00	100.00	300.00	42.34	12.59	23.52	0.02	0.00	1.58	71.48	8.38
7	86.00	160.00	120.00	45.07	7.65	15.51	0.02	0.00	9.89	69.48	97.33
8	86.00	160.00	300.00	41.90	7.51	11.45	0.02	0.00	11.74	68.05	98.02
9	32.36	130.00	210.00	41.14	13.37	21.33	0.03	0.00	1.31	88.95	10.45
10	99.64	130.00	210.00	45.03	9.77	19.46	0.03	0.00	6.17	66.31	78.51
11	66.00	79.55	210.00	40.76	9.31	28.12	0.01	0.00	0.98	88.03	4.59
12	66.00	180.45	210.00	53.99	9.82	7.19	0.00	0.00	16.65	62.32	100.67
13	66.00	130.00	58.64	40.09	9.39	27.57	0.01	0.00	2.00	70.78	10.10
14	66.00	130.00	361.36	44.03	10.01	21.81	0.00	0.00	4.92	69.97	20.40
15	66.00	130.00	210.00	41.86	12.47	20.89	0.03	0.00	2.96	85.13	32.19
16	66.00	130.00	210.00	41.51	12.74	21.07	0.04	0.00	2.97	85.39	31.66
17	66.00	130.00	210.00	42.43	12.96	21.58	0.03	0.00	2.91	85.32	31.86
18	66.00	130.00	210.00	41.15	12.57	22.23	0.05	0.00	3.26	85.37	31.97

Table 4.22: The regression coefficients and ANOVA of response surfaces for ionic liquid treatment of virgin *E. grandis*

Coefficient	Solid recovery	Glucan (SF)	Xylan (SF)	Lignin (SF)	Glucan (LF)	Xylan (LF)	Lignin (LF)	Glucan EH
Mean/Interc.	85.3124	41.89454	12.72060	21.5074	0.038653	-0.000410	3.019348	31.52564
(1)Ionic liquid conc. (%)(L)	-9.2059	3.48598	0.12937	-1.1927	0.003401	-0.009923	0.901757	24.20351
Ionic liquid conc. (%)(Q)	-5.5165	-0.44994	-1.09503	-1.3072	-0.001185	0.003666	0.279342	12.40512
(2)Temp (°C)(L)	-12.0702	3.96751	-1.26783	-11.3900	0.001962	0.003304	4.290795	70.28894
Temp (°C)(Q)	-7.2569	2.58408	-2.51509	-3.2438	-0.021665	0.003669	2.074010	18.17484
(3)Time (min)(L)	-1.3039	0.13254	-0.74403	-2.6343	-0.006027	0.007041	1.055224	6.58436
Time (min)(Q)	-10.6482	-1.17414	-2.41736	1.7274	-0.021301	0.003668	0.180703	-8.25893
1L by 2L	6.4965	-0.72236	-3.04991	-0.2994	-0.005575	-0.005640	0.662873	11.18813
1L by 3L	0.5885	-0.81304	1.04490	-0.8605	0.004565	-0.012019	-0.645030	-5.08163
2L by 3L	0.0590	-1.01677	-0.81008	-0.9149	-0.002969	0.010562	0.975928	5.14891
Lack of fit	70.605	85.3022	25.70842	9.7576	0.000318	0.000836	7.3467	1347.33
R^2	0.950	0.605	0.704	0.980	0.868	0.591	0.979	0.940
Sdt. Error	0.136	0.294	0.047	0.360	0.000	0.000	0.025	0.048

The aqueous ionic liquid treatment of virgin *E. grandis* gave the solid recoveries ranging from 62 to 89 %. The lowest solid recovery was obtained at the most severe treatment (at the highest temperature of 180.5°C). The highest temperature (180.5°C) is above the glass transition of lignin, so it does not only enhance carbohydrates dissolution and xylan degradation, but also gives high delignification (Li et al., 2011b). High solid recoveries corresponded to lower glucan digestibility while lower solid recoveries corresponded to high glucan digestibility.

The CCD statistical analysis (shown in ANOVA Table 4.22) was obtained by fitting the data into the second-order polynomial regression model. At 95% confidence interval, the lack of fit was significant for solid recovery, glucan retention, xylan retention, lignin dissolution, and glucan digestibility. The lack of fit was not significant for lignin retention, glucan dissolution, and xylan dissolution. The trend of lack of fit obtained in this treatment differs from the one obtained in aqueous ionic liquid treated of virgin SCB, in which glucan retention and xylan retention had non-significant lack of fit. However, this trend relates to the one obtained in aqueous ionic liquid treatment of wheat straw, in which glucan retention and xylan retention had significant lack of fit (Fu and Mazza, 2011b). Considering the R^2 values, which ranged from 0.59 to 0.98, the second-order regression model fairly fit the experimental results. The low R^2 values (0.59 for xylan dissolution, 0.61 for glucan retention and 0.70 for xylan retention) confirm lack of fit, and are accompanied by very small pure errors. Very low value of pure error for xylan dissolution (0.00) is related to limitations in determining amount of dissolved xylan. The range of R^2 values obtained for this treatment is comparable to the one obtained in aqueous ionic liquid treatment of virgin SCB (0.61 to 0.92).

From statistical analysis, it was observed that glucan retention was mainly influenced by both linear and quadratic terms of temperature, linear term of ionic liquid concentration, and quadratic term of time. Temperature appears to be the most significant factor for glucan retention. High temperatures tend to enhance glucan dissolution, thus reducing glucan retention. Higher ionic liquid concentrations tend to retain glucan in the solids. The higher ionic liquid concentrations render the reaction medium basic, thus limiting glucan dissolution. The quadratic terms of temperature and time mainly affect glucan dissolution. The longer reaction times and higher temperatures enhance glucan dissolution.

Xylan retention was affected by all terms and interactions of temperature, time and ionic liquid concentration, except for the linear term of ionic liquid (which was not statistically

significant). Higher reaction temperatures tend to solubilise significant amount of xylan, yielding the solids which have very low xylan content. Longer reaction times also enhanced xylan dissolution. Higher ionic liquid concentrations retain most xylan in solid fraction. The ability of high ionic liquid concentrations to favour xylan retention has been explained in Section 4.4.1, and is related to buffering effect of acetate ion in the [EMIM]OAc used (Brandt et al., 2011; Zhang et al., 2013a). Although none of the factors is statistically significant at 95 % confidence level, the linear terms of ionic liquid concentration and time, interactions of time with ionic liquid and with temperature tend to affect xylan dissolution to some extent.

Lignin retention and dissolution were mainly influenced by temperature (both linear and quadratic terms). The impacts of linear and quadratic terms of both time and ionic liquid concentration followed. As temperature increases, the delignification rate increases, thus leading to increase in concentration of lignin in the liquid fraction. Temperatures greater than glass transition temperature of lignin (>150°C) enhanced delignification to near complete (Li et al., 2011b). Longer reaction times and higher ionic liquid concentration also enhanced delignification, which resulted in lower lignin content in solid fraction. The similar trend of delignification has been observed on aqueous ionic liquid treatment of SCB (in this study) and of wheat straw (Fu and Mazza, 2011b).

The effect of temperature was the most significant factor in both solid recovery and glucan digestibility of solids, while time and ionic liquid concentration followed. As temperature increased, delignification and xylan dissolution increased, leading to lower solid recoveries and highly digestible solids. The effects of ionic liquid concentrations and longer reaction times also enhanced delignification and xylan dissolution, thus reducing solid recovery and improving glucan digestibility

The desirability plot of the CCD experimental results had to be performed in order to get the desirable treatment conditions (considered as a compromise) for all the components (glucan, xylan and lignin). The method for the determination of desirabilities shown in Table 4.25 was used in the analysis of CCD experimental results, which was carried out in STATISTICA 11.

Table 4.23: The approach used for determining desirabilities in aqueous ionic liquid fractionation of virgin *E. grandis* (Adapted from (Diedericks et al., 2012b))

Stream	Component	Goal	Min. conc.	Max. conc.
Liquid	Glucose	Min	48.18	0.0
	Xylose	Min	13.63	0.0
	Lignin	Max	0.0	27.48
Solid	Glucose	Max	0.0	48.18
	Xylose	Max	0.0	13.63
	Lignin	Min	27.48	0.0
	Desirability	Max	0.0%	100%

The desirability plot shown in Figure 4.29 was obtained from STATISTICA 11 analysis, and it provided the desirable treatment conditions of 32 % ionic liquid concentration, 180.45 °C of temperature and 210 min of time. The low ionic liquid concentration is attractive as it limits the viscosity challenges affiliated to high ionic liquid concentrations. The temperature of 180.45 °C is higher than glass transition temperature of lignin, so it is expected to enhance delignification, xylan dissolution, as well as xylan degradation to some extent (Li et al., 2011b). The desirable conditions obtained are different from the optimum conditions obtained on ionic liquid treatment of virgin SCB (shorter reaction time in *E. grandis* ionic liquid treatment), and ionic liquid treatment of wheat straw (49.5 % ionic liquid concentration, 158 °C and 216 min), but the reaction time is comparable to the one obtained in wheat straw treatment (Fu and Mazza, 2011b). The difference is attributed to different factor levels used, consideration of liquid fraction in this study which was not considered on wheat straw treatment, and desirability which was based on mass balance in this study while optimum conditions were based on fermentable sugars recovery in treatment of wheat straw (Fu and Mazza, 2011b).

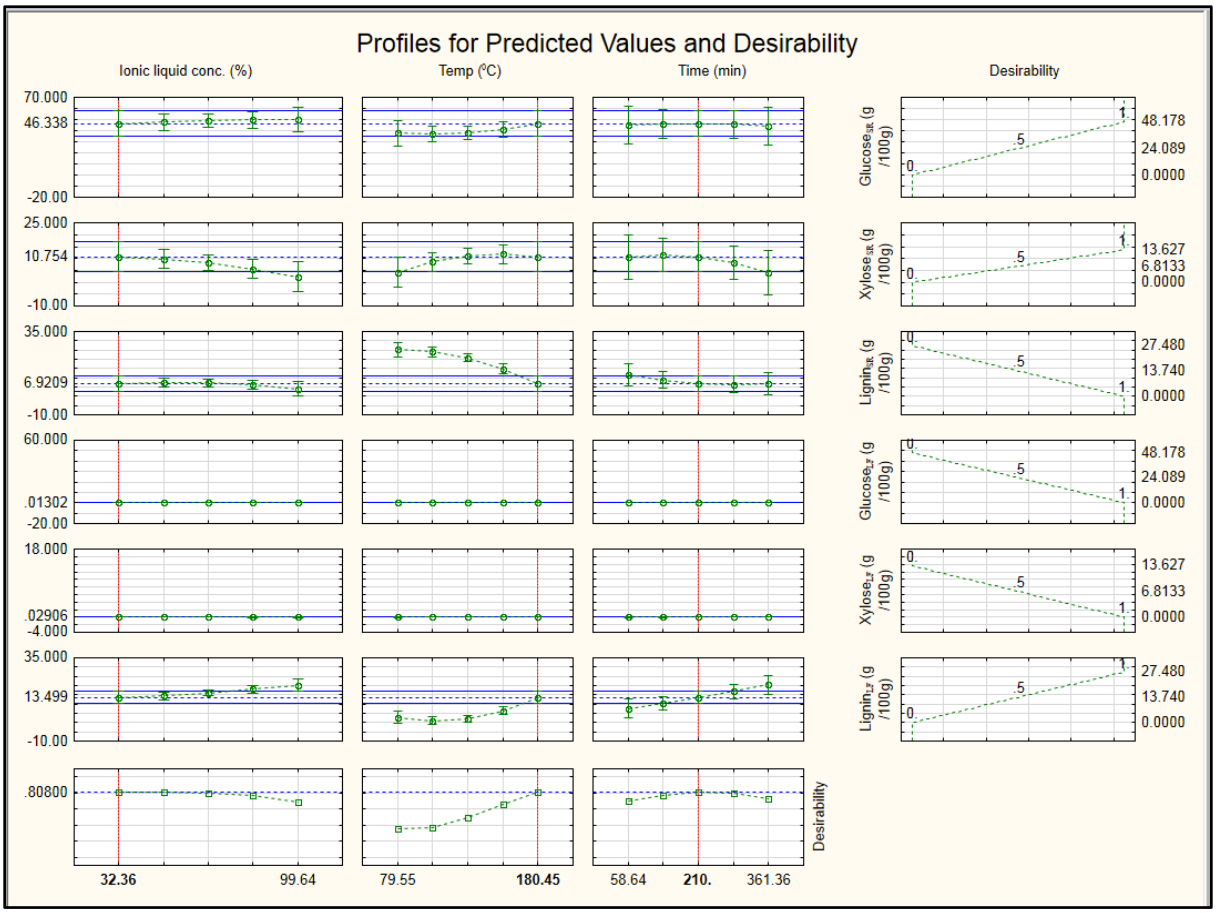

Figure 4.20: The desirability plot for aqueous ionic liquid treatment of virgin *E. grandis*

In ionic liquid fractionation of *E. grandis*, it was observed that treatment temperature significantly affected (high temperatures) enhanced cellulose (glucan), hemiceelulose (xylan) and lignin dissolutions. High ionic liquid concentrations limited glucan and xylan dissolutions. The obtained desirable conditions were later tested and compared with other approaches.

4.4.2.2. Optimisation of ionic liquid fractionation for NaOH pre-extracted *E. grandis*

The experimental results for the CCD are shown 4.24 for ionic liquid treatment of pre-extracted *E. grandis*. The solid recovery for ILs treatment of pre-extracted *E. grandis* is abbreviated as P-E and as V-E in relation to virgin *E. grandis* (in Table 4.24). The ANOVA table is presented in Table 4.25 for statistical analysis of data.

Table 4.24: The experimental results for ionic liquid treatment of pre-extracted *E. grandis*

Run	**IL conc. (%)**	**Temp (°C)**	**Time (min)**	**Solid fraction**			**Liquid fraction**			**Solid recovery (%)**		**Glucan enzymatic digestibility (%)**
				Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)	Glucan (g/100g)	Xylan (g/100g)	Lignin (g/100g)	P-E	V-E	
1	46.00	100.00	120.00	32.06	1.97	16.70	0.07	0.00	0.44	89.35	69.75	40.28
2	46.00	100.00	300.00	35.38	2.18	16.18	0.08	0.00	0.81	88.34	68.96	40.38
3	46.00	160.00	120.00	34.47	1.76	12.91	0.08	0.00	1.47	78.51	61.29	62.95
4	46.00	160.00	300.00	30.06	1.38	10.80	0.12	0.67	2.64	76.69	59.87	99.83
5	86.00	100.00	120.00	29.88	1.58	16.79	0.16	0.23	0.86	84.46	65.93	64.99
6	86.00	100.00	300.00	30.51	1.32	15.38	0.12	0.00	1.53	84.28	65.79	69.23
7	86.00	160.00	120.00	27.95	1.20	12.17	0.11	0.00	2.89	79.31	61.91	98.90
8	86.00	160.00	300.00	26.87	0.92	10.42	0.14	0.00	5.88	78.86	61.56	99.92
9	32.36	130.00	210.00	29.52	1.42	16.04	0.16	0.16	0.69	96.23	75.12	34.73
10	99.64	130.00	210.00	33.62	1.30	15.23	0.12	0.00	1.55	68.18	53.22	100.01
11	66.00	79.55	210.00	34.49	1.74	15.80	0.10	0.03	0.28	94.44	73.72	36.59
12	66.00	180.45	210.00	34.01	1.81	6.63	0.08	0.00	7.02	61.55	48.05	100.27
13	66.00	130.00	58.64	35.27	1.82	14.42	0.13	0.14	3.01	78.99	61.66	39.92
14	66.00	130.00	361.36	33.47	1.60	13.53	0.11	0.02	4.40	77.92	60.83	44.49
15	66.00	130.00	210.00	36.39	2.30	15.98	0.09	0.01	1.10	88.92	69.41	40.36
16	66.00	130.00	210.00	37.26	2.03	16.12	0.08	0.00	1.09	89.03	69.50	40.66
17	66.00	130.00	210.00	36.90	2.12	17.40	0.08	0.00	1.12	89.30	69.71	39.66
18	66.00	130.00	210.00	37.76	2.47	15.57	0.09	0.00	1.11	88.96	69.44	41.83

Table 4.25: The regression coefficients and ANOVA of response surfaces for ionic liquid treatment of pre-extracted *E. grandis*

Coefficient	Solid recovery	Glucan (SF)	Xylan (SF)	Lignin (SF)	Glucan (LF)	Xylan (LF)	Lignin (LF)	Glucan EH
Mean/Interc.	88.8824	37.18584	2.231989	16.24556	0.085936	-0.102218	1.147204	40.06838
(1)Ionic liquid conc. (%)(L)	-7.7865	-1.44412	-0.362473	-0.46495	0.016360	0.075153	1.061813	29.19881
Ionic liquid conc. (%)(Q)	-3.3247	-4.87551	-0.631189	-0.24345	0.033852	0.057172	-0.364910	23.92083
(2)Temp (°C)(L)	-12.9398	-1.35846	-0.245318	-5.00674	-0.000707	0.029669	3.012401	37.16972
Temp (°C)(Q)	-6.3030	-2.98478	-0.338188	-3.36917	0.000122	0.032789	1.427517	24.68272
(3)Time (min)(L)	-0.7713	-0.66570	-0.159038	-1.06746	0.001094	0.076148	1.103437	7.31088
Time (min)(Q)	-5.9791	-2.89716	-0.383135	-1.41555	0.020687	-0.225035	1.461861	6.13464
1L by 2L	2.9791	-0.66700	0.058039	-0.10403	-0.022125	-0.224170	0.881222	-4.37917
1L by 3L	0.5537	0.15907	-0.092191	-0.13366	-0.015561	0.224170	0.529828	-7.93129
2L by 3L	-0.2704	-2.35910	-0.149450	-0.48564	0.023372	-0.102218	0.780860	8.38935
Lack of fit	335.808	60.6622	0.452510	2.0979	0.004717	0.074745	5.98438	904.27
R^2	0.749	0.666	0.906	0.970	0.627	0.836	0.903	0.925
Sdt. Error	0.030	0.335	0.039	0.626	0.000	0.000	0.000	0.819

For ionic liquid treatment of pre-extracted *E. grandis*, the CCD statistical analysis (shown in Table 4.25) was obtained by fitting the data into the second-order polynomial regression model. At 95% confidence interval, the lack of fit was significant for solid recovery, glucan retention, glucan dissolution, xylan dissolution, lignin dissolution, and glucan digestibility. The lack of fit was not significant for xylan retention and lignin retention. When compared with the lack of fit in ionic liquid treatment of virgin *E. grandis*, the lack of fit is not significant for xylan retention in the treatment of pre-extracted *E. grandis* while significant in treatment of virgin *E. grandis*. The R^2 values in fitting the experimental data obtained in treatment of pre-extracted *E. grandis* into second-order regression model ranged from 0.61 to 0.92. Low R^2 values (0.63 for glucan dissolution, 0.67 for glucan retention and 0.75 for solid recovery) are accompanied by low values of pure error, especially glucan dissolution. The range of R^2 values obtained in this treatment is similar to the one obtained in treatment of virgin *E. grandis*.

In ionic liquid fractionation of pre-extracted *E. grandis*, glucan retention was mainly affected by ionic liquid concentration (both linear and quadratic terms were statistically significant). Temperature and time effects then followed. The extremes of ionic liquid concentration (lowest and highest) tend to enhance glucan dissolution, thus reducing the amount of retained glucan. The lower ionic liquid concentrations are coupled with higher temperatures, and the combination favours dissociation of water into hydroxyl ions, which catalysed glucan dissolution. Higher ionic liquid concentrations dissolve significant amount of glucan, and lots of antisolvent addition (washes of ionic liquid fraction) in order to precipitate all glucan becomes a challenge. The similar trend of ionic liquid concentration being the factor, which strongly affects glucan content, was observed in glucan dissolution.

Xylan retention was mostly affected by ionic liquid concentration (both linear and quadratic terms were statistically significant). The reaction time impact on xylan retention followed. With increasing ionic liquid concentration, xylan retention increased. The reasons for the preference of xylan retention have been provided in Section 4.4.1. For xylan dissolution, all of the treatment factors were statistically significant at 95 % confidence interval. As shown in Table 4.25, all interactions were mostly significant factors on xylan dissolution. Ionic liquid concentration, temperature and time (all terms) then followed. At high ionic liquid concentrations and low temperatures, at high ionic liquid concentrations and short reaction times, and at low temperatures and short reaction times, higher amounts of xylan were

detected. At those interactions, there is minimum degradation of xylan because the conditions are less severe.

Lignin retention was mostly affected by temperature (both linear and quadratic terms being statistically significant). At high temperatures (above glass transition temperature of lignin), there is enhanced delignification. Very high delignification results in low lignin content of solid fractions. The strong effect of temperature was confirmed by temperature being the most significant factor on lignin dissolution. Although all factors are statistically significant (at 95 % confidence interval) on lignin dissolution, temperature outweighs them. At high temperature, there is high delignification, thus high lignin content in liquid fraction.

Temperature was the most significant factor in solid recovery and glucan digestibility of the solids. The treatment at higher temperatures yielded low solid recoveries, but highly digestible solids. Both ionic liquid concentration and time needed to be coupled with higher temperatures in order to enhance delignification and xylan dissolution, which yielded lower solid recoveries but highly digestible solids.

The desirability plots of the CCD experimental results had to be performed in order to get the desirable treatment conditions (considered as a compromise) for all the components. The method for the determination of desirabilities shown in Table 4.26 was used in the analysis of CCD experimental results, which was carried out in STATISTICA 11.

Table 4.26: The approach used for determining desirabilities in aqueous ionic liquid fractionation of pre-extracted *E. grandis* (Adapted from (Diedericks et al., 2012b))

Stream	Component	Goal	Min. conc.	Max. conc.
Liquid	Glucose	Min	42.44	0.0
	Xylose	Min	6.35	0.0
	Lignin	Max	0.0	21.24
Solid	Glucose	Max	0.0	42.44
	Xylose	Max	0.0	6.35
	Lignin	Min	21.24	0.0
	Desirability	Max	0.0%	100%

The desirability plot shown in Figure 4.30 was obtained from STATISTICA 11 analysis, and it provided the desirable treatment conditions of 66 %w/w ionic liquid concentration, 180.45 °C of temperature and 210 min of time. The moderate ionic liquid concentration is attractive for limiting the viscosity challenges affiliated to high ionic liquid concentrations, and for protecting xylan from acidic hydrolysis and degradation. The temperature of 180.45°C is very

high, it is higher than glass transition temperature of lignin, so it is expected to enhance delignification, xylan dissolution, as well as xylan degradation to some extent (Li et al., 2011b). The ionic liquid concentration in treatment of pre-extracted *E. grandis* (66 %w/w, 180.5 °C and 210 min) is higher than the one obtained in treatment of virgin *E. grandis* (32 %w/w ionic liquid concentration, 180.5 °C and 210 min), while temperature and time are the same. Also at the similar temperature, higher ionic liquid was combined with shorter reaction time (58.64 min) in treatment of pre-extracted SCB. One could expect the similar trend to be observed in this treatment. However, the different types of biomass, which had different degrees of delignification and xylan dissolution, could have resulted in different trends.

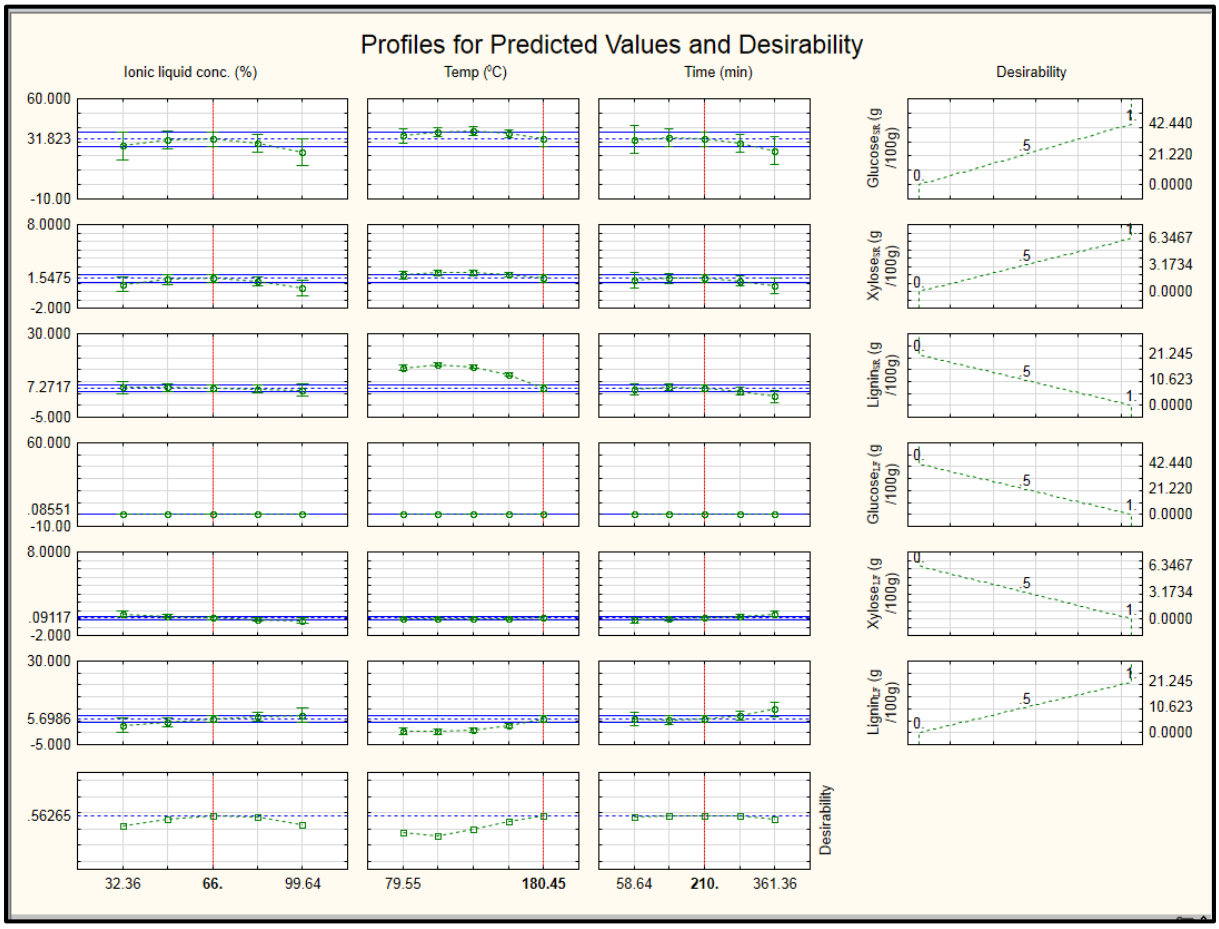

Figure 4.21: The desirability plot for aqueous ionic liquid treatment of pre-extracted *E. grandis*.

From ionic liquid fractionation of both virgin and pre-extracted *E. grandis* optimisation study, it is clear that temperature enhances delignification and glucan digestibility of recovered solid residues. At high temperature and low ionic liquid concentration, xylan was susceptible to degradation. Testing of desirable conditions on both virgin (32 %w/w ionic

liquid, 180.5 ºC and 210 min) and pre-extracted (66 %w/w ionic liquid, 180.5 ºC and 210 min) *E. grandis* was carried out in order to allow for clear understanding of impacts of ionic liquid fractionation on lignocellulose components, particularly xylan and lignin (glucan digestibility has been already been explored during optimisation experiments).

4.4.2.3. Ionic liquid treatments of *E. grandis* at the desirable conditions

The chemical compositions of the fractions obtained from aqueous ionic liquid fractionation of virgin *E. grandis* are shown in Table 4.27. The solid recovery following ionic liquid fractionation of *E. grandis* was found to be 55.86 %, and this recovery is lower than the range of 65.5 to 87.4 % obtained by other researchers as shown in Section 2.3.3. The lowest solid recovery (65.5 %) was obtained when the cholinium ionic liquid was used, and it was accompanied by significant xylan dissolution (38.3 %) and delignification (54.7 %), 82.9 % glucan retention in solid residue, while the obtained were fairly digestible with glucan digestibility of 58.0 % (An et al., 2015). The high solid recovery (87.4 %) was obtained when [EMIM]OAc ionic liquid was used (Wu et al., 2011). In that treatment significant glucan retention (90.4 %), lower delignification (10.1 %), lower xylan dissolution (16.2 %), but fairly digestible solids (68% which is higher than 58% obtained in low solid recovery) were observed (Wu et al., 2011). The difference in digestibility when using different ionic liquids might be related to the efficiency of imidazolium ionic liquids.

Table 4.27: The chemical compositions of liquid and solid fractions obtained from ionic liquid fractionation of virgin *E. grandis*

	Ionic liquid hydrolysate	**Solid residue**	**Mass balance (%)**
Solid recovery (%)		55.86 ± 1.90	
Glucose (g/100g)	3.04 ± 0.01	37.26 ± 0.85	83.7 ± 4.3
Xylose (g/100g)	3.13 ± 0.01	3.73 ± 0.09	50.3 ± 5.0
Lignin (g/100g)	15.10 ± 0.11	7.29 ± 1.56	81.5 ± 7.1

The determined lignin in liquid fraction was 15.10 (g/100g) based on dry raw biomass, which corresponds to 54.95 % of lignin in *E. grandis*. However, 73.47 % of lignin in original *E. grandis* was solubilised, so 18.52 % remained in liquid fraction following acid precipitation as both acid soluble lignin and degraded lignin products. The 73.47% lignin dissolution is higher than reported delignification values which range from 10.1 % (at mild treatment

conditions) to 63.0% (at stronger treatment conditions) shown in Section 2.3.3. The incomplete precipitation of lignin (although only 25% of solubilised lignin was not precipitated in this study) matches the observation in previous studies (Diedericks et al., 2012b; Karatzos et al., 2012b).

The xylan detected in liquid fraction was 3.13 (g/100g) based on dry raw biomass, which corresponds to 22.96 % of xylan in original *E. grandis*. However, 72.63 % of xylan in original *E. grandis* was solubilised, so 49.67 % of xylan was degraded during this treatment. The 72.63 % xylan dissolution is higher than the reported xylan dissolution values, which range from 12.3 to 61.9 %, as shown in Section 2.3.3. 12.3 % solubilised xylan was obtained when combination of high ionic liquid concentration (as received) with low temperature (90 °C) and short reaction time (90min) was used (Lee et al., 2009). 61.9 % xylan dissolution was obtained when a combination of high ionic liquid concentration (80 %) with high temperature (120 °C) and long reaction time (1320 min) was used (Brandt et al., 2011). When comparing the xylan dissolution ranges with the xylan dissolution obtained in this treatment, it can be seen that high temperatures and long reaction times lead to high xylan solubilisation.

The aqueous ionic liquid treatment of hardwood has been carried out using 80 % ionic liquid, 120 °C and 1320 min (Brandt et al., 2011). Although long reaction time was used, the lower temperature and high ionic liquid concentration used by Brandt et al, (2011) gave higher solid recovery in comparison to this treatment combination. The low solid recovery obtained in this treatment (combination of low ionic liquid concentration with high temperature,180.45 °C), is related to high delignification and xylan solubilisation at temperatures higher than transition temperature of lignin (Li et al., 2011b). As shown in Section 4.4.2.1, temperature leads to low solid recovery through enhanced delignification, xylan solubilisation, and glucan hydrolysis (to some extent). The glucan retention obtained in that aqueous ionic liquid treatment was 78.4 %, which is comparable to the 77.3 % obtained in this treatment (Brandt et al., 2011). The 22.7 % glucan dissolution (plus degradation) observed in this study is enhanced by high temperature and low ionic liquid concentration used. The xylan dissolution obtained in this study (72.6 %) is comparable to 61.9%, reported by Brandt et al. (2011). The lignin dissolution obtained in that aqueous ionic liquid treatment was 17.4%, which was far lower than 73.5% obtained in this study (Brandt et al., 2011). High xylan and lignin dissolutions obtained in this treatment are enhanced by high temperature and low ionic liquid

concentration treatment combination. In this study, very high glucan digestibility (98.6 %) of solid residues was achieved, as compared to 60 % reported by Brandt et al. (2011).

The mass balance of glucan, xylan and lignin were found to be 83.65±4.27, 50.33±4.98 and 81.48±7.11 % respectively. In aqueous ionic liquid treatment of hardwood, the researchers focused mainly on solid residue and its digestibility, which led to not preparing the mass balances (Brandt et al., 2011). When considering the non-aqueous ionic liquid treatment of hardwoods, it is evident that pre-treatment to obtain digestible solids for biofuels and/or high value chemicals production was key objective of most researchers as compared to fractionation for materials and chemicals production (see Section 2.3.3), so there were no mass balances prepared. When compared with the mass balances in section 4.4.1, in this treatment there was higher glucan loss. Xylan and lignin balances are higher in this treatment as compared to treatment of SCB. 4.6 % xylan balance and 6.7 % lignin balance increases are attributed to biomass type, as the treatment conditions were the same.

The lignin was acid precipitated from ionic liquid fraction, filtered, dried and considered as clean lignin. The hemicelluloses separation using alcohol precipitation was tried prior to lignin precipitation, but did not provide sufficient quantities for further characterisation.

The obtained lignin had S/G ratio of 5.56. There is an increase in the ratio when compared to the feedstock (S/G ratio of 4.35). The slight increase in S/G indicates that syringyl units are more reactive and easily hydrolysed than guaiacyl units, during ionic liquid treatment. This S/G ratio (5.56) is higher than the one obtained from ionic liquid treatment of birch wood at 110 °C for 720 min (1.14 obtained when using quantitative ^{13}C NMR and 1.81 obtained when using 2D-HSQ C NMR) and ionic liquid treatment of poplar at 110 °C for 960 min (1.24 obtained using DFRC method), and the difference might be attributed to different feedstocks and reaction conditions, especially treatment temperature which was higher than transition temperature of lignin (Kim et al., 2011; Wen et al., 2013a).

FTIR analysis *E. grandis* lignin (ionic liquid treatment) is presented in Appendix B, results indicated that the obtained lignin was oxidised. No carbohydrates-related peaks were observed, confirming that acid precipitation improved lignin purity.

FTIR analysis of ionic liquid treated solid residue indicated that the treatment resulted in partial deacetylation and delignification of virgin *E. grandis*.

Following the study of ionic liquid fractionation of virgin E. grandis, the effects of including alkaline post-extraction was explored, and is presented below.

The chemical compositions of the fractions obtained from ionic liquid fractionation plus NaOH post-extraction (at 80 °C, 0.5 M NaOH and 120 min) of *E. grandis* are shown in Table 4.28. The solid recovery following the treatment of *E. grandis* was found to be 50.00 %. The obtained solid recovery is lower than the one obtained in ionic liquid treatment alone (55.86 %). The decrease in solid recovery results from solubilisation of hemicelluloses and lignin in the alkaline post-extraction.

Table 4.28: The chemical compositions of liquid and solid fractions obtained from NaOH post-extraction of ionic liquid treated *E. grandis*

	Ionic liquid hydrolysate	**Solid residue**	**Mass balance (%)**	
			Based on virgin material	Based on ionic liquid treated material
Solid recovery (%)		50.00 ± 1.60		
Glucose (g/100g)	2.06 ± 0.04	33.55 ± 1.69	73.9 ± 4.5	95.6 ± 5.0
Xylose (g/100g)	0.83 ± 0.19	2.74 ± 0.09	26.2± 2.0	95.7 ± 6.1
Lignin (g/100g)	4.93 ± 0.11	7.10 ± 0.50	43.8 ± 1.9	165.0 ± 36.0

The detected lignin was 4.93 (g/100g) based on dry raw biomass, which corresponds to 17.9 % of lignin in virgin *E. grandis*, and 67.6 % of lignin in ionic liquid treated *E. grandis*. The high lignin balance (165.0 %) indicates that pseudo-lignin was formed during post-extraction (Tan et al., 2009).

The detected xylan in ionic liquid fraction was 0.83 (g/100g) based on dry raw biomass, which corresponds to 6.1 % of xylan in virgin *E. grandis*, and 22.3 % of xylan in ionic liquid treated *E. grandis*. The high xylan balance indicates that degradation and side reactions (pseudo-lignin formation) were minimal in this post-extraction. The mass balance of glucan, shown in Table 4.28, indicates that minimum glucan was solubilised in this post-extraction.

The hemicelluloses and lignin were separated using alcohol precipitation of hemicelluloses, and crude lignin from drying the residual liquor. The molecular weight of the extracted

hemicelluloses was found to be 14639 Da. This molecular weight is relatively lower than (49330-60760 Da) those reported for ionic liquid treatment of *Eucalyptus* (Xu et al., 2013). This molecular weight is comparable to the one obtained in ionic liquid treatment of bamboo, which is 10020 Da (Yang et al., 2013). However, when compared with the molecular weights obtained in alkaline extraction of hemicelluloses from hardwoods (shown in Section 4.2.2), this molecular weight is very low. The significant decrease in size of extracted hemicelluloses is attributed to cleavage of lignin-carbohydrates bonds and depolymerisation of hemicellulose during ionic liquid treatment plus NaOH post-extraction (Yang et al., 2013).

The neutral sugar composition of the extracted hemicellulose, mainly xylan (in relative percentages) was calculated the total detected neutral sugars shown in Appendix B. The neutral sugar composition is shown in Figure 4.31. Xylose was the dominant neutral sugar (67.0 % of neutral sugars), followed by galactose (17.7 %), and arabinose (15.3 %). The trend of high xylose content in the hemicelluloses obtained from alkaline post-extraction of ionic liquid treated solids was observed in hemicelluloses from ionic liquid treatment of *Eucalyptus*, whereby xylose ranged from 63.25 to 74.85 % and galactose ranged from 4.49 to 7.32 % have been observed (Xu et al., 2013). Although xylose was the dominant neutral sugar in ionic liquid fractionation of *Eucalyptus*, glucose (4.85 and 14.40 %) was second instead of galactose (Xu et al., 2013).

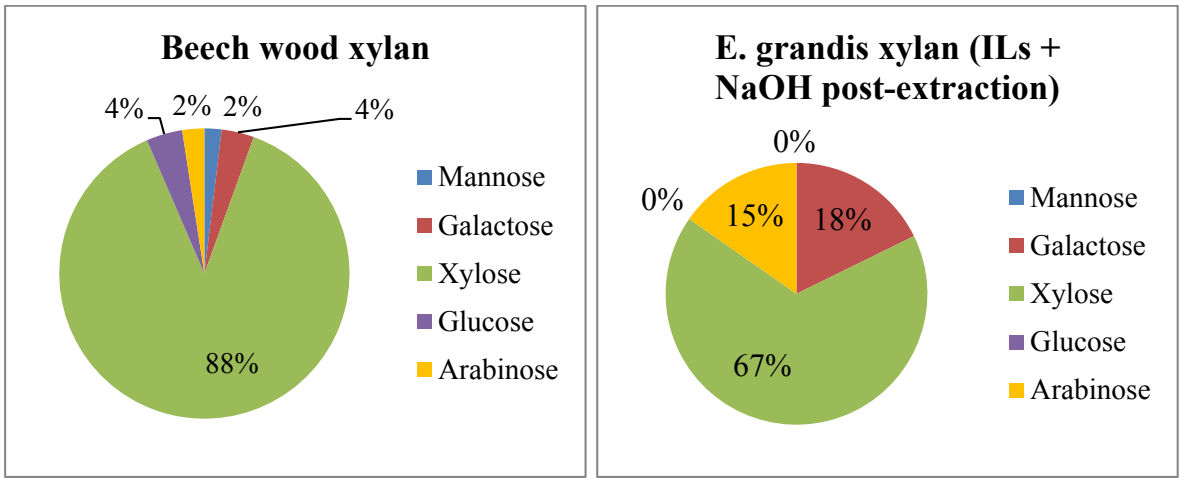

Figure 4.22: Neutral sugar compositions of *E. grandis* xylan (ionic liquid treatment plus NaOH post-extraction) and Beechwood xylan (commercial)

The acid insoluble lignin content of *E. grandis* xylan (ionic liquid treatment plus NaOH post-extraction) was determined gravimetrically (masses shown in Appendix C), and was found to be 4.63±0.03%. The lignin content obtained is comparable to the 3.54% obtained in

hemicellulose fraction from ionic liquid fractionation of SCB (Lan et al., 2011). Comparison with lignin content xylan from herbaceous material was used because xylan obtained from ionic liquid fractionation of Eucalyptus (Xu et al., 2013) was not analysed for lignin content. When compared with Beechwood xylan, *E. grandis* xylan (ionic liquid treatment plus NaOH post-extraction) has higher arabinose, but glucose, arabinose and mannose are comparable. This xylan also has lignin content that is comparable to that of Beechwood xylan (3.57 %).

FTIR analysis of *E. grandis* xylan (ionic liquid treatment plus NaOH post-extraction), shown in Appendix B, indicated that the obtained xylan was cleaner (low intensity of lignin peaks). The low intensity of lignin-related peaks is in agreement with low lignin content obtained when using wet chemical method.

FTIR analysis of *E. grandis* lignin (ionic liquid treatment plus NaOH post-extraction), shown in Appendix B, indicated that the obtained lignin was oxidised (strong intensity of conjugated carbonyl groups).

The glucan recovery in the solid residue, following ionic liquid and NaOH post-extraction of *E. grandis*, was 69.63 %. This recovery is lower than glucan recovery which ranges from 78.4 to 90.4 % as shown in Section 2.3.3. When compared to glucan recovery of 77.33 % (obtained in ionic liquid treatment only), this glucan recovery is low. This low glucan recovery shows that 7.7% of glucan was solubilised in NaOH post-extraction. The glucan digestibility of the solid residue was found to be 100.61 %. The 100.61 % enzymatic digestibility obtained in this treatment is slightly higher than the one obtained with ionic liquid treatment alone (98.55 %). The glucan digestibility of 100.61% is very good for biofuels and/or high value chemicals production. FTIR analysis of ionic liquid treatment plus NaOH post-extracted solid residue, in Appendix B, indicated that virgin *E. grandis* was completely deacetylated and highly delignified after treatment.

The ionic liquid treatment was also carried out on alkaline pre-extracted *E. grandis*. The chemical compositions of the fractions obtained from ionic liquid fractionation of *E. grandis* are shown in Table 4.29. The solid recovery following ionic liquid fractionation of pre-extracted *E. grandis* was found to be 57.22 %, and this recovery is higher than 55.86 % (obtained in ionic liquid treatment of virgin *E. grandis*). The solid recovery (57.22 %) obtained in this treatment is higher than the one obtained in ionic liquid treatment plus NaOH post-extraction (50.00 %). The increase in solid recovery from this treatment when compared

to ionic liquid treatment only and to ionic liquid plus NaOH post-extraction is attributed to different ionic liquid concentrations used (66 %w/w ionic liquid on pre-extracted material but 32 %w/w on virgin material). Higher ionic liquid concentration protected hemicelluloses and lignin from excessive dissolution and degradation, thus yielding higher solid recovery compared to low ionic liquid concentration treatments.

Table 4.29: The chemical compositions of liquid and solid fractions obtained from ionic liquid fractionation of pre-extracted *E. grandis*

	Ionic liquid hydrolysate	**Solid residue**	**Mass balance (%)**	
			Based on virgin material	Based on pre-extracted material
Solid recovery (%)		57.22 ± 2.00		
Glucose (g/100g)	3.62 ± 0.01	35.20 ± 1.25	80.57 ± 5.04	91.74 ± 3.34
Xylose (g/100g)	0.81 ± 0.01	5.13 ± 0.28	43.58 ± 6.76	93.54 ± 4.75
Lignin (g/100g)	9.69 ± 0.08	8.15 ± 0.21	64.92 ± 1.70	83.99 ± 3.26

The solubilised lignin (detected) was 9.69 (g/100g) based on dry raw biomass, which corresponds to 35.26 % of lignin in virgin *E. grandis*. But when comparing lignin solubilisation obtained to the one in alkaline pre-extracted solid, solubilised lignin is 45.62 %. When considering mass differences, 61.63 % lignin was solubilised, but 45.62 % was acid precipitated. The 61.23 % delignification is lower than 73.47 % lignin dissolution obtained with ionic liquid treatment of virgin *E. grandis*. The decrease in delignification could have been caused by lack of acid catalyst (absence of acetic acid from alkaline pre-extracted biomass, neutralising effect of any residual NaOH and buffering effect of ionic liquid used).

The solubilised xylan (detected) was 0.81 (g/100g) based on dry raw biomass, which corresponds to 5.94 % of xylan in *E. grandis*. But when comparing xylan solubilisation obtained to the one in alkaline pre-extracted solid, solubilised xylan is 12.76 %. The 12.76 % xylan dissolution is lower than 22.96 % xylan dissolution (72.63 % removed xylan) obtained with ionic liquid treatment of virgin *E. grandis*. Decrease in xylan dissolution in ionic liquid treatment following alkaline pre-extraction might be attributed to by lack of acid catalyst (absence of acetic acid from alkaline pre-extracted biomass, neutralising effect of any

residual NaOH and buffering effect of ionic liquid used), which limits catalysis of xylan dissolution and delignification.

The mass balance of glucan, xylan and lignin were found to be 80.57±5.04, 43.58±6.76 and 64.92±1.70 % respectively. The mass balances for ionic liquid fractionation of pre-extracted *E. grandis* were determined by relating the amount of components after ionic liquid treatment to the virgin biomass. But when comparing with the NaOH pre-extracted material the balances would be 91.47, 93.54 and 83.99 % for glucose, xylose and lignin respectively. The mass balance values obtained in this treatment are different from the values obtained in ionic liquid treatment of virgin *E. grandis*, which are 83.65, 50.33 and 81.48 % of glucan, xylan and lignin respectively. In this treatment glucan, xylan and lignin are reserved due to limited solubilisation.

The obtained lignin had S/G ratio of 2.57. There is slight decrease in the ratio when compared to the feedstock (S/G ratio of 4.35). The slight decrease in S/G ratio of the extracted lignin implies that guaiacyl units were highly extracted during ionic liquid fractionation of pre-extracted *E. grandis*. The extracted lignin might have low reactivity in downstream processing.

FTIR analysis of *E. grandis* lignin (NaOH pre-extraction plus ionic liquid treatment), shown in Appendix B, indicated that obtained lignin was oxidised. Absence of carbohydrates peaks indicated effective separation (using acid precipitation).

The glucan recovery in the solid residue, following NaOH pre-extraction plus ionic liquid treatment of *E. grandis*, was 73.06 % based on virgin *E. grandis*, but 82.94 % based on pre-extracted *E. grandis*. This recovery (82.94 %) is higher than the one obtained through ionic liquid treatment only (77.33 %). The increase in glucan retention might be attributed to higher ionic liquid concentration used in this treatment as compared to the one used in treatment of virgin *E. grandis*. The glucan digestibility of the solid residue was found to be 100.42 %. The glucan digestibility following this treatment is slightly higher than the one obtained for ionic liquid treated solids (98.55 %) and comparable to that of ionic liquid treatment plus NaOH post-extracted solids (100.61 %).

All ionic liquid fractionation approaches yielded highly digestible cellulose-rich solid residues. High quality cellulosic materials (highly digestible) indicate that ionic liquid fractionation of E. grandis can be applied in production of fermentation-based fuels and

chemicals. Temperature was the most significant factor, which enhanced digestibility of solid residue, delignification and xylan dissolution. However, significant xylan degradation was observed during ionic liquid fractionation of virgin *E. grandis*. High xylan degradation is undesirable, so coupling ionic liquid fractionation was considered to address the challenge. Coupling ionic liquid fractionation with alkaline pre-extraction, showed improved hemicellulose (xylan) preservation and was considered to be the best ionic liquid fractionation approach for *E. grandis*.

From this chapter, the conclusions that were drawn are:

- Alkaline pre-extraction of both SCB and E. grandis resulted in extraction of polymeric hemicellulose (mainly comprised of xylan).
- Alkaline pre-extraction of SCB yielded solid residues with good digestibilities.
- Organosolv fractionation of SCB and E. grandis resulted in xylan degradation.
- Organosolv fractionation of SCB and E. grandis yielded solid residues with fair digestibilities (insufficient quality for application in biofuels and high value chemicals production).
- Coupling organosolv fractionation with alkaline post-extraction (for both feedstocks) improved digestibility of solid residues, but increased xylan degradation further.
- Coupling organosolv fractionation with alkaline pre-extraction (for both feedstocks) improved digestibility of solid residues, and minimised xylan degradation.
- Ionic liquid fractionation of SCB and E. grandis resulted in xylan degradation.
- Ionic liquid fractionation of SCB and E. grandis yielded highly digestible solid residues.
- Coupling ionic liquid fractionation with alkaline post-extraction (for both feedstocks) increased xylan degradation further.
- Coupling ionic liquid fractionation method with alkaline pre-extraction reduced xylan degradation.

Ionic liquid fractionation was observed to outweigh organosolv fractionation, when they were applied on SCB (herbaceous material) E. grandis (hardwood). Further comparisons of the two fractionation methods are presented in Chapter 5.

5. Comparison of fractionation methods

To my knowledge, there is no comprehensive comparison of organosolv and ionic liquid fractionation methods on SCB and *E. grandis*, which was considered in this study. The study was focused on comparing the ionic liquids and organosolv fractionation processes in terms of extraction and separation efficiencies. The comparison includes yields, mass balances, and quality of the cellulose, hemicellulose and lignin products. In this study all lignocellulose components are considered, in order to processes' efficiencies, which are essential in biorefinery approach. The comparison of organosolv and ionic liquid fractionations of both feedstocks in terms of operational challenges is also included.

In literature, some experimental studies on comparing ionic liquid fractionation with other pre-treatment methods (like aquasolv, dilute acid and alkaline pre-treatments) have been carried out for cellulose digestibility, hemicellulose quality and lignin quality (Li et al., 2010; Nguyen et al., 2010; Fu and Mazza, 2011a; 2011b; Li et al., 2011a; Yoon et al., 2011; Geng and Henderson, 2012; Sun et al., 2012; Sun et al., 2013; Yang et al., 2013). Ionic liquid fractionation gave highly digestible cellulose, high purity lignin, and polymeric hemicelluloses. Organosolv fractionation has been experimentally compared with other pretreatment methods (like aquasolv and alkaline pre-treatments), with cellulose digestibility and lignin quality as main aspects, but nothing about hemicellulose quality. Although organosolv process gave fairly digestible cellulose and high purity lignin, it yielded oligomeric and monomeric hemicelluloses, which were often susceptible to further hydrolysis and degradation. As for the experimental comparison of organosolv and ionic liquid fractionation methods, there is no work reported, but the literature available for comparison of organosolv and ionic liquid fractionation methods is in the review papers, which indicated that both organosolv and ionic liquid fractionation methods yield high purity and high quality lignin (Agbor et al., 2011; Menon and Rao, 2012; Espinoza-Acosta et al., 2014). Auto- and/or alkaline catalysed organosolv process tends to yield fairly digestible cellulose, while ionic liquid process often yields highly digestible cellulose. Organosolv process yields oligomers and monomers, while ionic liquid process mostly yields polymeric and oligomeric hemicelluloses. The limited experimental comparison of organosolv and ionic liquid fractionation methods has motivated for this study.

Hemicellulose is the component of lignocellulose which is more susceptible to hydrolysis and degradation, and is often underutilised in pulp and paper industries, and most biorefinery

approaches are considering hemicellulose pre-extraction (Al-Dajani and Tschirner, 2008; Mendes et al., 2009; Chimphango, 2010; Júnior et al., 2010; Walton et al., 2010; Gomes, 2012; Postma, 2012; Vena, 2013; Joubert, 2015). Hemicelluloses obtained through alkaline pre-extraction are mostly polymeric. Similarly, in pretreatment processes, the focus has been directed mainly on cellulose digestibility (particularly for biofuels production), with recently increasing interest on lignin applications, but limited consideration of hemicelluloses. In ionic liquid fractionation approach, alkaline post-extraction of hemicelluloses has been employed at several times (Lan et al., 2011; Sun et al., 2012; Sun et al., 2013; Xu et al., 2013; Yang et al., 2013), but alkaline pre-extraction of hemicelluloses has not been extensively explored (Geng and Henderson, 2012). As preservation of hemicelluloses (mainly polymers and oligomers) was one of the major targets of this study, alkaline catalysis, and alkaline pre- and/or post-extraction of hemicellulose seemed attractive. However, alkaline catalysis is not mostly preferred as it increases recovery costs (solvent and catalyst) as compared to easier recycling and reuse of alkali in alkaline extraction (Sannigrahi and Ragauskas, 2013). So coupling fractionation methods with alkaline pre- and/or post-extraction seems to be promising approach, if alkaline conditions are desired. In this study, coupling organosolv and ionic liquid fractionation methods with alkaline pre- and/or post-extraction was studied in order to obtain the better approach.

The two fractionation methods, organosolv and ionic liquids, are compared in sections 5.1 and 5.2. The comparison is based on fractionation goals for biorefinery approach. The goals include; high yields of fractions (cellulose, hemicellulose and lignin), high purity of fractions, highly digestible cellulose, minimum degradation of fractions (particularly hemicellulose), allow for recovery of each fraction, cost effectiveness (robustness to various feedstocks, low capital and operating costs, and regeneration of energy chemicals and enzymes), as well as commercial scalability (Alvira et al., 2010; Zhu et al., 2010; Agbor et al., 2011; Menon and Rao, 2012; Vancov et al., 2012). The comparison based on yields, purity and quality of cellulose, hemicellulose and lignin is covered by section 5.1. Section 5.2 compares the two fractionation methods in terms of operational challenges, which include recovery of fractions, robustness, capital and operational costs, as well as scalability.

5.1. Fractionation efficiency based on mass balances, yields and quality of cellulose, hemicellulose and lignin

The yields, mass balances and glucan digestibility for the fractionation approaches are shown in Tables 5.1and 5.2 for SCB and *E. grandis* respectively.

Alkaline pre-extraction of hemicelluloses, when coupled with organosolv and/or ionic liquid fractionation methods is the best approach, as compared to alkaline post-extraction of hemicelluloses.

As shown in Table 5.1, alkaline pre-extraction of hemicelluloses from SCB resulted in 71 % xylan solubilisation, which was also detected in the liquid fraction. The extracted xylan in SCB corresponds to the one obtained at similar conditions (1.5 M NaOH, 65 °C and 92 min), 69 % extracted xylan which was reported by Vena (2013). As for alkaline pre-extraction of hemicelluloses from *E. grandis*, it resulted in 55 % xylan solubilisation, which was also detected in liquid fraction. The 55 % extracted xylan from *E. grandis* (in this study) is higher than the values obtained by Vena (2013), which ranged from 8.5 to 16.0 %. The difference in values of extracted xylan can be related to reporting of xylan recovered (precipitated or membrane separation coupled with freeze drying) by Vena (2013) and limitation of hemicellulose extraction as pulp was to be used in pulp and paper applications.

As shown in Tables 5.1 and 5.2, when both organosolv and ionic liquid fractionation methods were coupled with alkaline post-extraction, the trend of low xylan (quantified as xylose) balances were observed for treatments of both SCB (65.2 and 36.2 %for organosolv and ionic liquid respectively) and *E. grandis* (60.0 and 49.2 % for organosolv and ionic liquid respectively). These xylan balances are lower than those obtained without alkaline extraction coupling for both methods and feedstocks, so the decrease indicates further hydrolysis and degradation by alkaline post-extraction. The xylan balances for ionic liquid fractionation coupled with alkaline post-extraction for both feedstocks could not be compared, as the studies did not consider mass balances but were concerned about quality of the fractions (Lan et al., 2011; Sun et al., 2012; Sun et al., 2013; Xu et al., 2013; Yang et al., 2013).

Coupling organosolv and ionic liquid fractionation methods with alkaline pre-extraction, the trend of low xylan balances (when based on virgin materials) were observed for treatments of both SCB (30.3 and 25.1 % for organosolv and ionic liquid respectively) and *E. grandis* (41.9 and 43.6 % for organosolv and ionic liquid respectively). However, when these balances are

based on xylan content of alkaline pre-extracted materials, the xylan balances are greater than 80 % (which are considered as good) for SCB (97.8 and 81.2 % for organosolv and ionic liquid respectively) and E. grandis (86.8 and 93.5 % for organosolv and ionic liquid respectively). In their study of coupling alkaline pre-extraction with ionic liquids, Geng and Henderson (2012) did not consider mass balances, but considered enzymatic hydrolysis. So the comparison on mass balances for alkaline pre- and/or post-extraction coupling with organosolv and ionic liquid fractionation methods also seems new.

Alkaline post-extraction resulted in lower molecular weight hemicelluloses (which are shown in Table 5.3), which can be attributed to cleavage of LCCs by either organosolv or ionic liquids, than molecular weight of hemicelluloses obtained from alkaline pre-hydrolysis (Yang et al., 2013). The molecular weights of hemicellulose obtained from alkaline pre-extraction (34298 and 55991 Da for SCB and *E. grandis* respectively) are in agreement with those reported in literature for herbaceous/agricultural residues (32793-40400 Da) and for hardwoods (14500-99960 Da) (Sun et al., 2000; Sun, and Sun, 2002; Höije et al., 2005; Xu et al., 2006; Gomes, 2012; Postma, 2012; Júnior et al., 2013; Vena, 2013; Wei et al., 2013). The molecular weights of hemicelluloses obtained from ionic liquid fractionation coupled with alkaline post-extraction (11040 and 14659 Da for SCB and *E. grandis* respectively) are in agreement with those obtained in treatment of bamboo (10020-16110 Da) (Yang et al., 2013). As for glucan balances, alkaline pre-extraction approach when coupled with organosolv and/or ionic liquid fractionation methods gives good glucan retention in the solid residue than alkaline post-extraction approach.

The denotations in Tables 5.1 and 5.2 are: SF: solid fraction, LF: liquid fraction, MB: mass balance, NaOH +: pre-extraction, + NaOH: post-extraction, ILs: ionic liquid fractionation, W1 & W2: washing approach 1 & 2

Table 5.1: The mass balances of different fractionation processes for SCB

Material	Solid recovery (%)	Glucan (%)	Xylan (%)	Lignin (%)	Glucan digestibility (%)
Virgin (W1)	100	42.62 ± 1.73	22.79 ± 0.34	22.42 ± 0.43	—
Virgin (W2)	100	40.70 ± 0.69	20.26 ± 0.39	21.17 ± 0.02	3.71 ± 0.14
NaOH: SF	59.31 ± 0.86	38.47 ± 1.60	6.27 ± 0.19	8.28 ± 0.30	80.14 ± 0.01
: LF		0.38 ± 0.02	14.42 ± 1.19	13.92 ± 0.00	
: MB		95.45 ± 4.18	102.22 ± 6.16	104.87 ± 1.35	
ILs: SF	45.43 ± 1.60	36.53 ± 0.80	5.50 ± 0.15	5.31 ± 0.25	101.21 ± 1.34
: LF		0.40 ± 0.01	3.77 ± 0.12	10.53 ± 0.01	
:MB		90.74 ± 1.88	45.76 ± 2.81	74.82 ± 1.58	
ILs + NaOH: SF	45.04 ± 1.50	32.90 ± 0.59	4.54 ± 0.27	4.26 ± 0.63	100.94 ± 0.55
: LF		0.79 ± 0.10	-	3.63 ± 0.00	
: MB		82.78 ± 2.04	22.41 ± 1.26	37.27 ± 2.98	
NaOH + ILs: SF	46.09 ± 1.70	28.52 ± 1.29	4.31 ± 0..47	4.59 ± 0.40	100.73 ± 0.24
:LF		1.14 ± 0.07	0.78 ± 0.05	4.99 ± 0.05	
: MB		72.87 ± 4.41	25.12 ± 9.43	45.25 ± 4.18	
Organosolv: SF	66.74 ± 0.21	39.25 ± 0.71	7.79 ± 0.21	14.79 ± 0.48	63.02 ± 0.16
: LF		0.66 ± 0.04	8.05 ± 0.04	9.55 ± 0.25	
: MB		93.64 ± 4.43	69.50 ± 2.00	108.56 ± 2.93	
Organosolv+NaOH: SF	43.38 ± 1.71	35.17 ± 0.56	5.98 ± 0.09	1.47 ± 0.09	101.10 ± 0.52
: LF		0.42± 0.05	0.84 ± 0.94	17.63 ± 1.58	
: MB		83.50 ± 3.64	65.24 ± 6.50	85.19 ± 7.23	
NaOH+Organosolv: SF	41.08 ± 0.60	27.56 ± 0.46	5.89 ±0.07	5.25 ± 0.03	100.41 ± 0.04
: LF		0.04 ± 0.00	0.24 ± 0.01	2.72 ± 0.07	
: MB		67.81 ± 2.83	30.26 ± 2.24	37.65 ± 1.01	

Table 5.2: The mass balances of different fractionation processes for *E. grandis*

Material	Solid recovery (%)	Glucan (%)	Xylan (%)	Lignin (%)	Glucan digestibility (%)
Virgin	100	48.18 ± 1.87	13.63 ± 0.66	27.48 ± 0.32	1.20 ± 0.12
NaOH: SF	78.06 ± 2.67	42.44 ± 0.73	6.35 ± 0.12	21.24 ± 0.78	66.09 ± 0.14
: LF		0.99 ± 0.02	7.55 ± 0.27	7.19 ± 0.21	
: MB		90.14 ± 4.23	104.51 ± 5.30	103.46 ± 3.08	
ILs: SF	55.86 ± 1.90	37.26 ± 0.85	3.73 ± 0.09	7.29 ± 1.56	98.55 ± 0.27
: LF		3.04 ± 0.01	3.13 ± 0.01	15.10 ± 0.11	
:MB		83.65 ± 4.27	50.33 ± 4.98	81.48 ± 7.11	
ILs + NaOH: SF	50.00 ± 1.60	33.55 ± 1.69	2.74 ± 0.09	7.10 ± 0.50	100.61 ± 0.57
: LF		2.06 ± 0.04	0.83 ± 0.19	4.93 ± 0.11	
: MB		73.91 ± 4.53	26.19± 2.00	43.78 ± 1.92	
NaOH + ILs: SF	57.22 ± 2.00	35.20 ± 1.25	5.13 ± 0.28	8.15 ± 0.21	100.42 ± 0.62
:LF		3.62 ± 0.01	0.81 ± 0.01	9.69 ± 0.08	
: MB		80.57 ± 5.04	43.58 ± 6.76	64.92 ± 1.70	
Organosolv: SF	60.62 ± 0.56	38.61 ± 0.93	4.81 ± 0.33	14.93 ± 0.54	45.70 ± 0.31
: LF		0.14 ± 0.03	3.85 ± 0.03	10.18 ± 0.14	
: MB		80.43 ± 4.56	63.54 ± 6.16	91.38 ± 2.52	
Organosolv+NaOH: SF	40.27 ± 1.37	36.21 ± 1.06	4.25 ± 0.23	1.91 ± 0.19	79.37 ± 0.24
: LF		0.37 ± 0.04	0.08 ± 0.04	15.48 ± 0.88	
: MB		75.92 ± 3.68	31.77 ± 2.28	63.28 ± 1.31	
NaOH+Organosolv: SF	58.77 ± 2.01	37.82 ± 0.04	5.09 ± 0.05	13.34 ± 0.04	100.82 ± 0.09
: LF		0.28 ± 0.02	0.42 ± 0.04	7.23 ± 0.03	
: MB		79.08 ± 3.88	41.89 ± 4.95	74.85 ± 1.19	

Table 5.3: Molecular weight, molecular number and polydispersity values of hemicelluloses

Hemicellulose	Molecular weight (Da)	Molecular number (Da)	Polydispersity (Mw/Mn)
Larchwood arabinogalactan	19418	17116	1.13
Beechwood xylan	24812	15069	1.65
SCB Xylan (ILs + NaOH post-extraction)	11040	11040	1.00
E. grandis xylan (ILs + NaOH post-extraction)	14659	11402	1.29
SCB Xylan (Organosolv + NaOH post-extraction)	8148	6429	1.27
E. grandis xylan (Organosolv + NaOH post-extraction)	8970	7274	1.23
SCB xylan (NaOH pre-extraction)	34298	17790	1.93
E. grandis xylan (NaOH pre-extraction)	55991	35466	1.58

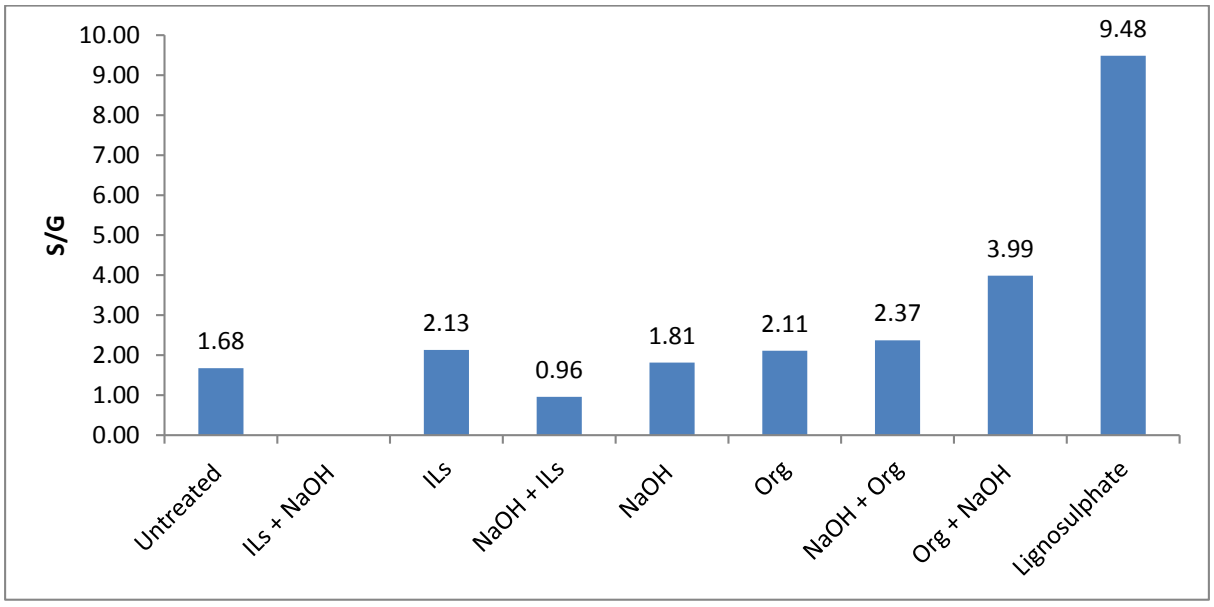

Figure 5.1: The proportions of syringyl and guaiacyl units in different SCB samples: ILs (ionic liquid), Org (organosolv), NaOH (alkaline pre-extracted), NaOH+ (alkaline pre-extraction plus), + NaOH (plus alkaline post-extraction) and Lignosulphate (calcium lignosulphate)

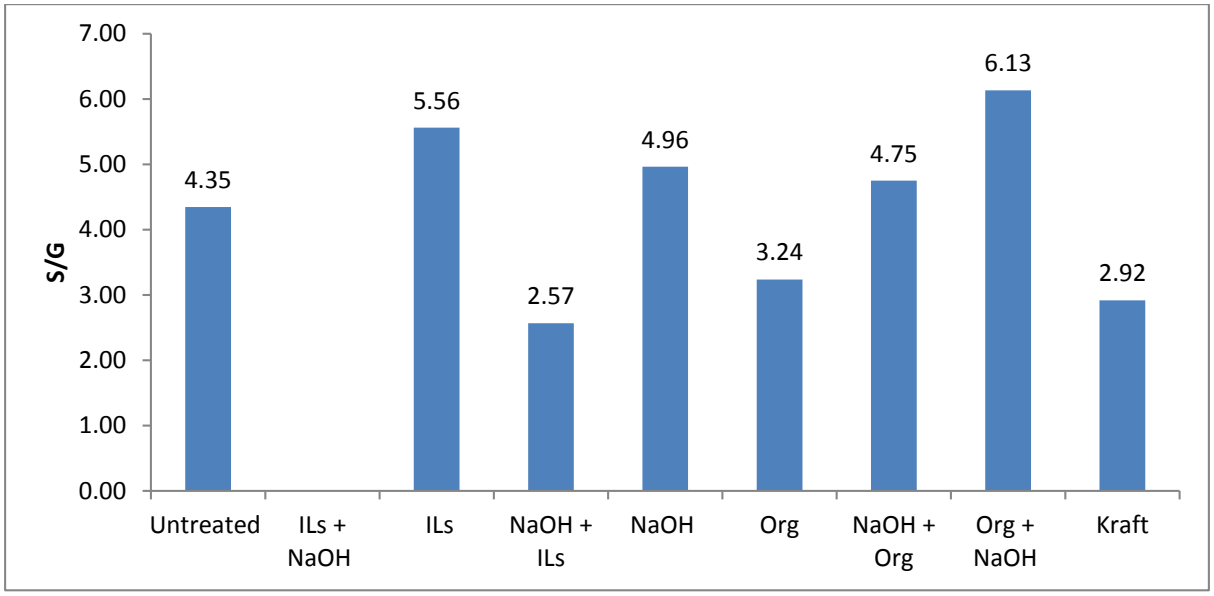

Figure 5.2: The proportions of syringyl and guaiacyl units in different *E. grandis* lignin samples: ILs (ionic liquid), Org (organosolv), NaOH (alkaline pre-extracted), NaOH+ (alkaline pre-extraction plus), + NaOH (plus alkaline post-extraction) and Kraft (from Kraft pulping)

Both organosolv and ionic liquid fractionation methods applied were severe (further hydrolysed and degraded solubilised hemicelluloses). However, ionic liquid (at moderate/appropriate conditions) fractionation method outweighs organosolv, in terms of cellulose digestibility, hemicellulose yields and quality, but the two yield lignins of similar properties. Tables 5.1, 5.2 and 5.3, together with Figures 5.1 and 5.2 show the quality of fractions (cellulose, hemicellulose and lignin) obtained from both organosolv and ionic liquid fractionation of SCB and *E. grandis*.

Table 5.4 was then developed to summarise the information provided by earlier tables and figures, and to enable easy comparison of the two fractionation methods on both feedstocks. The criteria used to assess the fractionation methods are; ≥80 % cellulose yield (in relation to cellulose content in virgin materials), ≥70 % cellulose purity (as purity is not the only factor affecting digestibility, but other factors like porosity, DP and crystallinity), ≥80 % glucan digestibility, ≥70 % hemicellulose yield (solubilised and detected hemicellulose in liquid fraction, process that degrades more than 70 % of solubilised hemicellulose is not effective for fractionation purpose), ≥80 % hemicellulose purity (for both solubilised and recovered by either precipitation or membrane separation techniques), mostly polymeric and oligomeric hemicelluloses are desirable, ≥70 % lignin yield (both solubilised and recovered), ≥80 %

lignin purity (for applications in valuable chemicals, high purity lignins are desired), and high quality lignin (low average molecular weight, good homogeneity, and low condensed structures). The criteria have been based on fractionation goals in literature (Alvira et al., 2010; Zhu et al., 2010; Agbor et al., 2011; Menon and Rao, 2012; Vancov et al., 2012). The green colour in Table 5.4 indicates values which satisfactory according to the criteria which has been mentioned, while the red colour indicates values which are below the limits.

Ionic liquid fractionation of both SCB and *E. grandis* show 50 % of satisfaction, while organosolv fractionation of both SCB and *E. grandis* show 37.5 % satisfaction. The factors which mainly affected the outputs of selection criteria are hemicellulose yields and purity, as well as lignin purity. The poor hemicellulose recoveries imply that the solubilised hemicelluloses were further hydrolysed and degraded. The hydrolysis and degradation of hemicelluloses solubilised in organosolv fractionation can be attributed to the severity of organosolv, autocatalysed organosolv uses high temperatures (185-210 °C) and at these temperatures solubilised hemicelluloses are susceptible to degradation. The similar trend (of poor balances of hemicelluloses) has been reported for autocatalysed organosolv, and solubilised hemicelluloses were mainly oligomers and monomers (Huijgen et al., 2011; Huijgen et al., 2012; Wildschut et al., 2013). High temperature (180.5 °C), long reaction time (210 min) and low ionic liquid concentration (32 %) rendered severe conditions, which further hydrolysed and degraded solubilised hemicelluloses.

As mentioned in chapter 4, the ionic liquid fractionation optimisations were significantly affected by hemicellulose (xylose) quantification method. The quantification method required acid hydrolysis, but acid hydrolysis was not effective due to buffering effect of acetate ion in the ionic liquid (Brandt et al., 2011). As the result, solubilised hemicellulose (xylan) was not quantified (as xylose was not detected), even in mild conditions. However, ionic liquid fractionation at moderate and mild conditions preserves hemicelluloses and outweighs organosolv (Alvira et al., 2010; Agbor et al., 2011; Menon and Rao, 2012; Vancov et al., 2012; Espinoza-Acosta et al., 2014). For organosolv fractionation, cellulose purity and digestibility were also low. The low to fair cellulose digestibility in autocatalysed organosolv treated materials has been observed by in literature, and is related to lack of acid catalyst, which facilitates further decrystallisation and depolymerisation of cellulose (Huijgen et al., 2011; Huijgen et al., 2012; Wildschut et al., 2013). For ionic liquid fractionation, lignin yields were low because of incomplete precipitation of solubilised lignin, as acid soluble and

degraded lignin could not be precipitated (Diedericks et al., 2012b), but higher for organosolv because the liquid fraction (containing all solubilised lignin) was analysed using UV-Vis spectroscopy. The purity of both hemicelluloses and lignin are not high because the compositions of liquid fraction were used for calculating proportions of hemicellulose and lignin.

S/G ratios of all lignin samples, the lignins were greater than 1.00, indicating high syringyl units. The amount of syringyl unitss in organosolv lignin obtained from SCB was increased to 1.26 times those in virgin SCB. The amount of syringyl units in organosolv lignin obtained from *E. grandis* was decreased to 0.74 times those in virgin *E. grandis*. The amount of condensed structures in ionic liquid lignin obtained from SCB was increased to 1.27 time those in virgin SCB. The amount of condensed structures in ionic liquid lignin obtained from *E. grandis* was increased to 1.28 times those in virgin *E. grandis*. Although all the lignins are condensed (S/G >1), the change in the amount of condensed structures in lignin from both organosolv and ionic liquid fractionations were not greater than twice those in virgin materials, so the increase was considered less significant. Very high amount of condensed structures in lignin tends to reduce lignin reactivity (Toledano et al., 2010; Vishtal and Kraslawski, 2011; Panagiotopoulos et al., 2013; Wells et al., 2013), but in this study, the amount of condensed structures in lignins is not very high. Thus the reactivity of recovered lignins, in relation to those of lignins in untreated feedstocks, might not be significantly lowered.

Table 5.4: The comparison of organosolv and ionic liquid fractionation methods on SCB and *E. grandis*, based on yield, purity and quality of fractions

Material	Process	Cellulose			Hemicellulose			Lignin		
		Yield	Purity	E.H	Yield	Purity	MW	Yield	Purity	S/G ratio
SCB	Org	92.1	63.5	63.0	39.7	44.1	—	42.6	52.3	2.11
	ILs	89.8	77.2	101.2	18.6	25.7	—	49.7	71.6	2.13
E. grandis	Org	80.1	66.2	45.7	28.2	27.2	—	37.0	71.8	3.24
	ILs	77.3	77.2	98.6	23.0	14.7	—	54.9	71.0	5.56

5.2. Comparison of Fractionation methods in terms of operational challenges

Ionic liquid and organosolv fractionation methods are compared for process efficiency in terms of robustness to different feedstocks, reaction conditions, extraction efficiency (recoveries and quality of products), separation efficiency (separation of solid-liquid fractions and lignin-hemicellulose mixtures), costs (equipment and chemicals), and scalability (pilot and commercial).

For biorefinery approach, ionic liquid fractionation method outweighs organosolv fractionation method. Table 5.5 presents the comparison of ionic liquid fractionation and organosolv fractionation methods in terms of extraction and separation efficiencies, cost effectiveness and scalability. Ionic liquid fractionation method is more robust to different feedstocks, often uses moderate reaction conditions, has high extraction efficiency, and has moderate to high separation efficiency, when compared to organosolv fractionation method. For biorefinery approach, it is essential to have high yields and quality fractions, to minimise hemicellulose and lignin degradation, and to have cost effective and easily scalable process (Alvira et al., 2010; Zhu et al., 2010; Agbor et al., 2011; Menon and Rao, 2012; Vancov et al., 2012).

As shown in Table 5.5, autocatalysed organosolv fractionation method fractionated SCB into average quality fractions, but fractionated *E. grandis* into low quality fractions. Cellulose digestibility was 63 % for organosolv treated SCB and 46% for organosolv treated *E. grandis*, and it indicated that organosolv fractionation performed better on SCB (herbaceous/agricultural residues) than on *E. grandis* (hardwoods). Hemicellulose (xylan) degradation was observed in organosolv fractionation of both SCB and E. grandis, while the lignin quality was good for organosolv fractionation of both feedstocks. In order to improve cellulose digestibility of autocatalysed organosolv treated materials, either increasing severity (which is undesirable as it would degrade hemicelluloses and lignin further) or coupling with alkaline extraction of hemicellulose (particularly pre-extraction, as it would lower amount of hemicelluloses exposed to degradation) could be applied (Huijgen et al., 2011; Huijgen et al., 2012; Wildschut et al., 2013). Autocatalysed organosolv is not robust to different types of feedstocks (when digestible cellulose is desired for production of biofuels and value added chemicals), and this observation differs from what was mentioned in literature reviews of organosolv process (Alvira et al., 2010; Zhu et al., 2010; Agbor et al., 2011; Menon and Rao,

2012; Vancov et al., 2012), mainly because the reviews considered acid-catalysed organosolv process.

Ionic liquid fractionation, for both SCB and *E. grandis* resulted in highly digestible (≥98 %) digestible solid residues. Ionic liquid fractionation of both SCB and *E. grandis* yielded high quality lignin, but hemicelluloses were significantly degraded. The high hemicellulose degradation is attributed to severe treatment conditions (very high temperature, 180.5 °C and very low ionic liquid concentration, 32 %w/w), the similar trend of hemicellulose degradation has been reported for ionic liquid treatments using temperatures ≥170 °C (Tan et al., 2009; Li et al., 2011). However, when moderate ionic liquid fractionation conditions are used, high cellulose digestibility, high quality lignin recovery, and hemicellulose preservation are achieved (Lee et al., 2009; Sun et al., 2009; Arora et al., 2010; Doherty et al., 2010; Li et al., 2010; Brandt et al., 2011; Fu and Mazza, 2011a; 2011b; Li et al., 2011a; Wu et al., 2011; Audu et al., 2012; Diedericks et al., 2012b; Hou et al., 2012; Karatzos et al., 2012b; Hou et al., 2013; Li et al., 2013; Shi et al., 2013; Socha et al., 2014; An et al., 2015). Organosolv lignin has been observed to have higher sugar contamination, higher ash content, and broader polydispersity than ionic liquid lignin, and the differences are affiliated to lower selectivity of organosolv fractionation as compared to ionic liquid fractionation (Vishtal and Kraslawski, 2011; Espinoza-Acosta et al., 2014).

Solvent recovery, recycle and reuse are essential and possible for both organosolv and ionic liquid fractionation, as the ability to recycle the solvent can reduce operating costs (Tan et al., 2009; Zhao et al., 2009; Alvira et al., 2010; Zhu et al., 2010; Agbor et al., 2011; Lan et al., 2011; Audu et al., 2012; Menon and Rao, 2012; Vancov et al., 2012; Brandt et al., 2013; An et al., 2015). Ethanol recovery following organosolv process is achieved through distillation, but ionic liquid recovery and recycling require optimisation (in order to reduce ionic liquid losses), as solubilised lignin and hemicellulose removal has to be improved prior to concentrating ionic liquids (Tan et al., 2009; Zhao et al., 2009; Alvira et al., 2010; Zhu et al., 2010; Agbor et al., 2011; Lan et al., 2011; Audu et al., 2012; Menon and Rao, 2012; Vancov et al., 2012; Brandt et al., 2013; An et al., 2015). Ethanol organosolv requires significant pressure control as the solvent is vaporises and poses explosion hazard at the operating temperature (Zhao et al., 2009; Harmsen et al., 2010; Huijgen et al., 2010). Ionic liquid fractionations do not require significant pressure control as ionic liquids have high temperature stability and negligible vapour pressures (FitzPatrick et al., 2010; Harmsen et al.,

2010; Stark, 2011). Although ionic liquids show good extraction and robustness to different types of feedstocks, the cost of ionic liquids are very high (Klein-Marcuschamer et al., 2011; Stark, 2011; Brandt et al., 2013; George et al., 2015). This high cost of ionic liquids has limited the scaling of ionic liquid fractionation process to only 6 litres capacity (Li et al., 2013). Organosolv has been used in pulping and pre-treatment for biofuels production at both demonstration and commercial scale, but it has not been applied globally due to need for optimising the process for production of high quality products (particularly hemicelluloses, as lignin has been proven to be high value and marketable) (Arato et al., 2005; Pan et al., 2005; Zhu et al., 2010; Sannigrahi and Ragauskas, 2013; Espinoza-Acosta et al., 2014; de Wild et al., 2015).

The separation of solid fraction from liquid fraction was cheaper and simpler for organosolv (as water and filtration were used) but costly for ionic liquid (as antisolvent like ketone:alcohol:water mixture or water with homogenising, and filtration are involved). Use of ketone:alcohol:water mixture as antisolvent is required to minimise the gel formation, without which blenders and homogenisers have to be used (Li et al., 2013). The separation of hemicellulose from lignin in organosolv liquor was challenging as alcohol precipitation could not work (solubilised hemicelluloses were alcohol soluble). The separation can be achieved by using other separation techniques like size exclusion chromatography, membrane technology and sub/supercritical antisolvent precipitation (Ebringerova and Heinze, 2000; Sun and Tomkinson, 2003; Sun et al., 2005; Haimer, et al, 2008; Willför et al., 2008; Xu et al., 2008; Haimer, 2010; Ren and Sun, 2010; Toledano et al., 2010; Peng et al., 2012). Introducing any of the mentioned alternative separation techniques, which might require costly sample preparation, leading to increased the operational costs. Ionic liquid fractionation applies alcohol precipitation as the separation method for hemicelluloses. Therefore, separation of hemicelluloses from lignin in liquid fraction might not pose operational challenge.

Ionic liquid fractionation posed a challenge of quantifying solubilised hemicelluloses, as ionic liquid buffered acid digestion (wet chemistry method). Inline FTIR and electrophoresis are other alternatives to monitor hemicellulose concentration, although they might increase capital cost (da Costa Lopes et al., 2013b; Hyvärinen et al., 2014). It is also worth considering sensitivity of the two fractionation methods to process changes. Ionic liquid is robust to various types of feedstocks, while autocatalysed organosolv gets limited by low

cellulose digestibility (when applied on hardwoods), as mentioned earlier in this section. As shown in Table 5.5, ionic liquid covers wide range of solvent concentration, temperatures, and time, while ranges are narrower for organosolv process, so rendering organosolv to be sensitive to process condition changes.

It was concluded that:

i. Ionic liquid fractionation method outweighs organosolv process, in terms of extraction and separation efficiencies (yield, purity and quality of fractions).
ii. Ionic liquid fractionation coupled with alkaline pre-extraction is the best treatment combination for extracting significant amount of hemicelluloses and preserving them in polymeric form, while also yielding high quality cellulose and lignin fractions.
iii. Comparison of organosolv and ionic liquid fractionation methods require further testing (scalability tests for ionic liquid fractionation) and techno-economic models development (which were not covered in this study due to high cost of ionic liquids and time constraints).

Table 5.5: Comparison of organosolv and ionic liquid fractionation methods in terms of extraction and separation efficiencies, as well as costs and demonstration and/or commercial scale status (based on this work, as well as other studies presented in Section 2.3 (Arato et al., 2005; Pan et al., 2005; Zhao et al., 2009; Klein-Marcuschamer et al., 2011; Stark, 2011; Brandt et al., 2013; Li et al., 2013; de Wild et al., 2015; George et al., 2015))

Method	**Reaction conditions**	**Robustness to different feedstocks**		**Extraction efficiency (product quality)**			**Separation efficiency**		**Costs**		**Scale-up**
		SCB	*E. grandis*	Cellulose	Hemicellulose	Lignin	Solid-liquid fractions	Lignin-hemicellulose mixture	Solvents	equipment	Demo/Commercial
Organosolv	35-70 %w/w ethanol, 180-210 °C, 0.5-1.5 hours	Fair fractionation method for SCB and other herbaceous/agricultural residues	Non-acid catalysed organosolv not effective for fractionating lignocellulose into high quality fractions	High recoveries, fair purity and low to fair digestibility (non-acid catalysed)	Oligomers and monomers (which are often susceptible to further hydrolysis and degradation)	Good quality lignin	Filtration and water washes required	Challenging as alcohol precipitation of solubilised hemicelluloses is not effective, so other methods like membrane technology and/or sub/supercritical solvent precipitation could be considered for biorefinery approach using organosolv process	Organic solvents (ethanol, methanol, other alcohols, ketones, polyols, organic acids), which are affordable at large quantities Need for solvent recovery and reuse	Costly equipment (pressure controlled reactors and corrosion resistant reactors for acid organosolv)	Both demonstration and commercial scale
Ionic liquid	5-100 %w/w ionic liquid, 90-190 °C, 0.5-22 hours	Good fractionation method for SCB and other herbaceous/agricultural residues	Good fractionation method for *E. grandis* and other hardwoods	Highly digestible cellulose	Polymers and oligomers (monomers at severe conditions)	Good quality lignin	Antisolvents, homogenisers (to break gels), filtration and washing	Alcohol precipitation for polymers and oligomers	Very costly (on kg basis). Need for solvent recovery and reuse	Affordable equipment	Few attempts on pilot scale tests

6. Conclusions and Recommendations

6.1. Conclusions

The aim of the study was to compare organosolv and ionic liquid fractionation methods on SCB and *E. grandis*, in terms of extraction and separation efficiencies, as well as operational challenges.

Ionic liquid fractionation method outweighed organosolv fractionation method. Ionic liquid fractionation method yielded highly digestible solids (≥98 %) for treatment of both SCB and *E. grandis*, while organosolv fractionation yielded low (46 % for *E. grandis* and 63 % for SCB). Both fractionation methods yielded high quality lignin. At the fractionation conditions used, ionic liquid fractionation was severe, and did not preserve hemicellulose. However, at moderate conditions, ionic liquid fractionation method preserves hemicelluloses and outweighs organosolv process. Ionic liquid fractionation method is more robust to different types of feedstocks than organosolv fractionation process.

In the case of separating solid-liquid streams, organosolv process utilised filtration and water washes, while ionic liquid process utilised antisolvents (for carbohydrates) and filtration. As for hemicellulose-lignin mixture separation, ionic liquid process used alcohol precipitation of hemicelluloses (cheaper approach), while organosolv process could not use alcohol precipitation, but required use of other techniques like membrane separation (which are relatively costly). Therefore, it can be concluded that ionic liquid fractionation method outweighs organosolv fractionation method, in terms of separation efficiency (as water can also be used as antisolvent).

Organosolv process uses affordable solvents, but most of requires costly equipment with pressure controls, as most organic solvents are volatile and cause pressure build-up. Ionic liquid process uses affordable equipment, but it has the challenge of costly solvents, which have even limited the scale-up. The clear distinction of the best method could not be reached.

When combining fractionation methods with either alkaline pre- or post-extraction, the combinations with alkaline pre-extractions were the most attractive. Alkaline pre-extraction combinations outweighed alkaline post-extraction combinations, as in alkaline pre-extraction there was significant extraction of high quality hemicelluloses, and the residual solids when fed to organosolv and ionic liquid processes did not experience significant hemicellulose

degradation. When virgin materials were fed to organosolv and ionic liquid processes, significant amount of hemicelluloses was exposed to harsh conditions and degraded, so alkaline post-extraction was fed with minimum hemicelluloses.

Ionic liquid fractionation coupled with alkaline pre-extraction is the best fractionation approach. The method extracts and preserve significant amount of hemicelluloses in polymeric form. Thus, allowing for application of recovered hemicelluloses in biopolymer-based biorefineries.

6.2. Recommendations

During ionic liquid fractionations with acetate-based ionic liquid concentrations ≥66%, wet chemical method (which involved acid digestion) did not work for solubilised xylan quantification (the pH of the post-acid hydrolysis read ≥5). It is therefore recommended that other quantification techniques, which do not require acid digestion, like inline FTIR and electrophoresis be studied for xylan detections in ionic liquid fractions (when ≥66% of acetate-based ionic liquid concentrations are used). Use of other quantification techniques might affect the determined concentrations of sugars in ionic liquid streams, and that might require correlation of the obtained results to those obtained in this study.

Ionic liquid fractionation method outweighs organosolv fractionation method. It is therefore recommended that several demonstration/pilot scale tests be carried out using ionic liquid fractionation method so that the feasibility of ionic liquid fractionation of lignocellulose in terms of techno-economics can be established. In addition, the solvent recovery and recycle optimisation studies for ionic liquid fractionations also need to be considered, so that the effect of solvent recyclability can be reflected on techno-economics.

To allow for further comparison of the effectiveness of organosolv to ionic liquid on fractionation of lignocellulose, the applications of obtained fractions need to be tested experimentally.

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Appendix A: Selection of ionic liquid

The pH values of the two different types of ionic liquids shown in Table A.1 were used to establish the choice of ionic liquid to use in the study. The acidic ionic liquid, [BMIM]MeSO4 could not be used in this study as it would not favour hemicelluloses' preservation.

Table A.0.1: The screening of ionic liquids based on pH values

Ionic liquid	Batch number	Conc. (g/L)	pH
[BMIM]$MeSO_4$	S87545-239	1.0164	3.62
		1.0603	3.62
		1.0624	3.63
	S87545-239	1.0363	3.70
		1.0338	3.68
		1.0323	3.64
	S87545-339	1.0793	3.64
		1.0760	3.65
		1.0786	3.64
	STB81080J9	1.0516	4.22
		1.0531	4.17
		1.0414	4.19
[EMIM]OAc	S82749-159	1.0497	6.40
		1.0394	6.33
		1.0381	6.37
	S72294-159	1.0173	5.91
		1.0182	5.9
		1.0139	5.91
	S82749-159	1.0099	6.05
		1.0096	6.08
		1.0028	6.10
	S82749-159	1.0029	6.08
		1.0039	6.11
		1.0192	6.12
	STBC3627V	1.0190	6.08
		1.0144	6.10
		1.0140	6.14

Appendix B: FTIR analyses of feedstocks and fractions

Table B. 0.1: FTIR analyses of virgin and treated SCB, as well as its fractions

Material	Band positions (cm^{-1})										
	3380	2900	1730	1630-1695	1600-1460	1420-1309	1240	1160	1030	895	832
Commercial Beechwood xylan	***	***	*	*	***	**	*	**	***	**	*
Virgin: W2	***	***	***	*	***	***	***	**	***	**	**
NaOH: Cellulose-rich solid (residue)	***	***	*	*	**	***	***	**	***	**	*
: Hemicellulose fraction	***	***	*	*	***	***	*	**	***	**	*
: Lignin fraction	***	*	*	***	***	**	*	*	*	*	*
ILs: Cellulose-rich solid (residue)	***	***	*	*	**	**	**	**	***	**	*
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	**	*	*	*	*	*	*
ILs + NaOH: Cellulose-rich solid (residue)	***	***	*	*	**	**	**	**	***	**	*
: Hemicellulose fraction	***	***	*	*	**	**	**	**	***	**	*
: Lignin fraction	***	*	*	***	***	**	*	*	*	*	*
NaOH + ILs: Cellulose-rich solid (residue)											
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	**	*	*	*	*	*	*
Organosolv: Cellulose-rich solid (residue)	***	***	**	*	**	***	***	**	***	*	*
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	***	**	**	*	*	*	*
Organosolv + NaOH: Cellulose-rich solid (residue)											
: Hemicellulose fraction	***	***	*	*	***	***	***	**	***	*	**
: Lignin fraction	***	*	*	***	***	**	*	*	*	*	*
NaOH + Organosolv: Cellulose-rich solid (residue)	***	***	*	*	**	***	***	**	***	**	*
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	***	**	**	*	*	*	*

* indicates absence of peak, ** indicates low intensity peak, and ***indicates strong intensity peak

Table B. 0.2: FTIR analyses of virgin and treated *E. grandis*, as well as its fractions

Material	Band positions (cm^{-1})										
	3380	2900	1730	1630-1695	1600-1460	1420-1309	1240	1160	1030	895	832
Commercial Beechwood xylan	***	***	*	*	***	***	*	**	***	**	*
Virgin	***	***	***	*	***	***	***	***	***	**	**
NaOH: Cellulose-rich solid (residue)	***	***	*	*	***	***	***	***	***	**	**
: Hemicellulose fraction	***	***	*	*	***	***	*	**	***	**	*
: Lignin fraction	***	*	*	***	***	*	*	*	*	*	*
ILs: Cellulose-rich solid (residue)	***	***	**	*	**	**	**	**	***	**	*
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	**	*	*	*	*	*	*
ILs + NaOH: Cellulose-rich solid (residue)	***	***	*	*	**	**	**	**	***	**	*
: Hemicellulose fraction	***	***	*	**	**	**	**	**	***	**	*
: Lignin fraction	***	*	*	***	***	**	*	*	*	*	*
NaOH + ILs: Cellulose-rich solid (residue)											
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	**	*	*	*	*	*	*
Organosolv: Cellulose-rich solid (residue)	***	***	**	*	***	***	***	**	***	**	*
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	***	**	*	*	*	*	*
Organosolv + NaOH: Cellulose-rich solid (residue)	***	***	*	*	**	**	**	**	***	**	*
: Hemicellulose fraction	***	***	*	*	***	***	***	***	***	*	**
: Lignin fraction	***	*	*	***	***	**	*	*	*	*	*
NaOH + Organosolv: Cellulose-rich solid (residue)											
: Hemicellulose fraction											
: Lignin fraction	***	*	*	***	***	*	*	*	*	*	*

* indicates absence of peak, ** indicates low intensity peak, and ***indicates strong intensity peak

Appendix C: Analyses of hemicelluloses

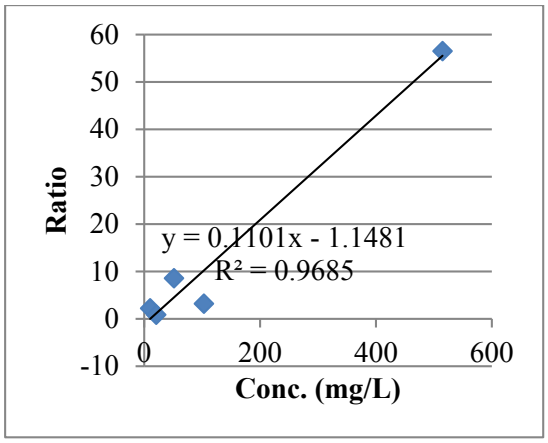
The compositions of different hemicelluloses were determined as shown below. These include gravimetric determination of lignin content, calibration of sugar standards used in GC-MS and data obtained from GC-MS.

Table C.0.1: Gravimetric determination of acid insoluble lignin in hemicelluloses

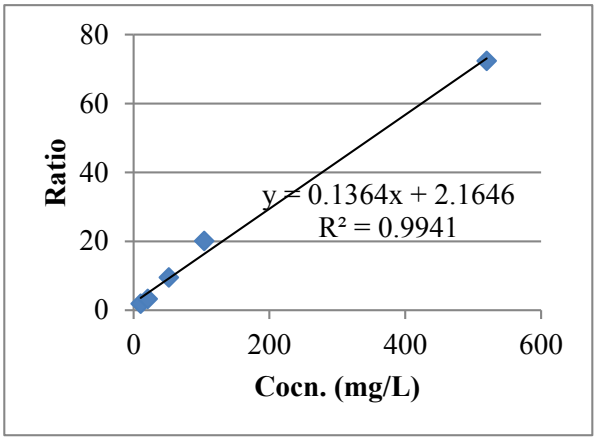
Sample	Mass (g)	crucible (g)	crucible + IR (g)	IR (g)	Crucible +ash (g)	Ash (g)	AIL	% AIL
SCB (ILs + NaOH post-extraction)	0.0308	45.4415	45.4471	0.0056	45.4459	0.0044	0.0012	3.90
SCB (ILs + NaOH post-extraction)	0.0318	29.2343	29.2396	0.0053	29.2383	0.0040	0.0013	4.09
E. grandis (ILs + NaOH post-extraction)	0.0322	29.6914	29.6964	0.0050	29.6949	0.0035	0.0015	4.66
E. grandis (ILs + NaOH post-extraction)	0.0326	29.3780	29.3832	0.0052	29.3817	0.0037	0.0015	4.60
SCB (Organosolv + NaOH post-extraction)	0.0316	43.3634	43.3771	0.0137	43.3686	0.0052	0.0085	26.90
SCB (Organosolv + NaOH post-extraction)	0.0317	29.1658	29.1794	0.0136	29.1709	0.0051	0.0085	26.81
E. grandis (Organosolv + NaOH post-extraction)	0.0323	29.3589	29.3778	0.0189	29.3685	0.0096	0.0093	28.79
E. grandis (Organosolv + NaOH post-extraction)	0.0321	29.6551	29.6735	0.0184	29.6645	0.0094	0.0090	28.04
SCB NaOH pre-extraction	0.0332	39.4970	39.5011	0.0041	39.4971	0.0001	0.0040	12.05
SCB NaOH pre-extraction	0.0333	30.3038	30.3080	0.0042	30.3040	0.0002	0.0040	12.01
E. grandis pre-extraction	0.0346	45.8336	45.8389	0.0053	45.8341	0.0005	0.0048	13.87
E. grandis pre-extraction	0.0351	29.2544	29.2595	0.0051	29.2546	0.0002	0.0049	13.96

For the calibration curves for sugars shown below, the Ratio is sample area divided by internal standard area.

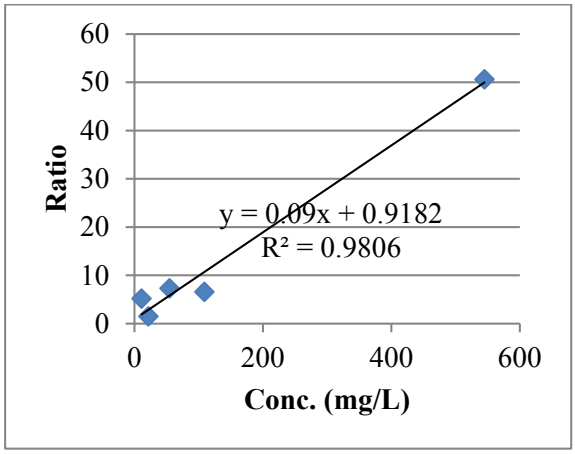
(a)



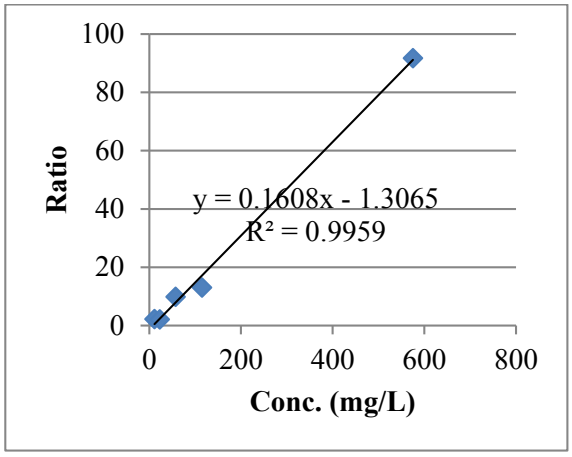
(b)



(c)



(d)



(e)

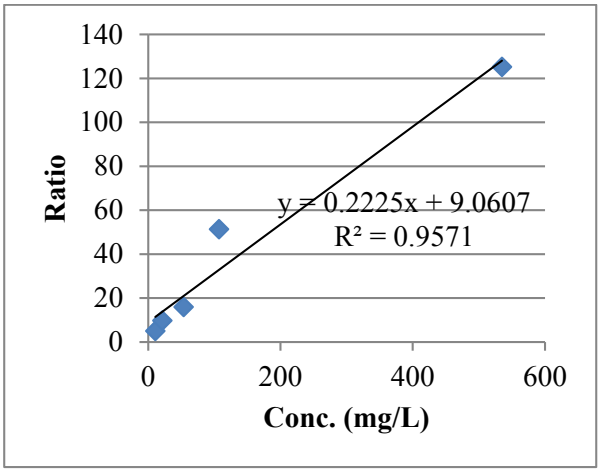


Figure C.0.1: The calibration curves for standard sugars used in compositional determination of hemicelluloses through GC-MS analyses, (a) is arabinose, (b) is mannose, (c) is galactose, (d) is xylose and (e) is glucose.

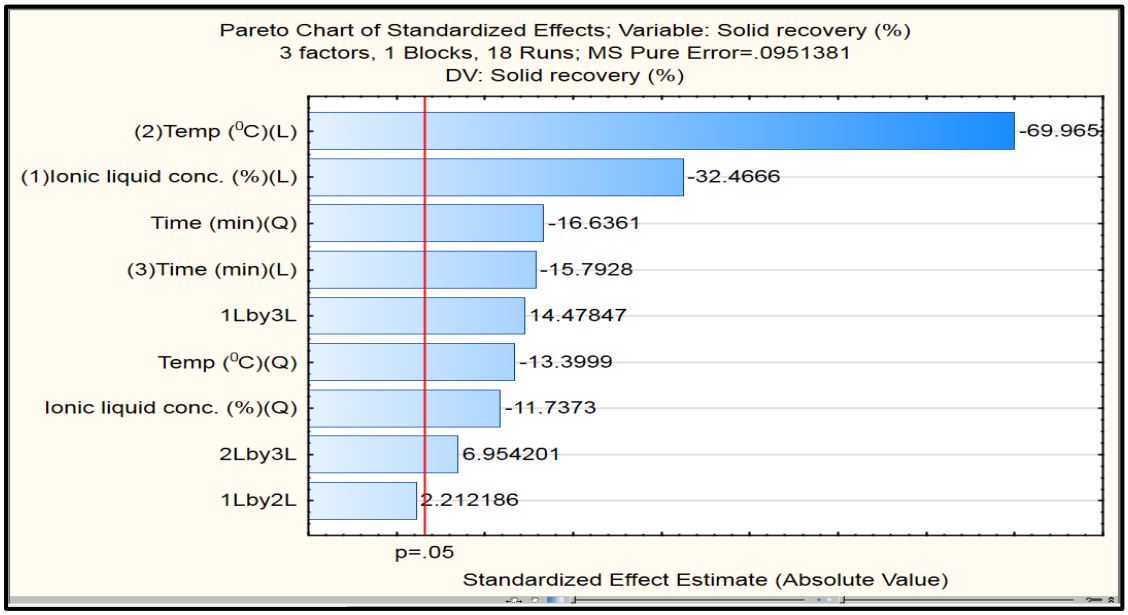
Table C.0.2: Neutral sugar composition of hemicelluloses

Sample	Mannose	Galactose	Xylose	Glucose	Arabinose
Larch Arbino-Galactan	0.00	88.26	1.79	0.00	9.96
Beechwood xylan	1.91	3.62	87.99	4.02	2.46
SCB (ILs + NaOH post-extraction)	1.70	3.39	81.37	4.41	9.13
E. grandis (ILs + NaOH post-extraction)	0.00	17.73	66.96	0.00	15.31
SCB (Organosolv + NaOH post-extraction)	0.00	0.00	78.49	10.13	11.38
E. grandis (Organosolv + NaOH post-extraction)	0.00	0.00	88.92	0.00	11.08
SCB NaOH pre-extraction	0.00	2.41	74.43	1.78	21.38
E. grandis pre-extraction	1.07	5.95	87.32	3.48	2.19

Appendix D: Pareto charts and surface plots (ionic liquid optimisation)

Appendix D presents the Pareto charts and response surface plots for optimisation of ionic liquid fractionation of SCB and *E. grandis*. The Pareto charts are used to mark the most significant figures at the given probability value. The most significant factors have their bars being greater than the probability line, while least significant factors have their bars smaller than the probability line. The response surface plot indicates the relationship of factor variables with response variables, when different models are fitted.

(a)



(b)

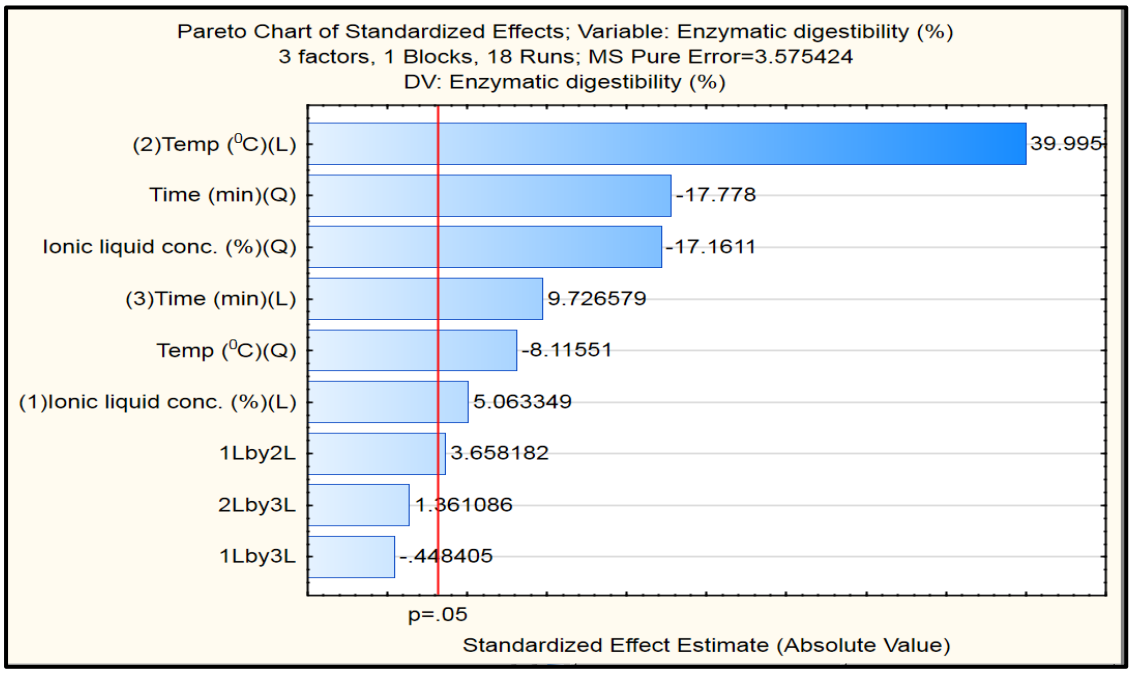
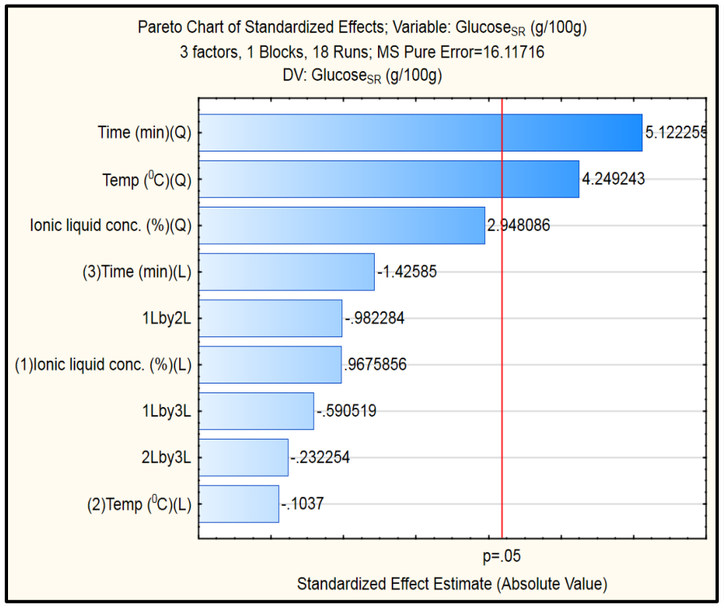
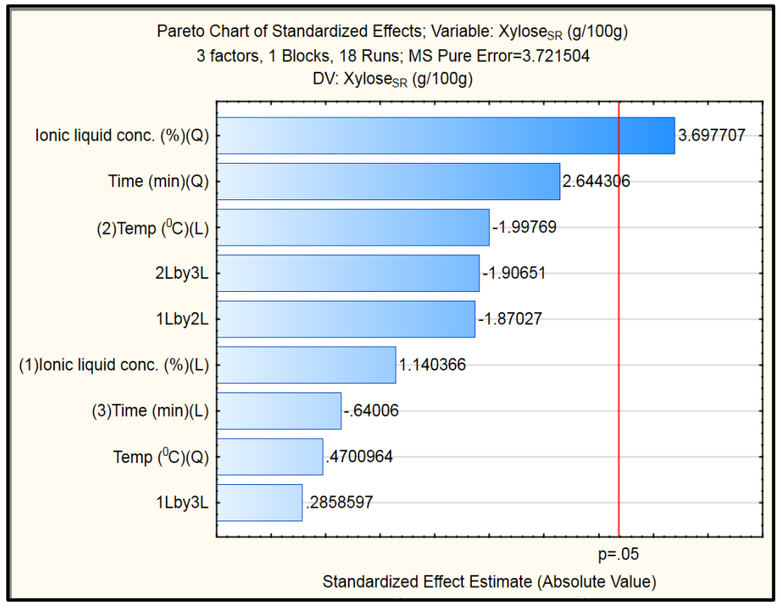
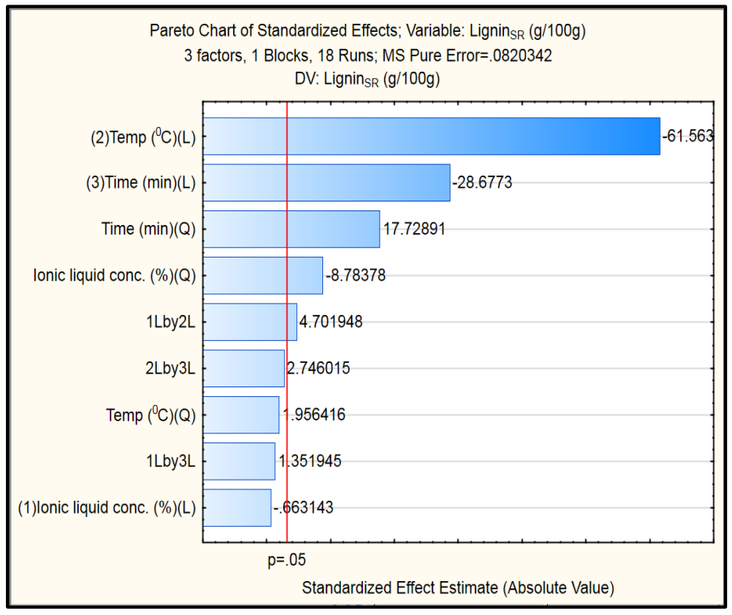


Figure D.0.2 The impact of ionic liquid treatment of virgin bagasse, (a) solid recovery, and (b) glucan digestibility

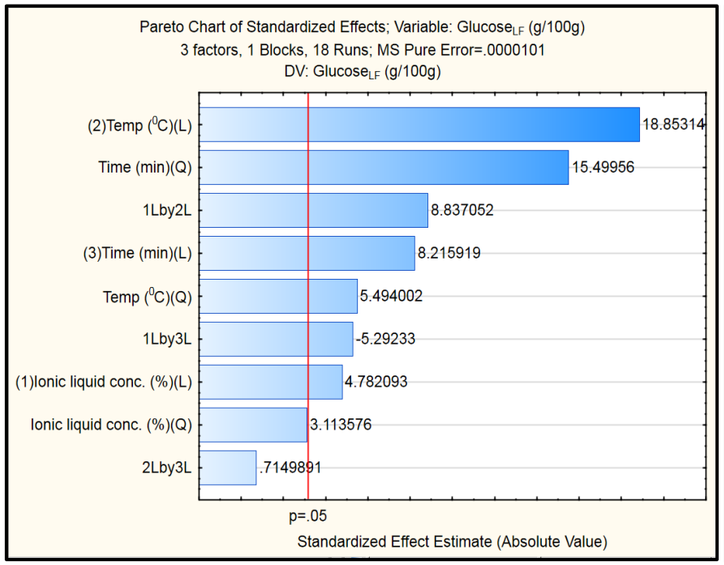
(a) (b) (c)

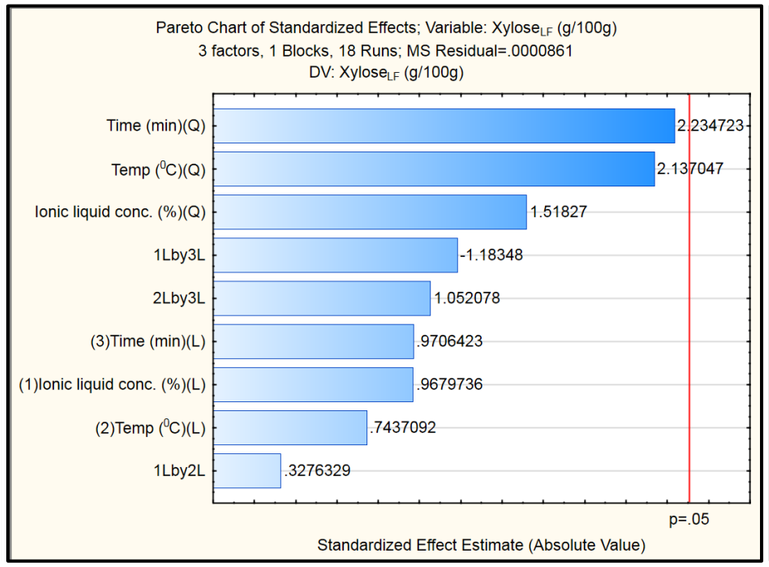






(d) (e) (f)





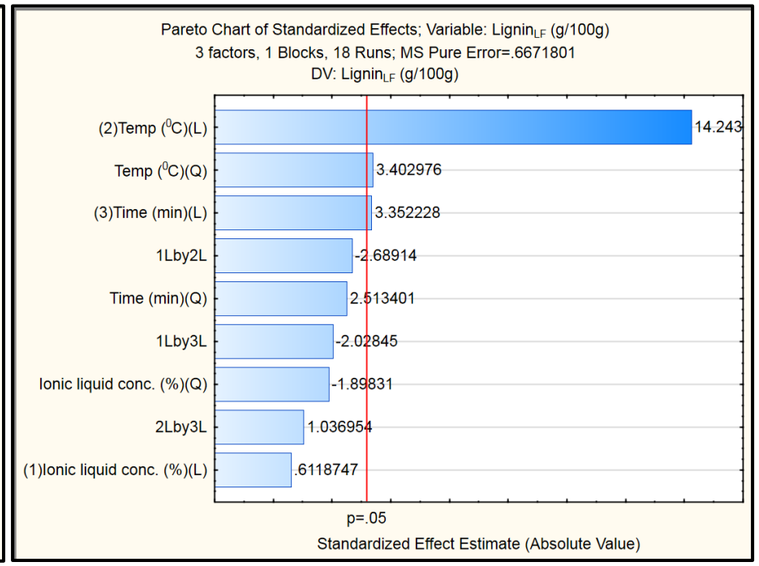
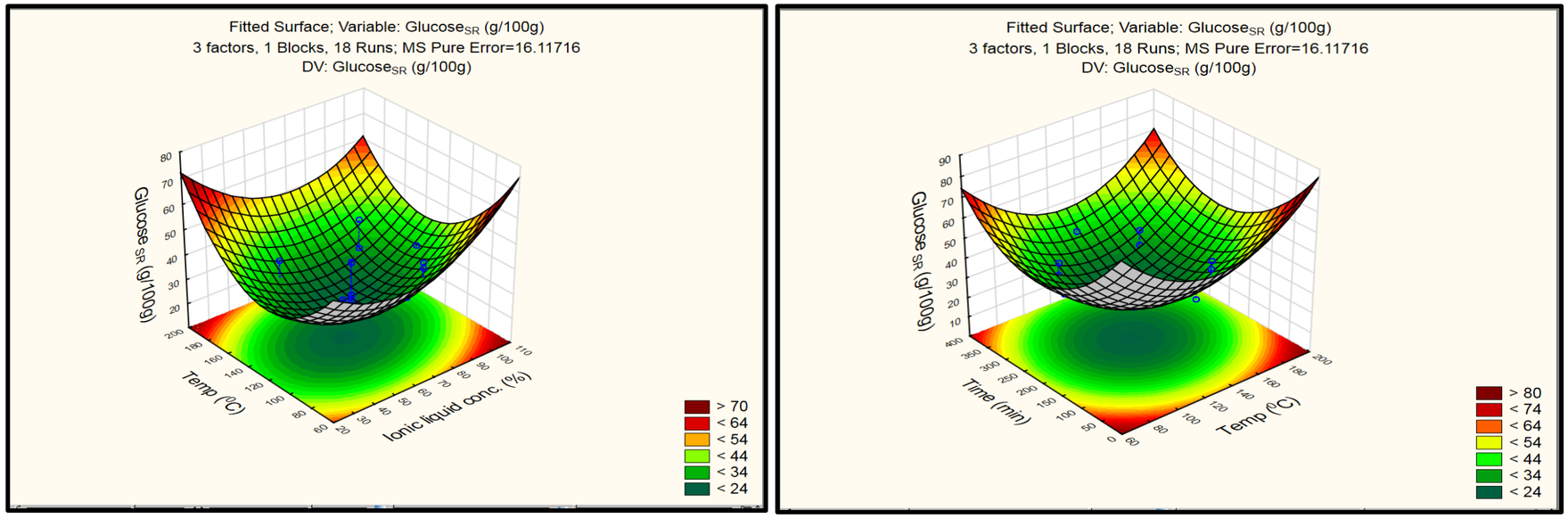


Figure D.0.3: The impact of ionic liquid treatment of virgin bagasse, (a) glucan retained, (b) xylan retained, (c) lignin retained, (d) glucan dissolved, (e) xylan dissolved, and (f) lignin dissolved

(a)

(b)



(c)

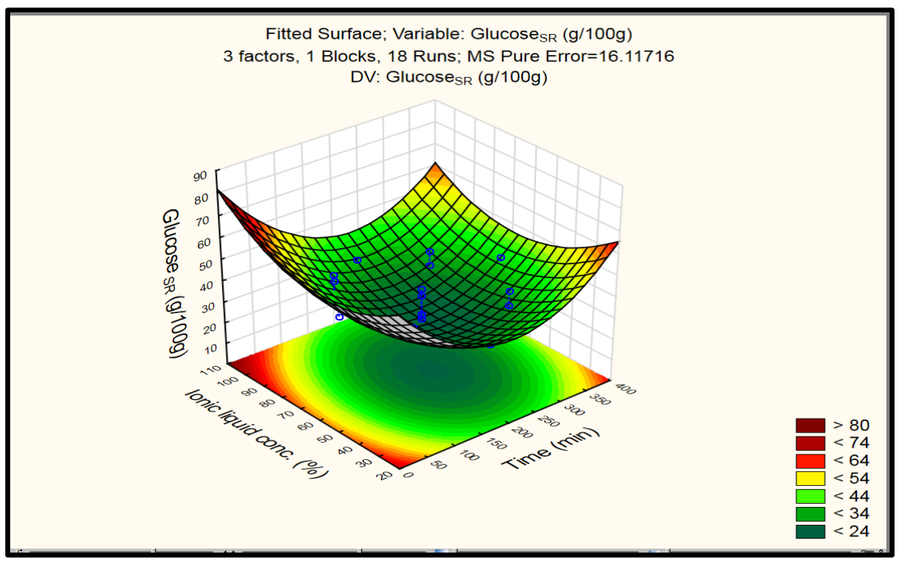
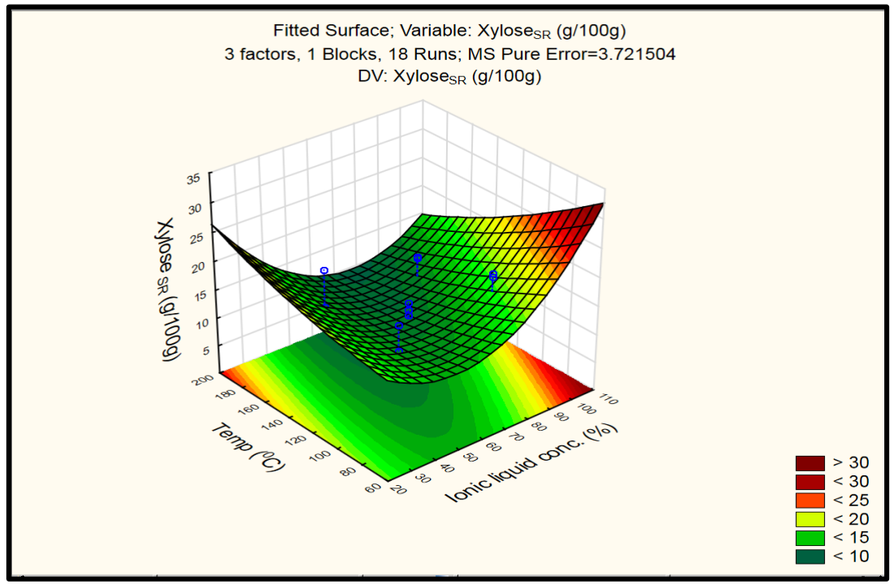
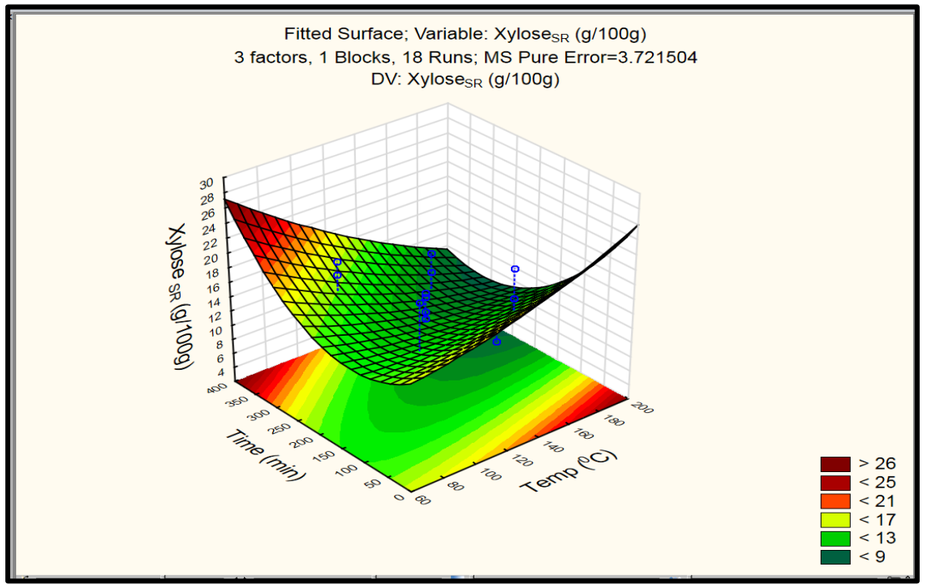


Figure D.0.4: Glucan retention (in virgin bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

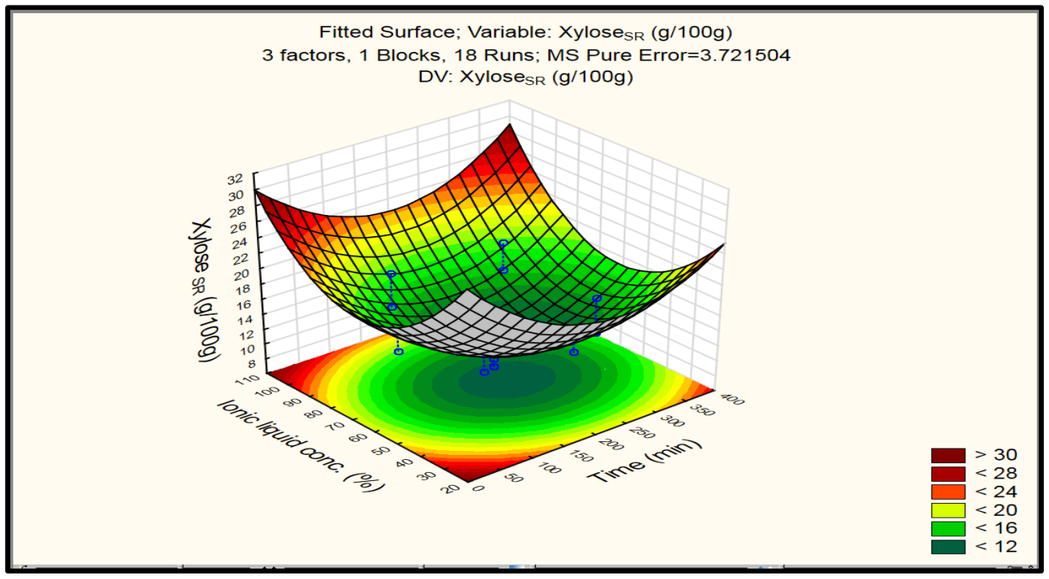
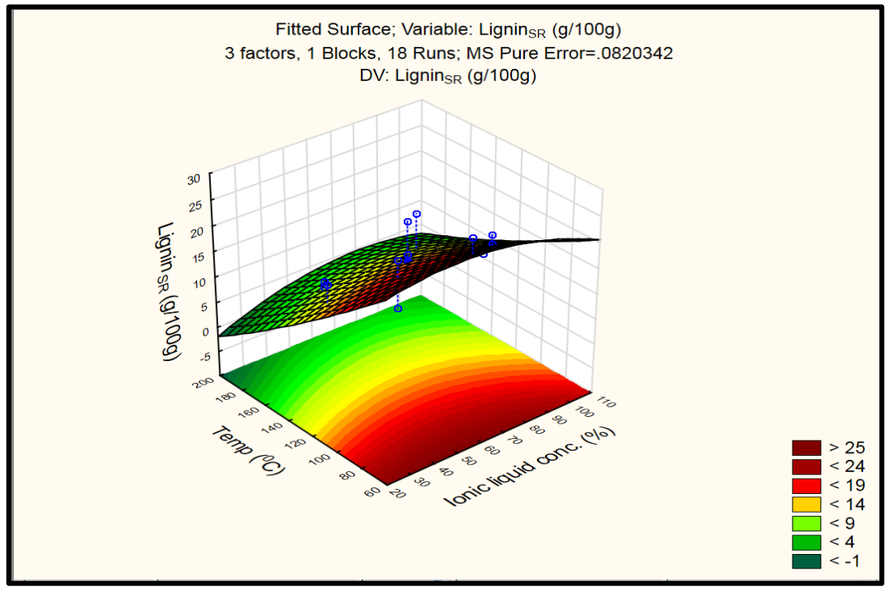
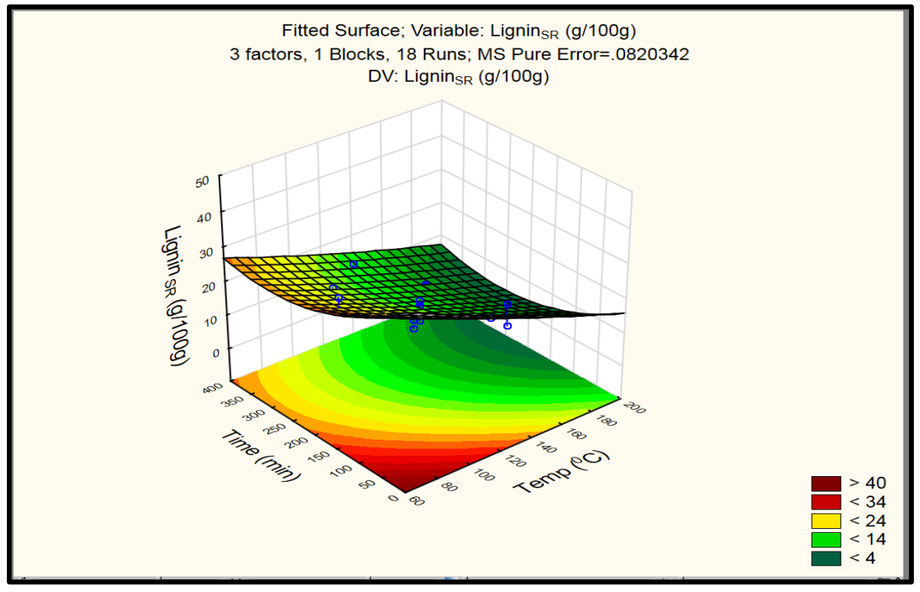


Figure D.0.5: Xylan retention (in virgin bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

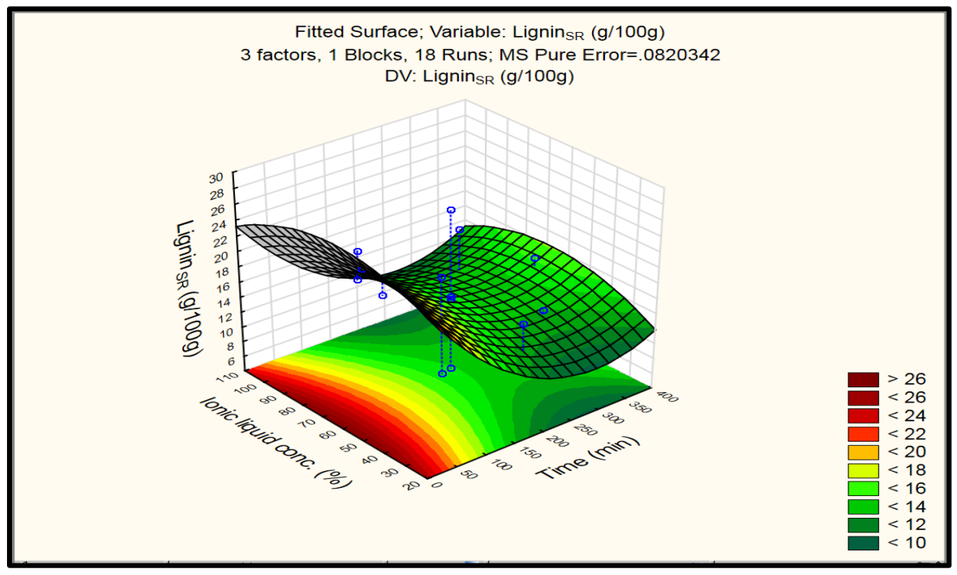
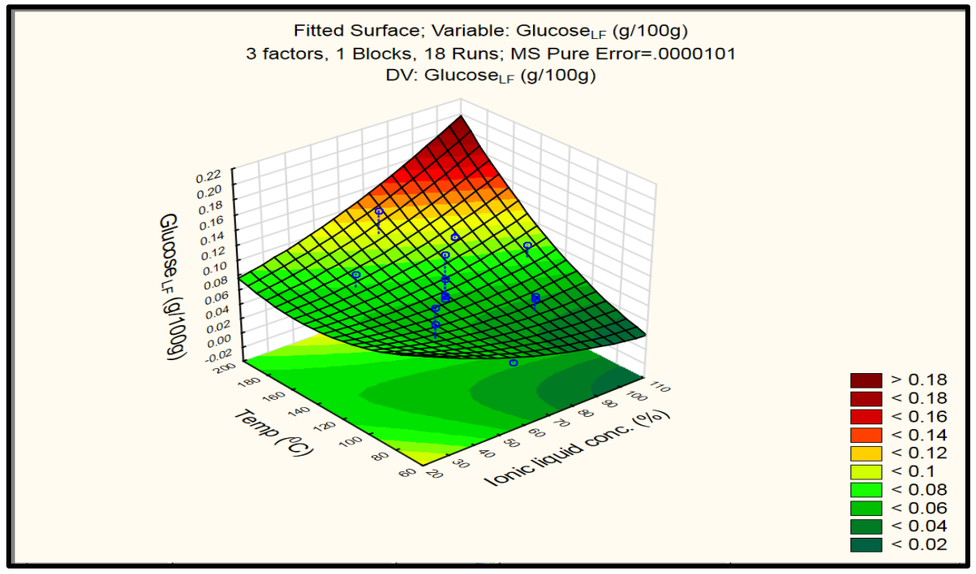
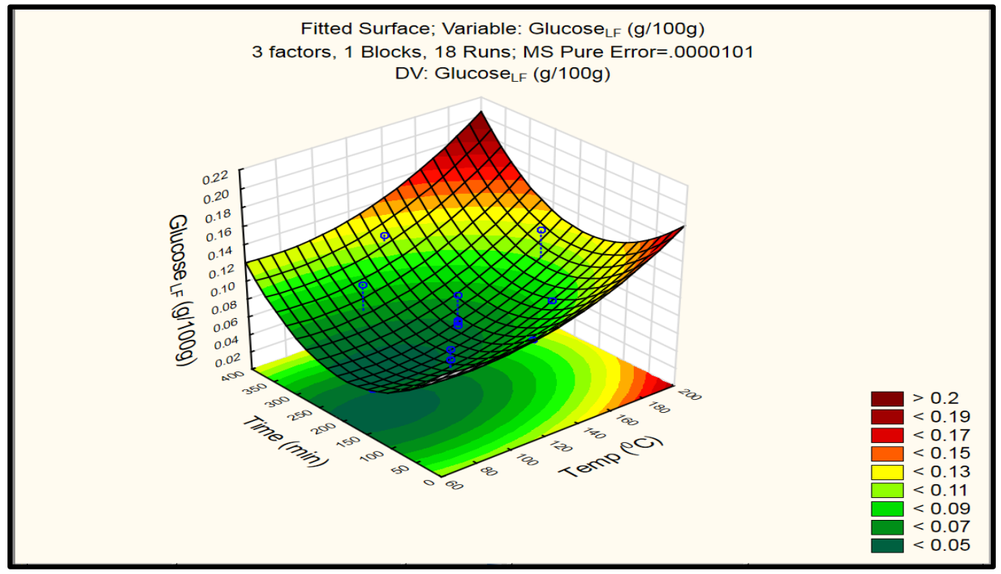


Figure D.0.6:Lignin retention (in virgin bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

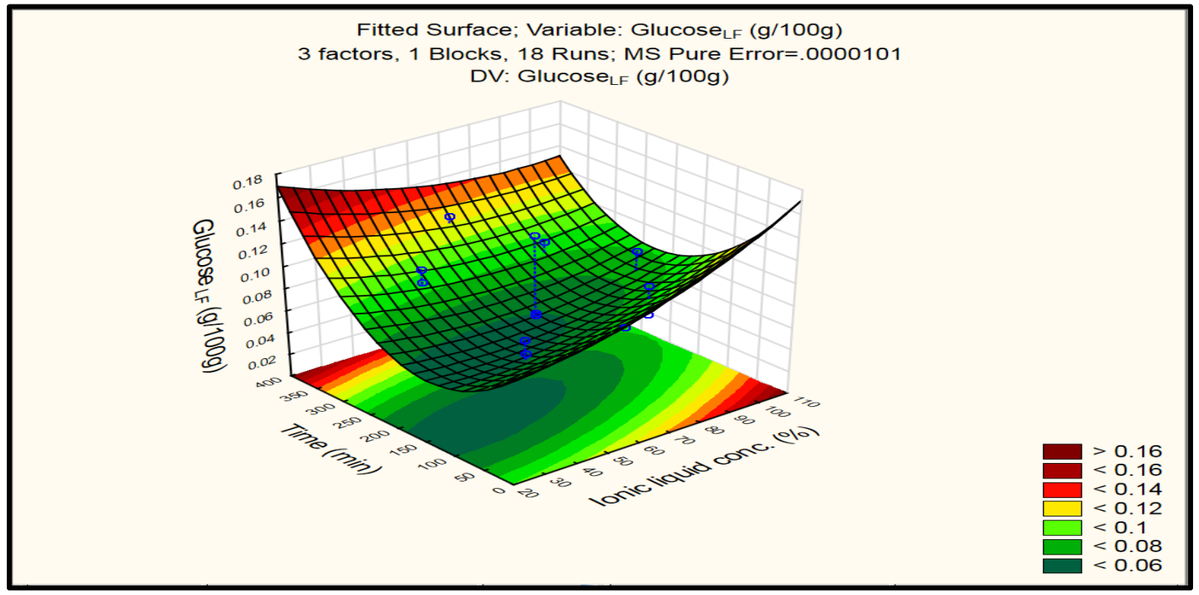
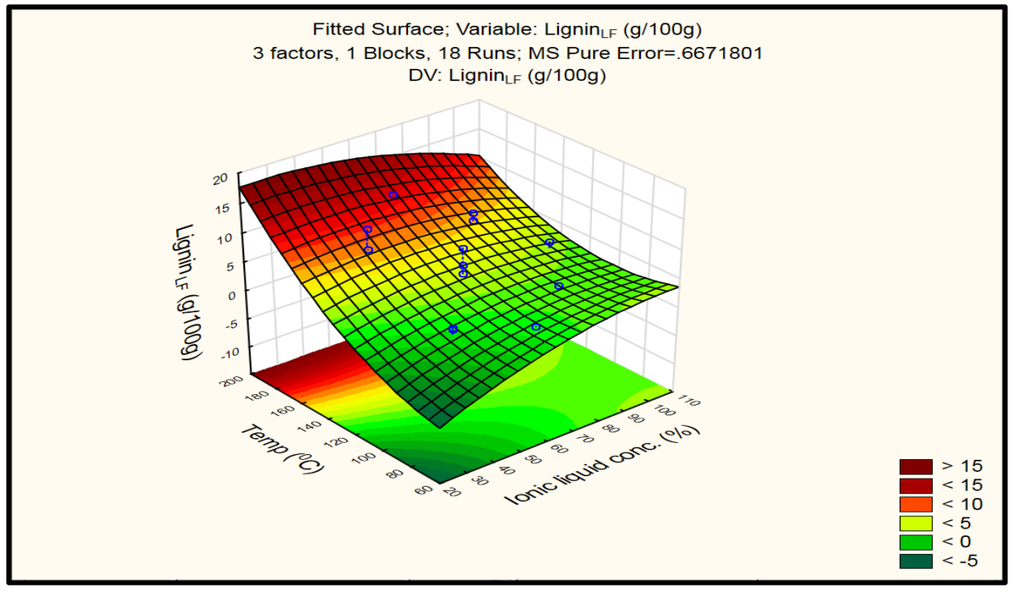
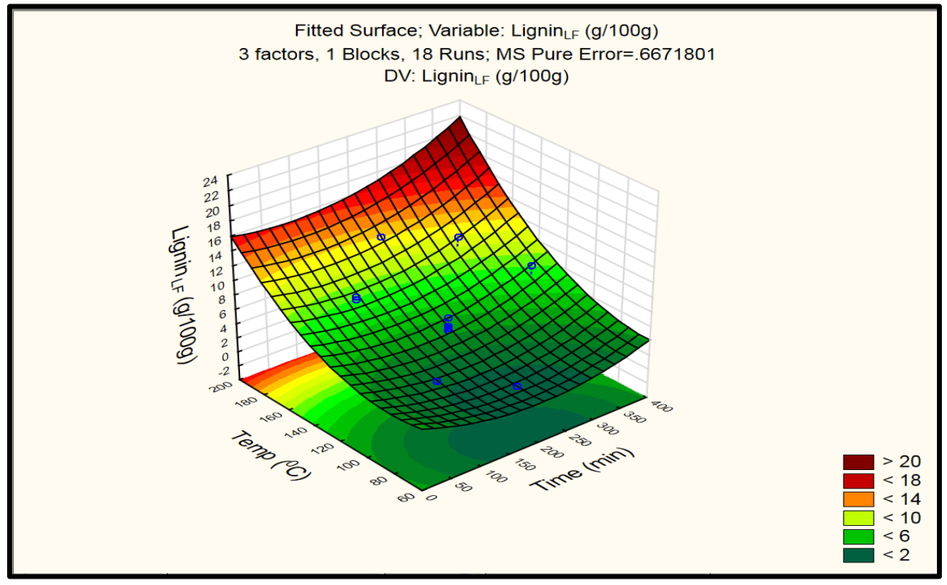


Figure D.0.7: Glucan dissolution (in virgin bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

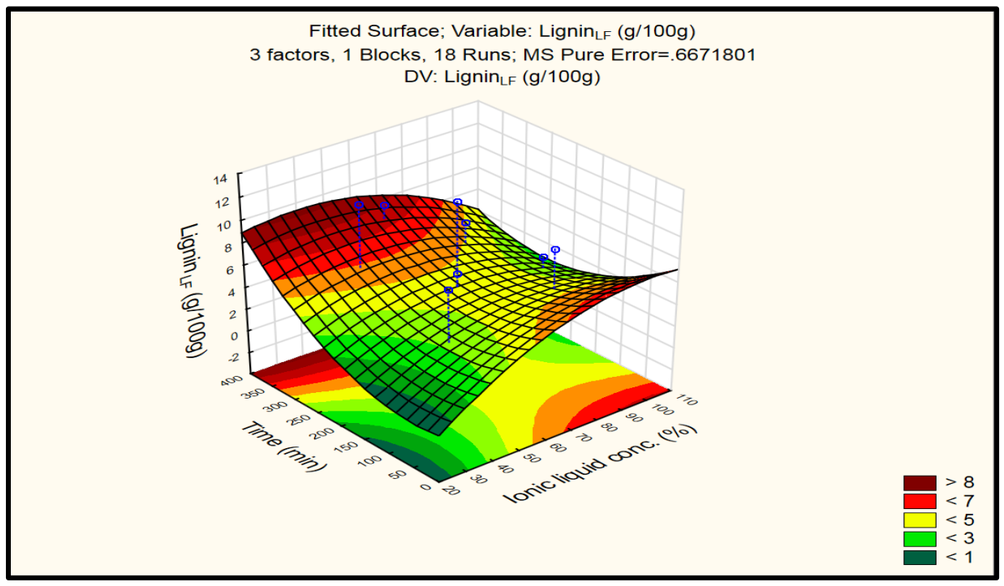
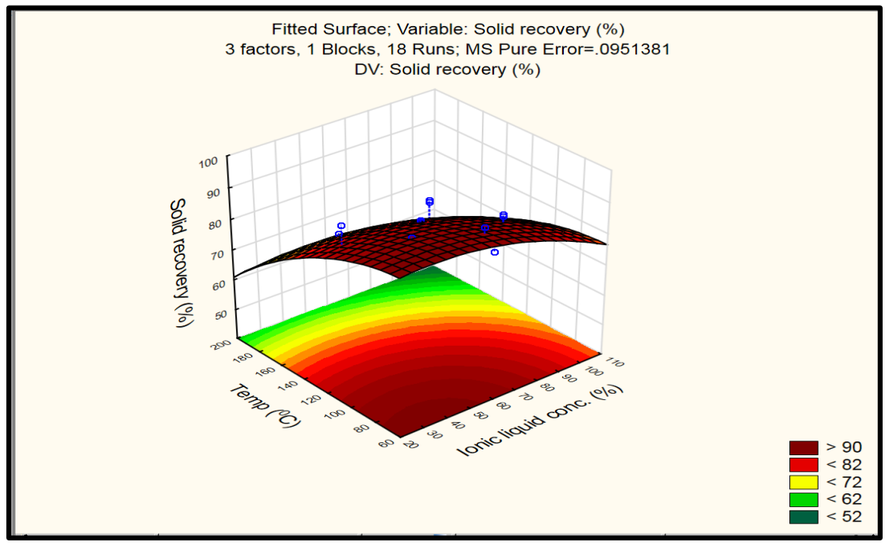
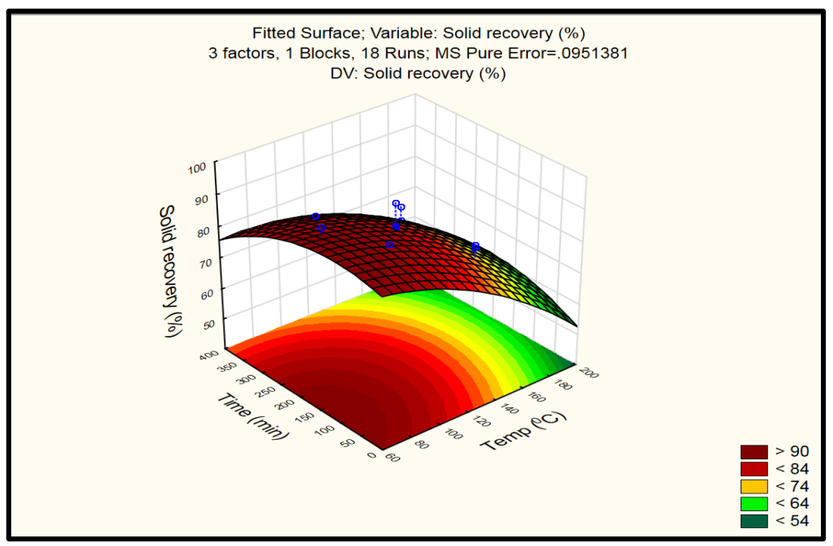


Figure D.0.8: Lignin dissolution (in virgin bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

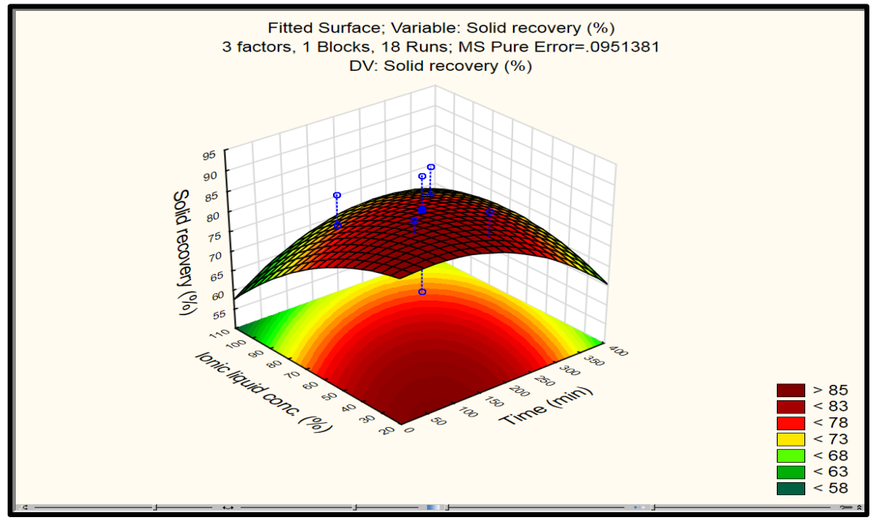
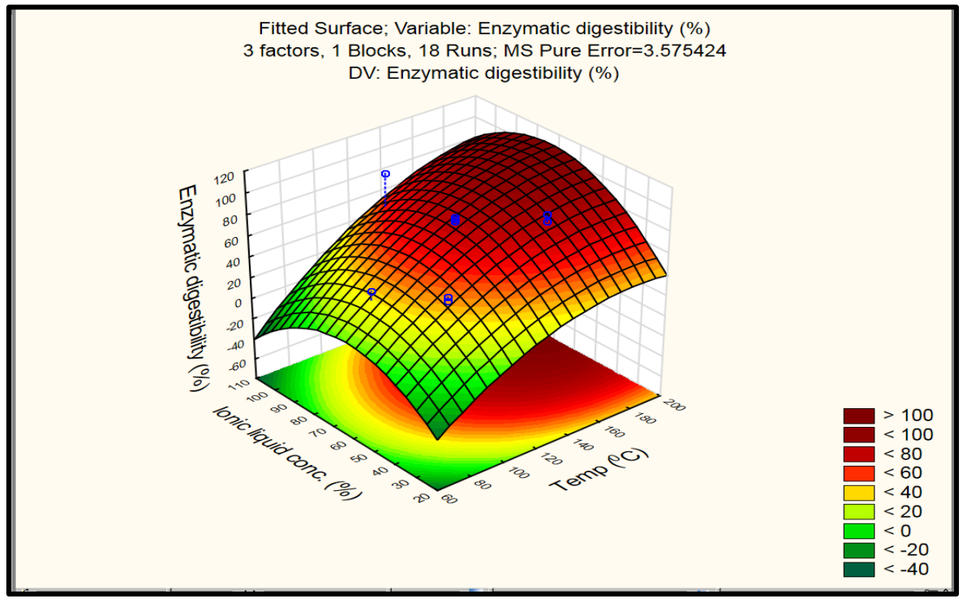
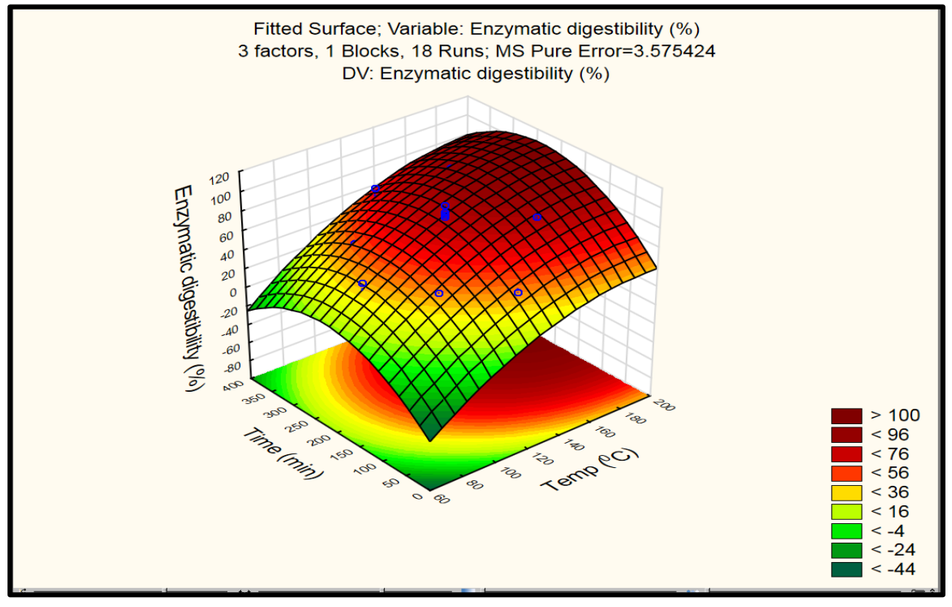


Figure D.0.9: Solid recovery (in virgin bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

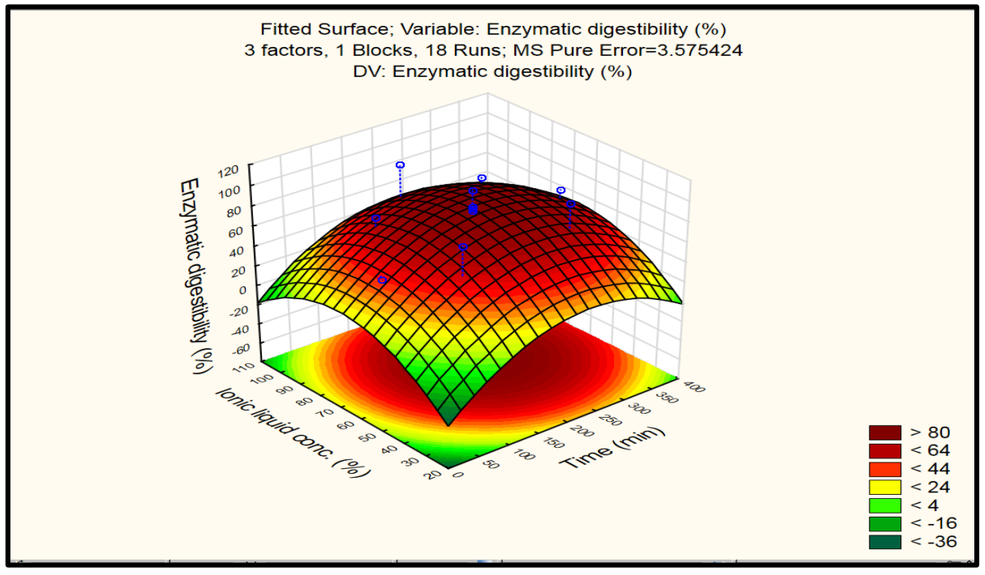
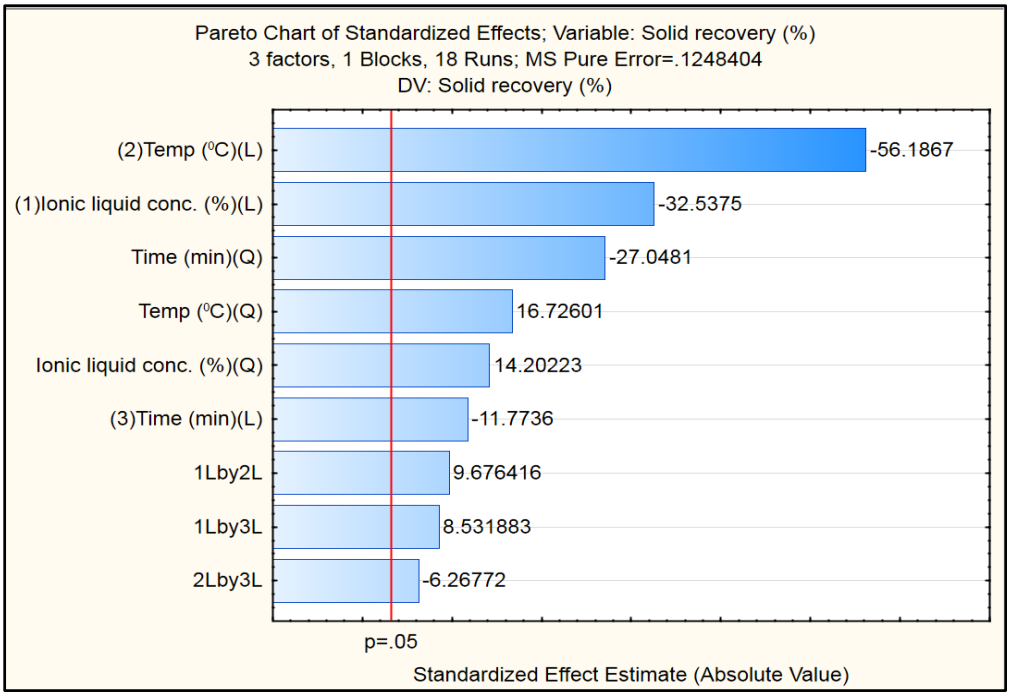


Figure D.0.10: Glucan digestibility (in virgin bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)

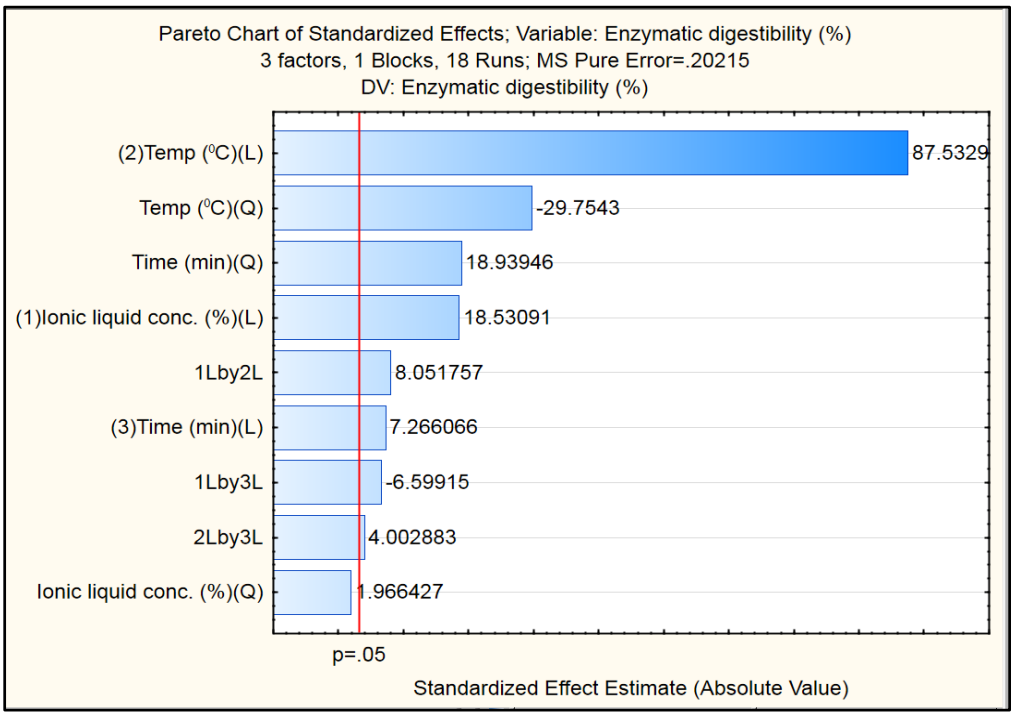
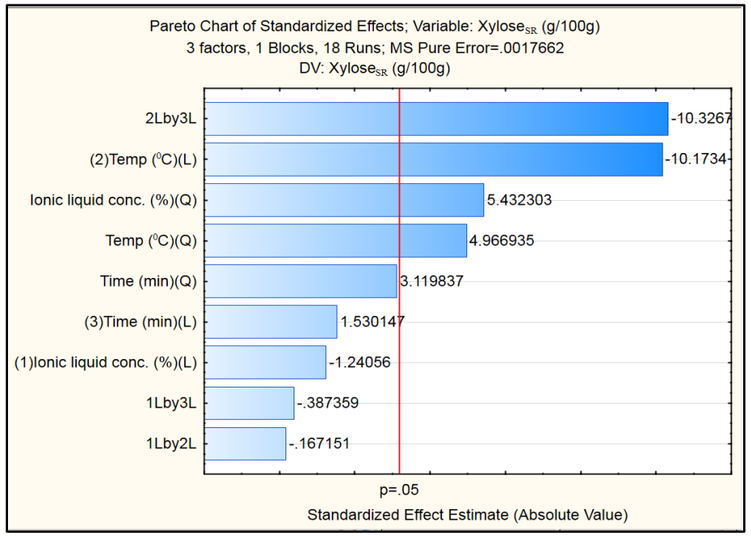
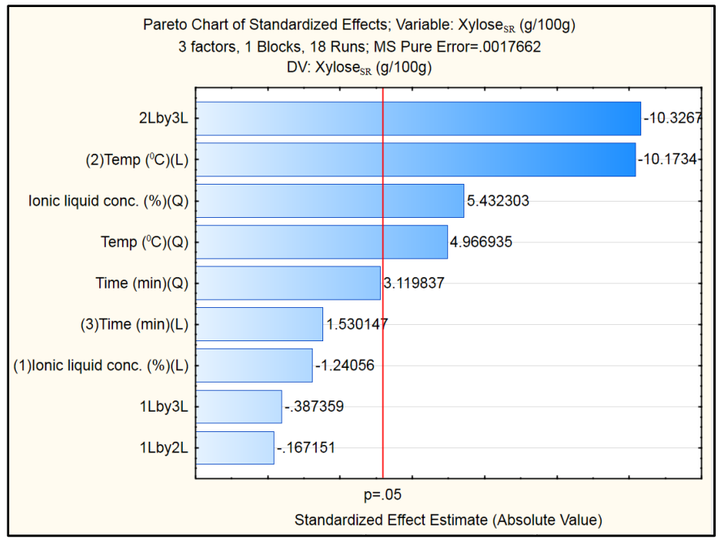


Figure D.0.11: The impact of ionic liquid treatment of pre-extracted bagasse, (a) solid recovery, and (b) glucan digestibility

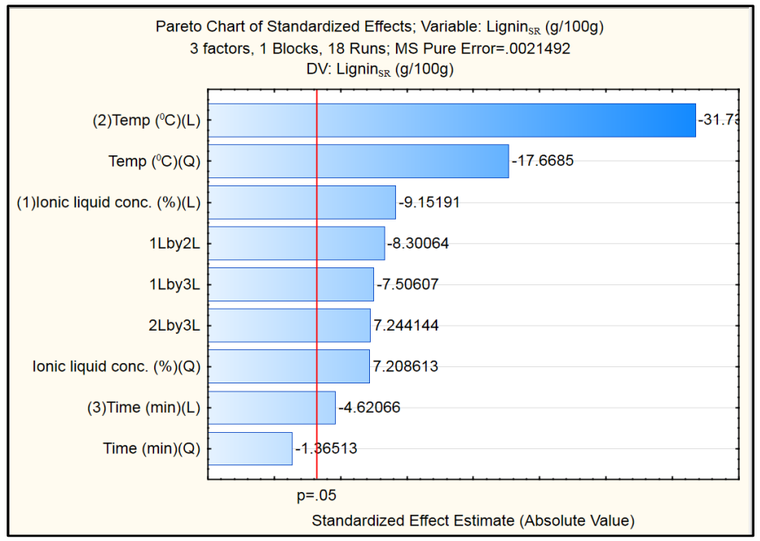
(a)



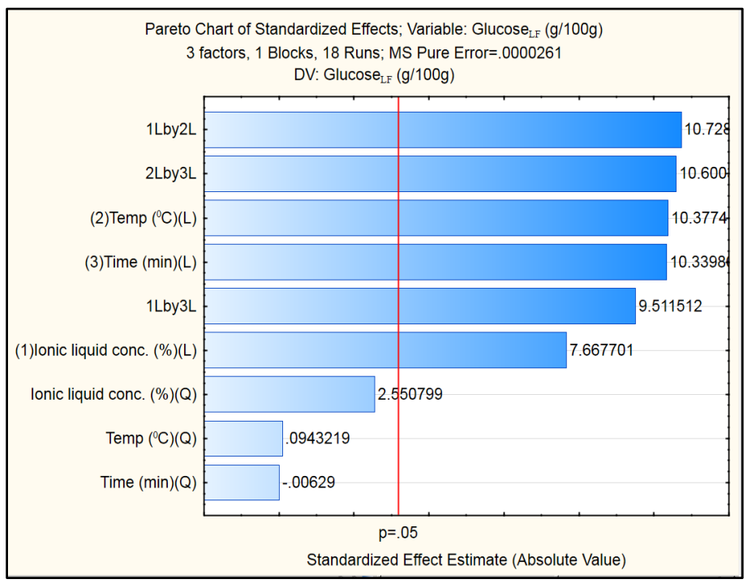
(b)



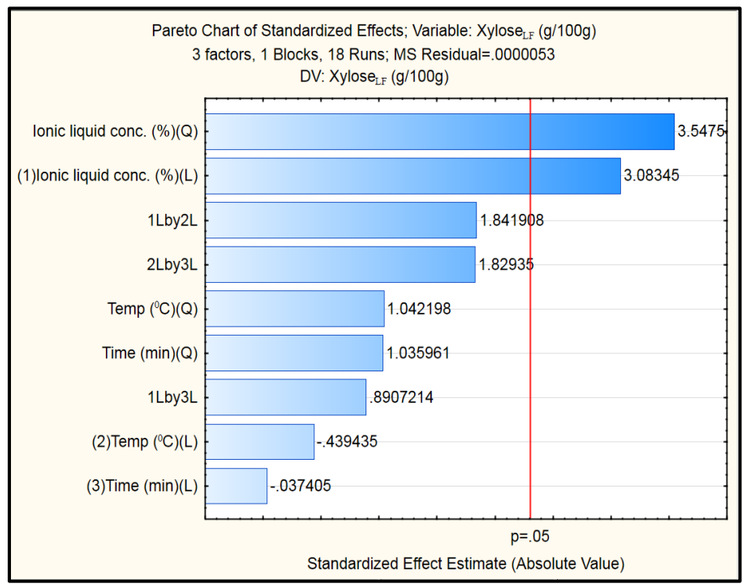
(c)



(d)



(e)



(f)

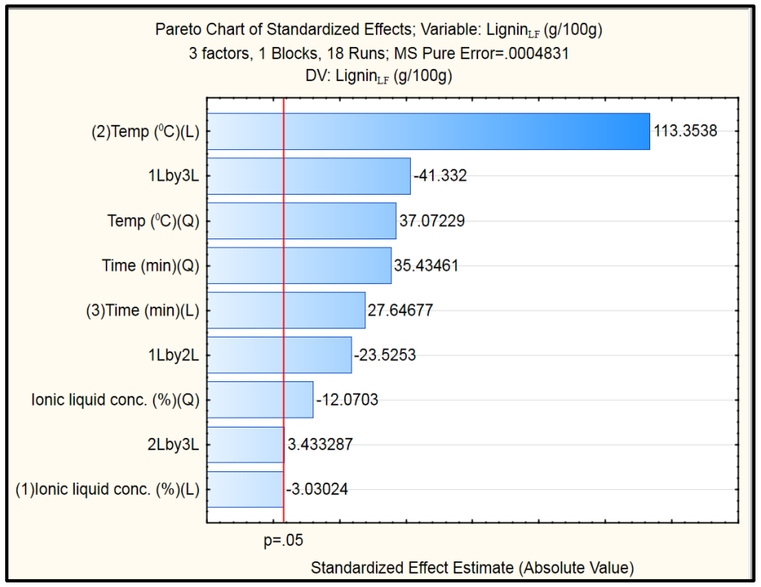
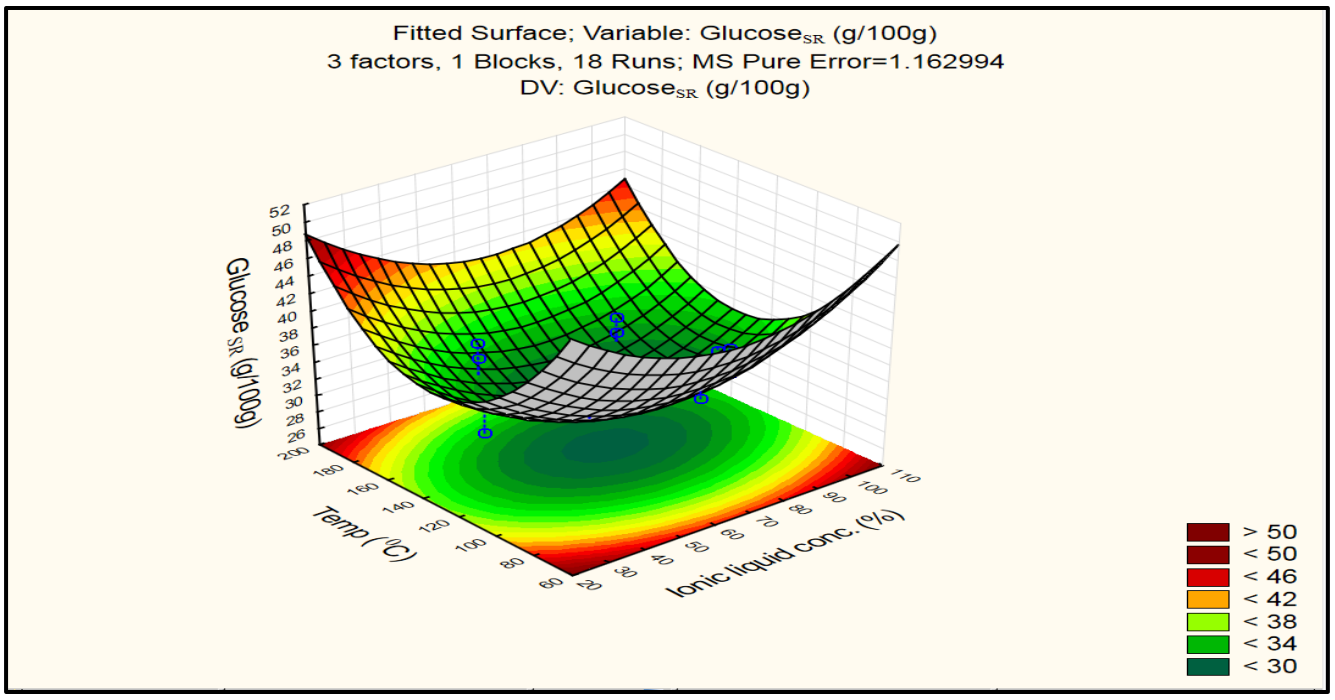
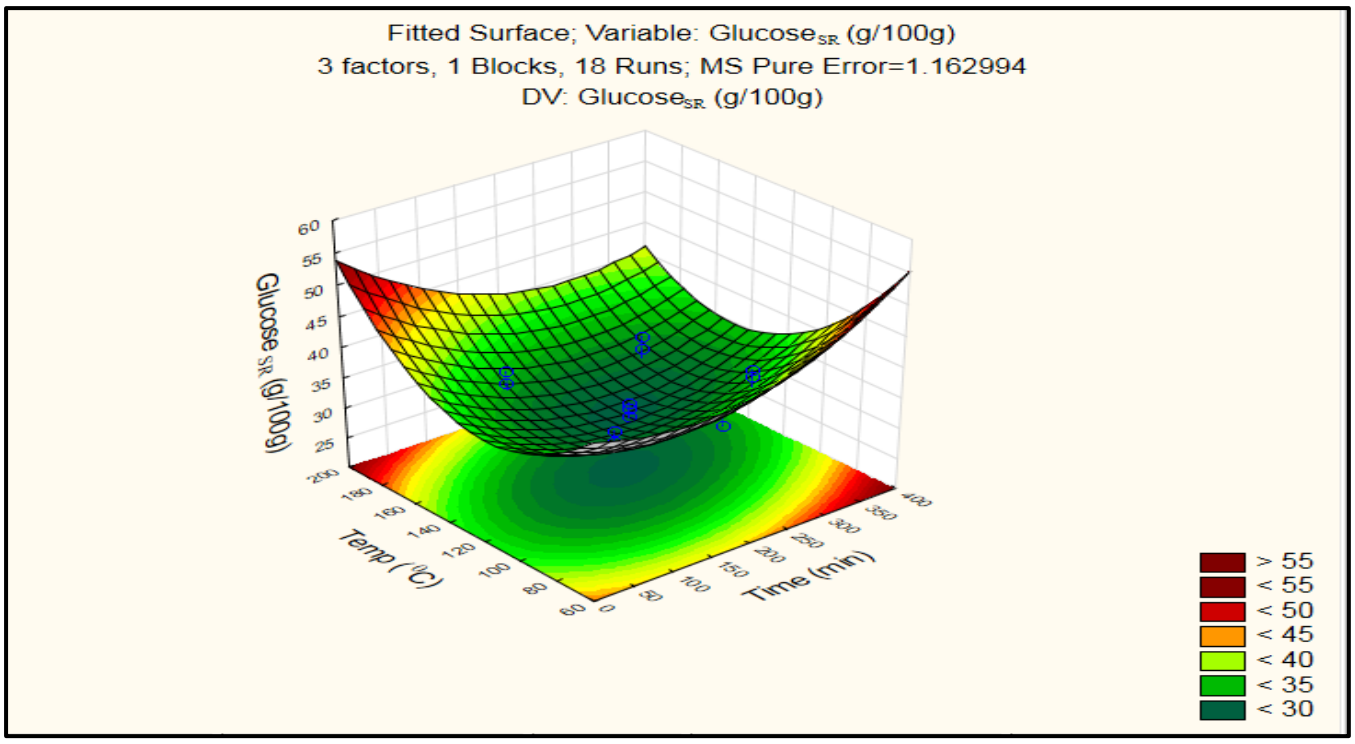


Figure D.0.12: The impact of ionic liquid treatment of pre-extracted bagasse, (a) glucan retained, (b) xylan retained, (c) lignin retained, (d) glucan dissolved, (e) xylan dissolved, and (f) lignin dissolved

(a)



(b)



(c)

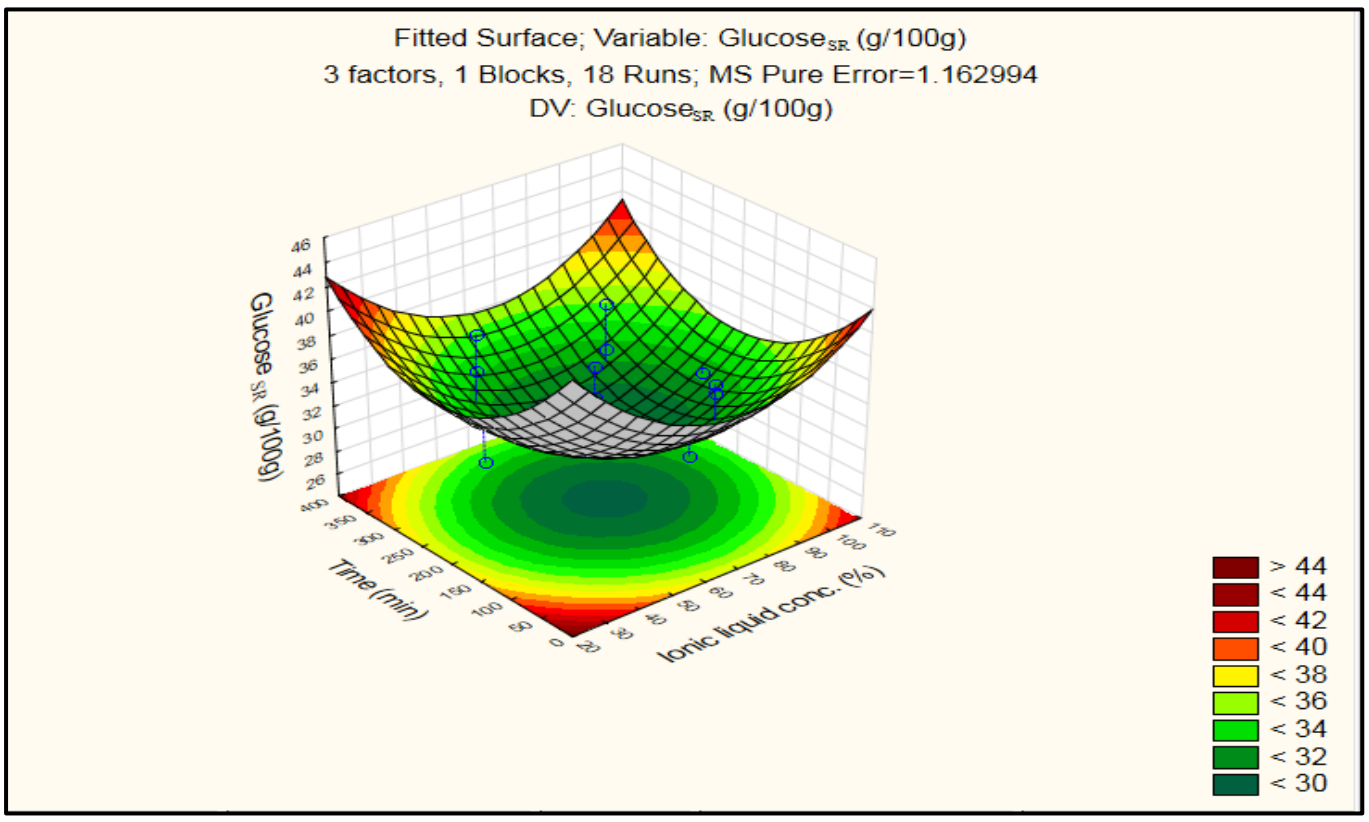
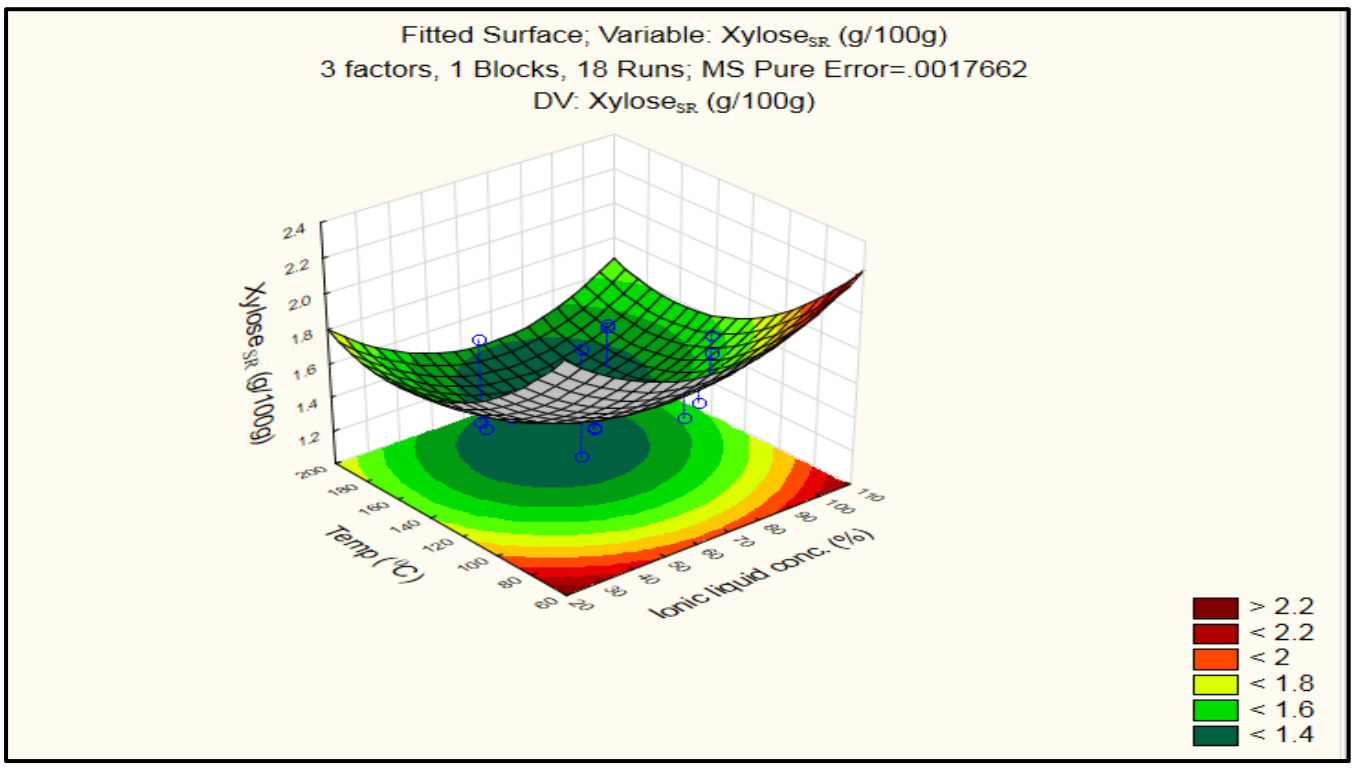
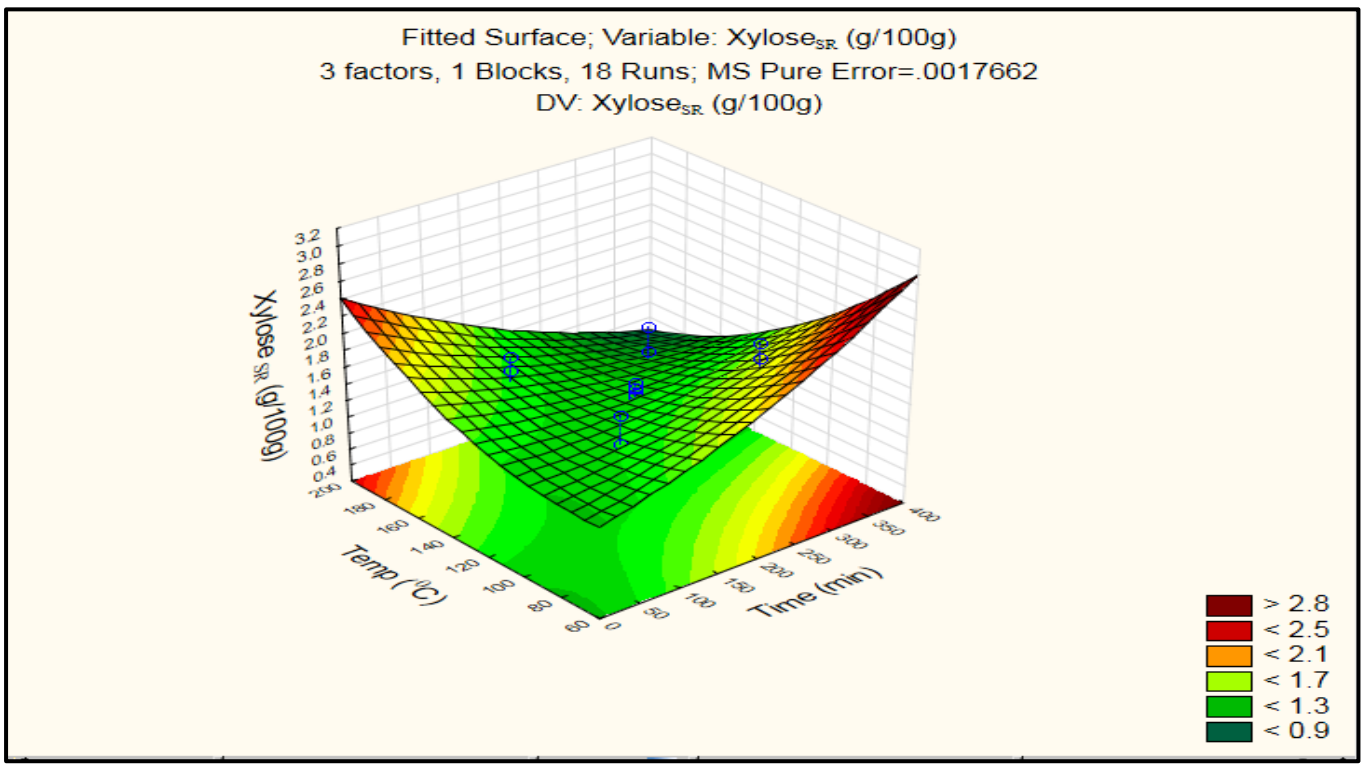


Figure D.0.13: Glucan retention (in pre-extracted bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

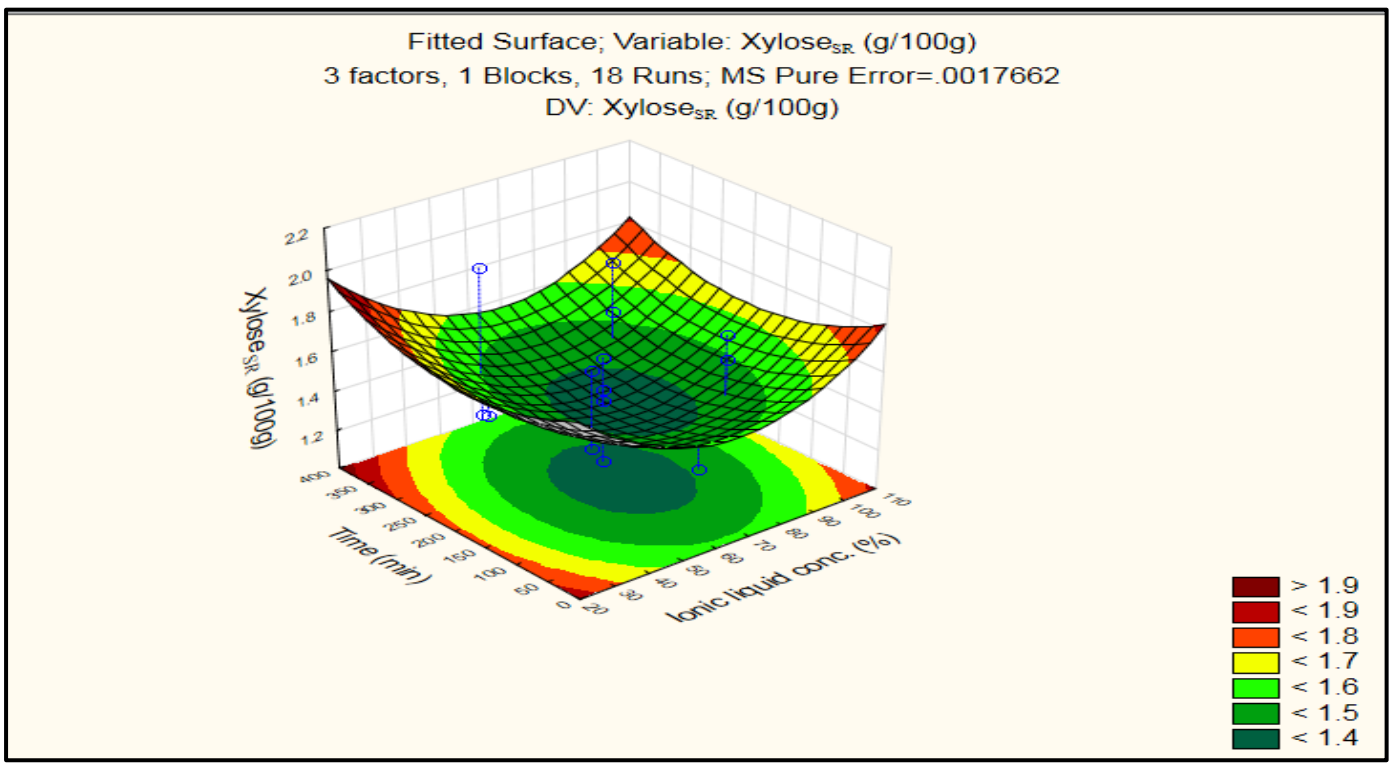
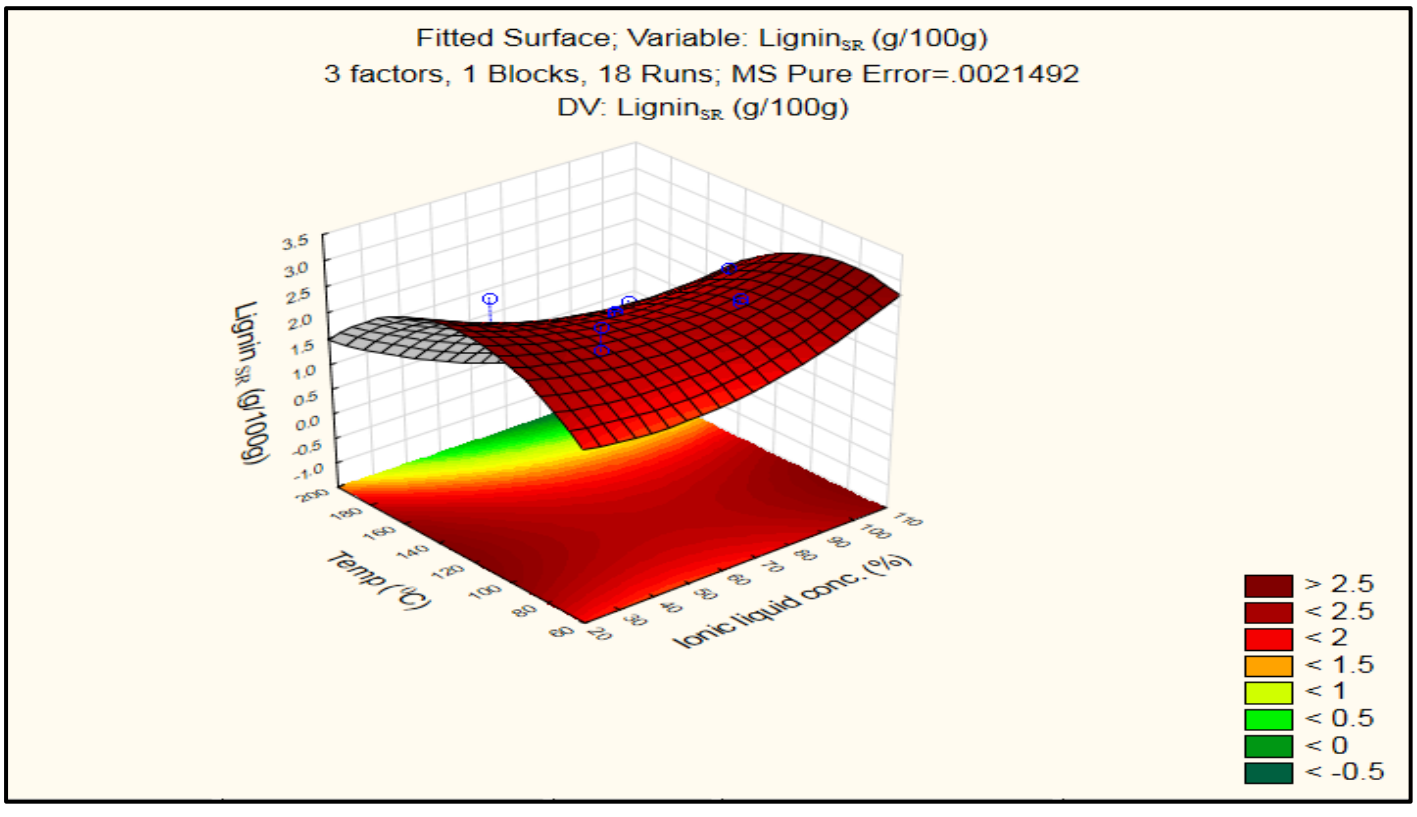
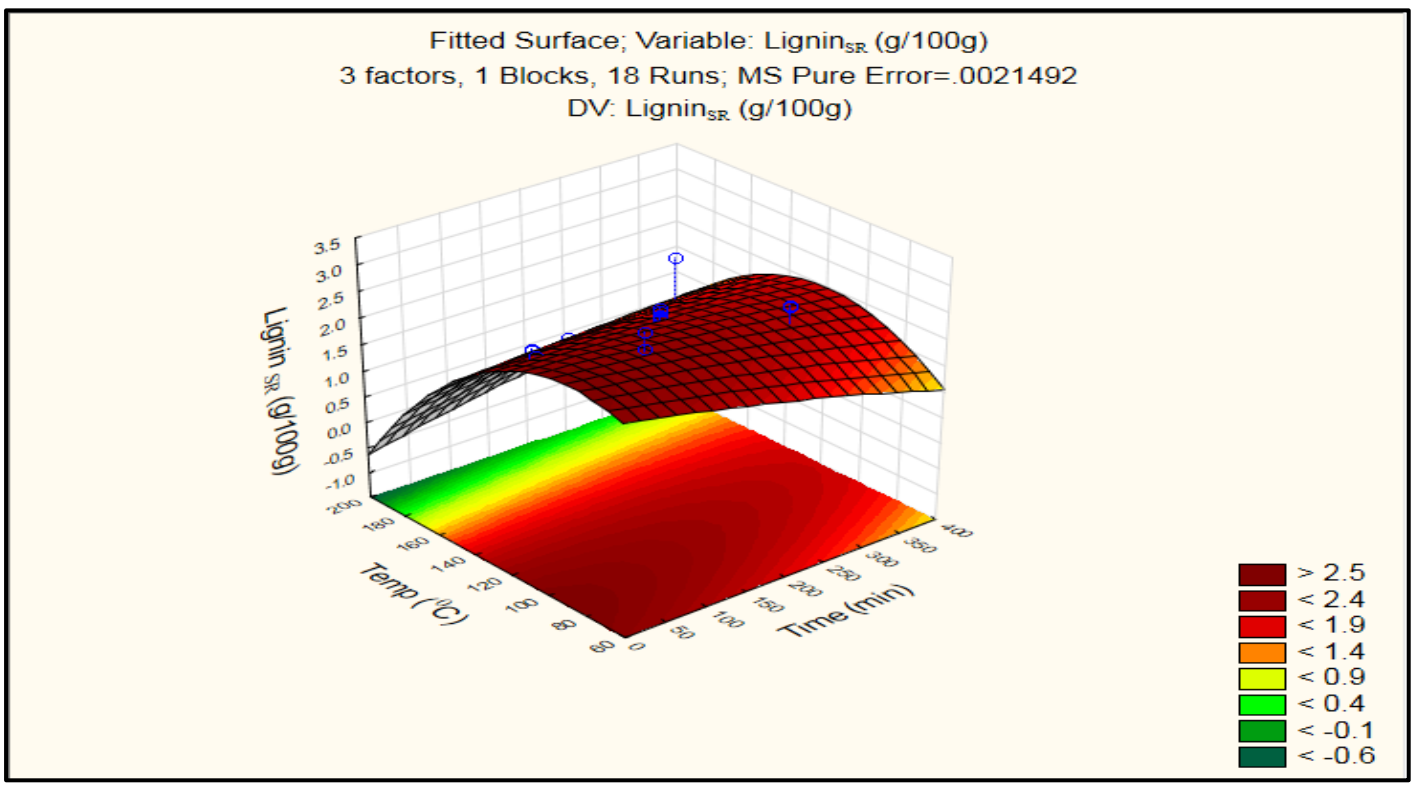


Figure D.0.14: Xylan retention (in pre-extracted bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

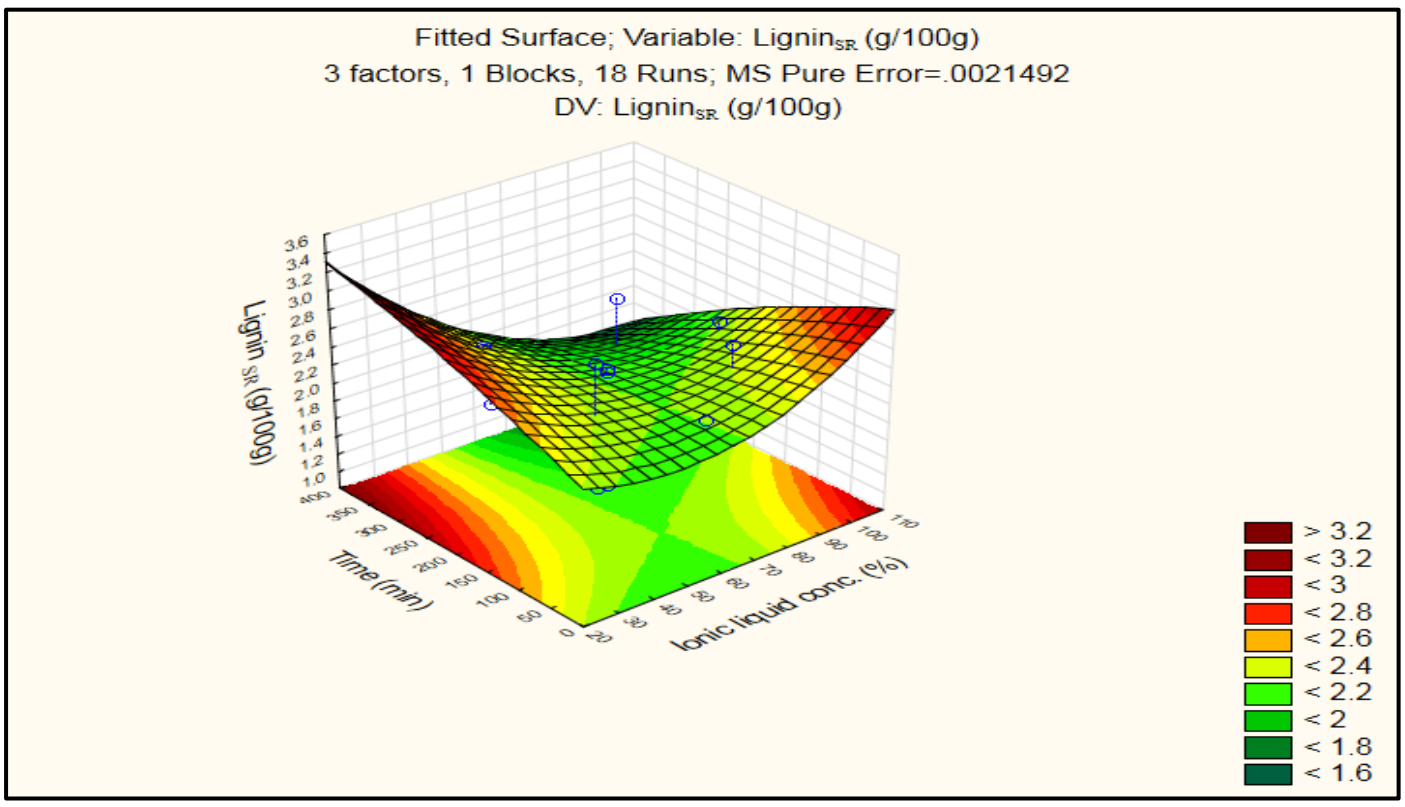
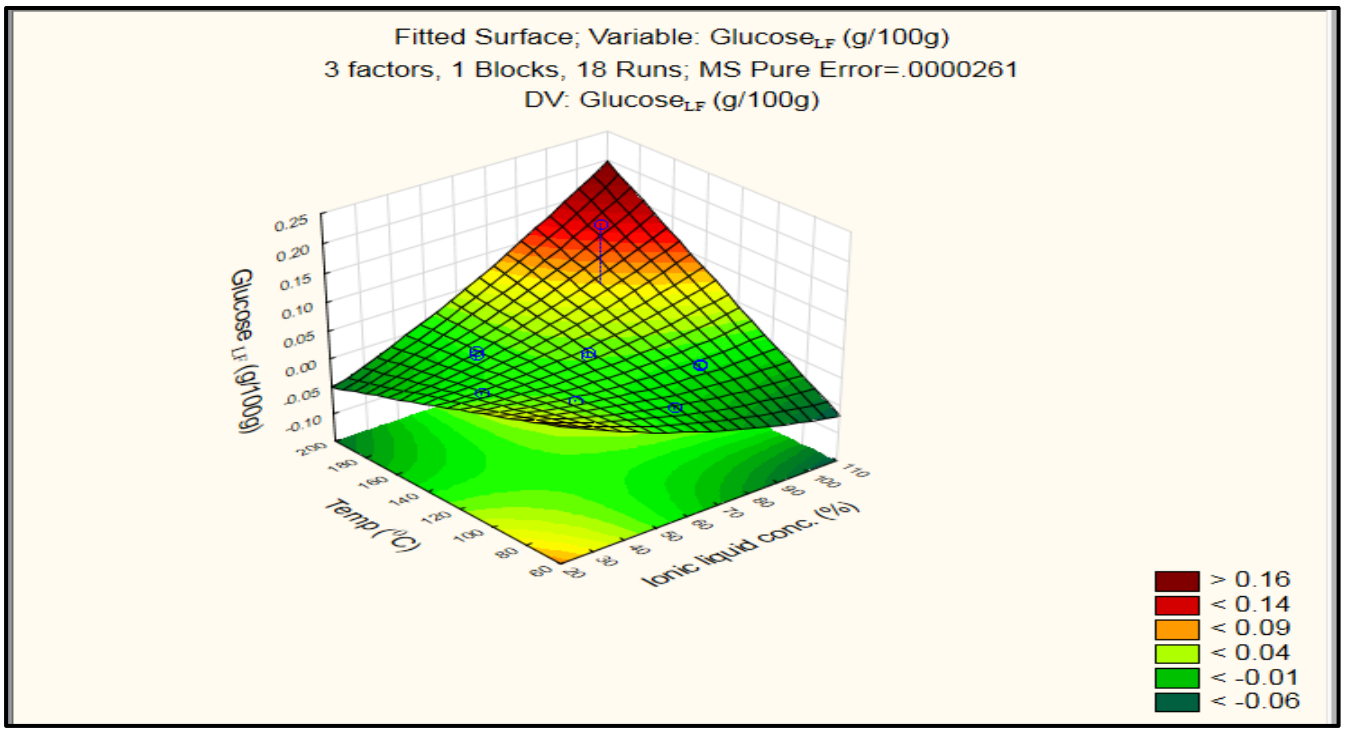
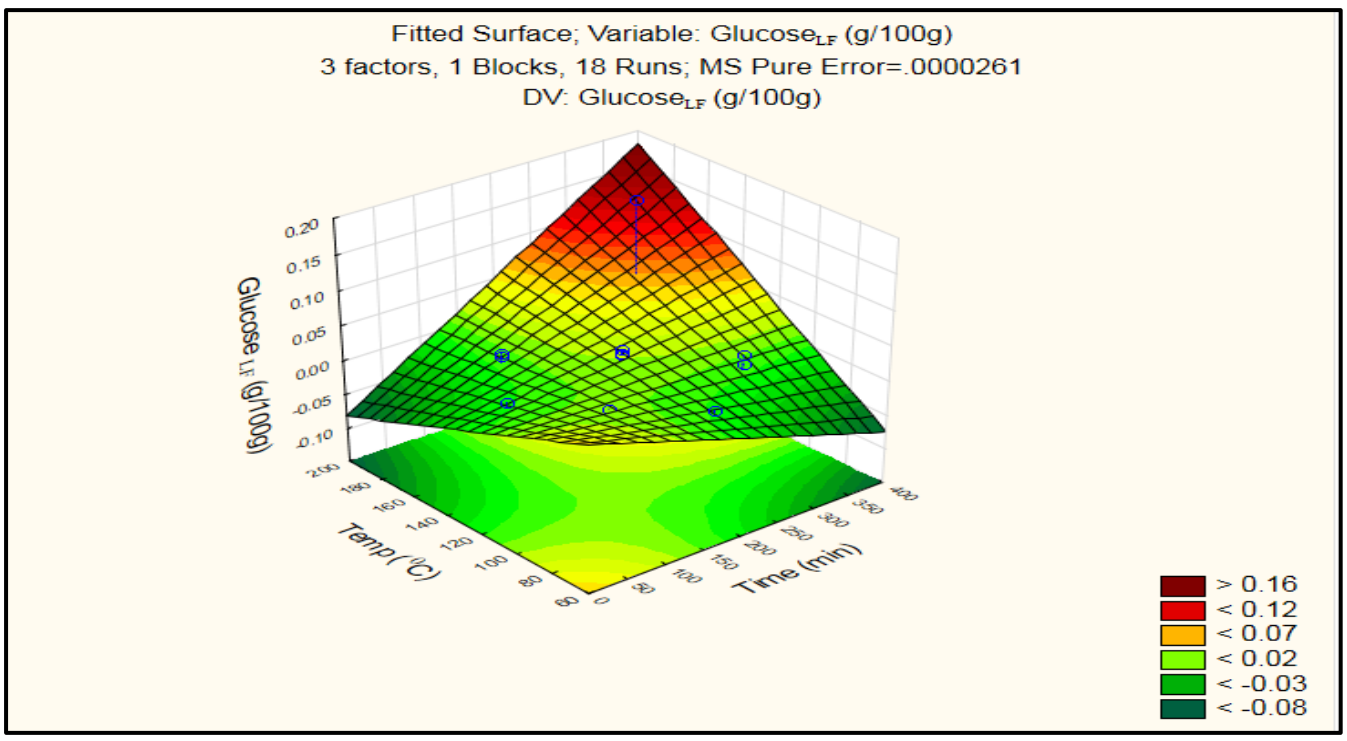


Figure D.0.15: Lignin retention (in pre-extracted bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

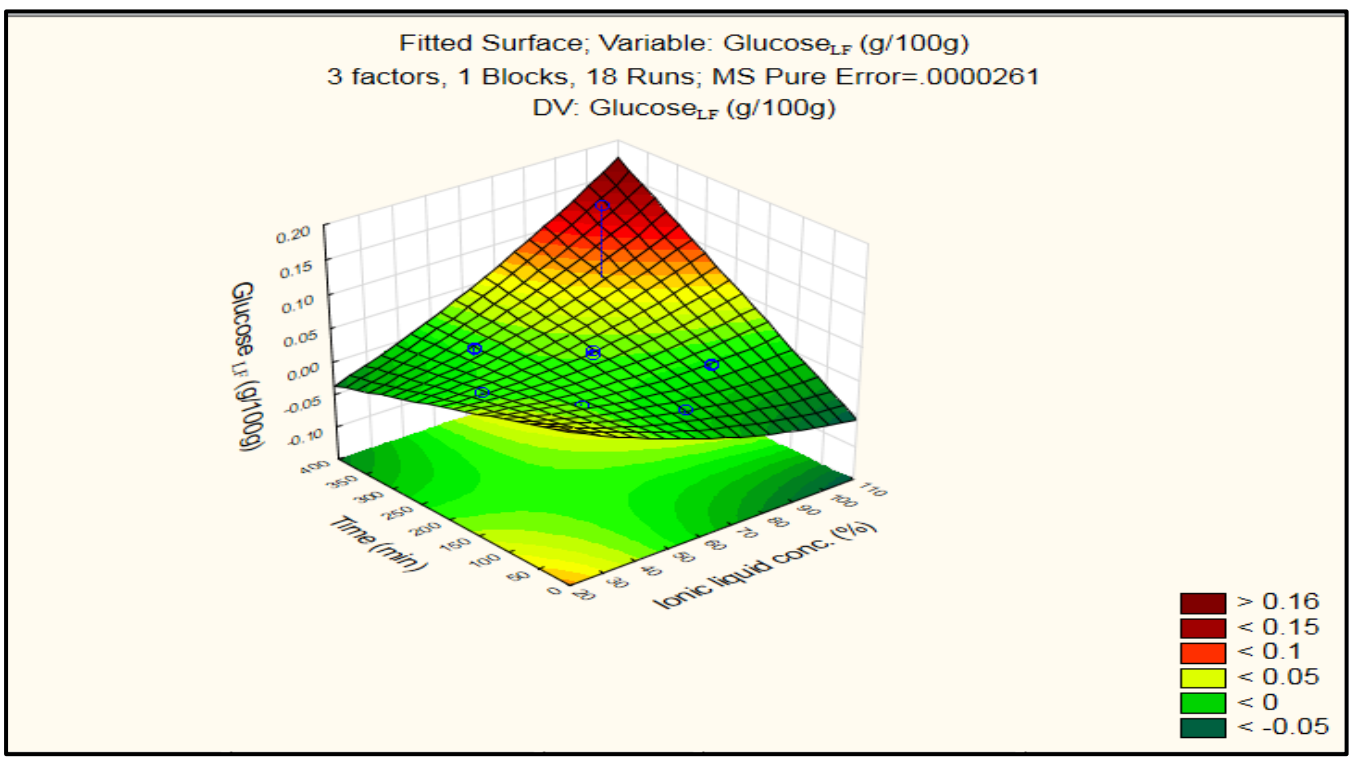
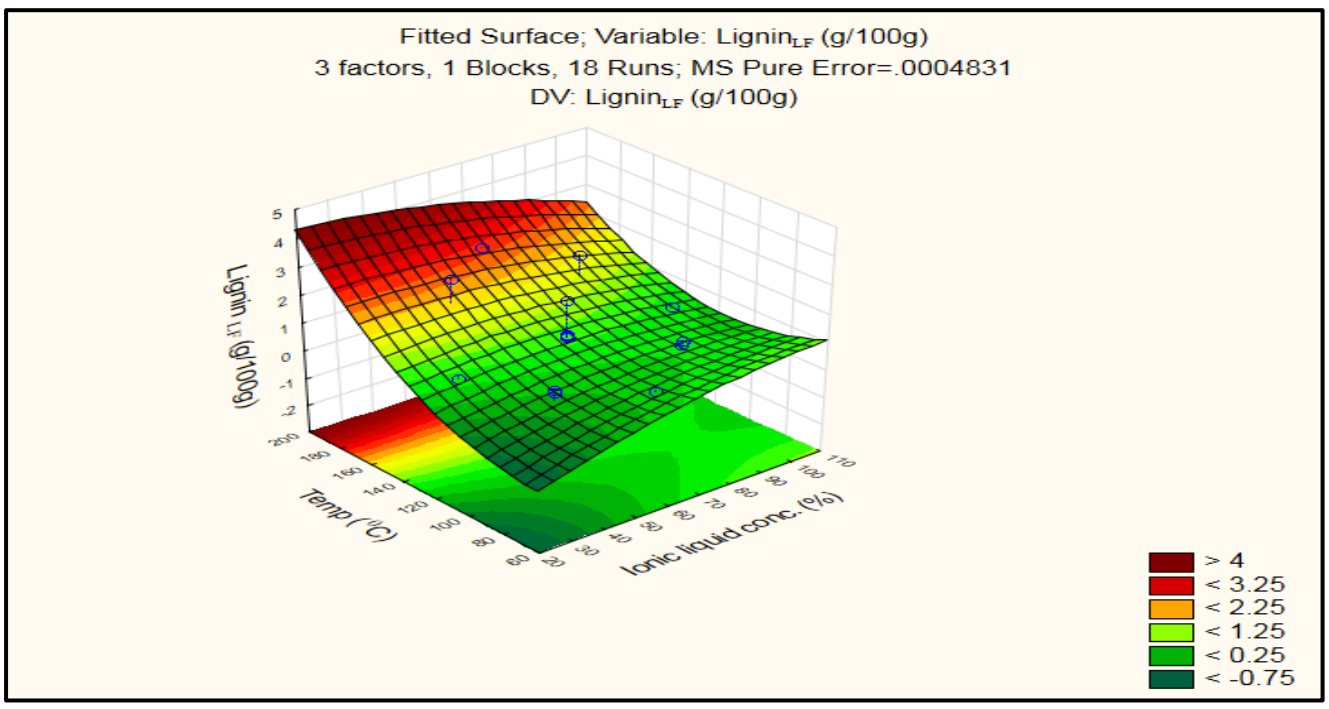
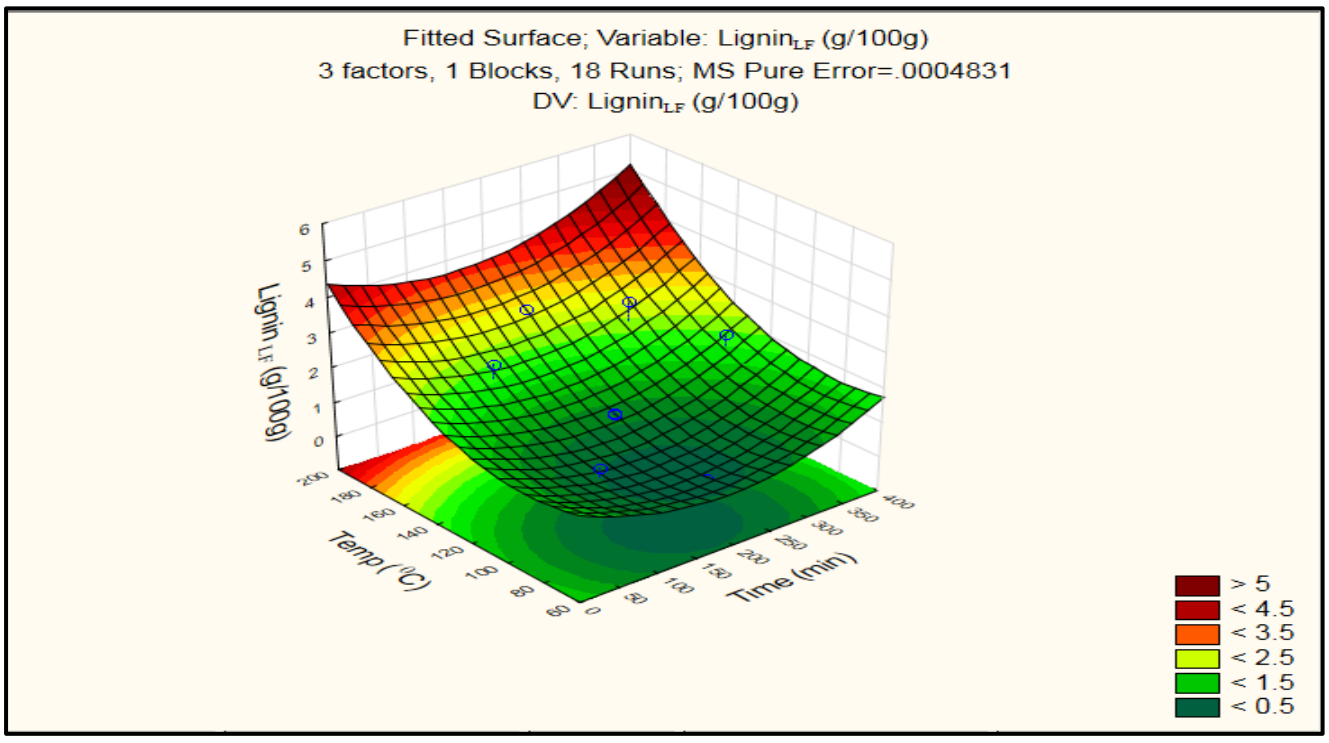


Figure D.0.16: Glucan dissolution (in pre-extracted bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

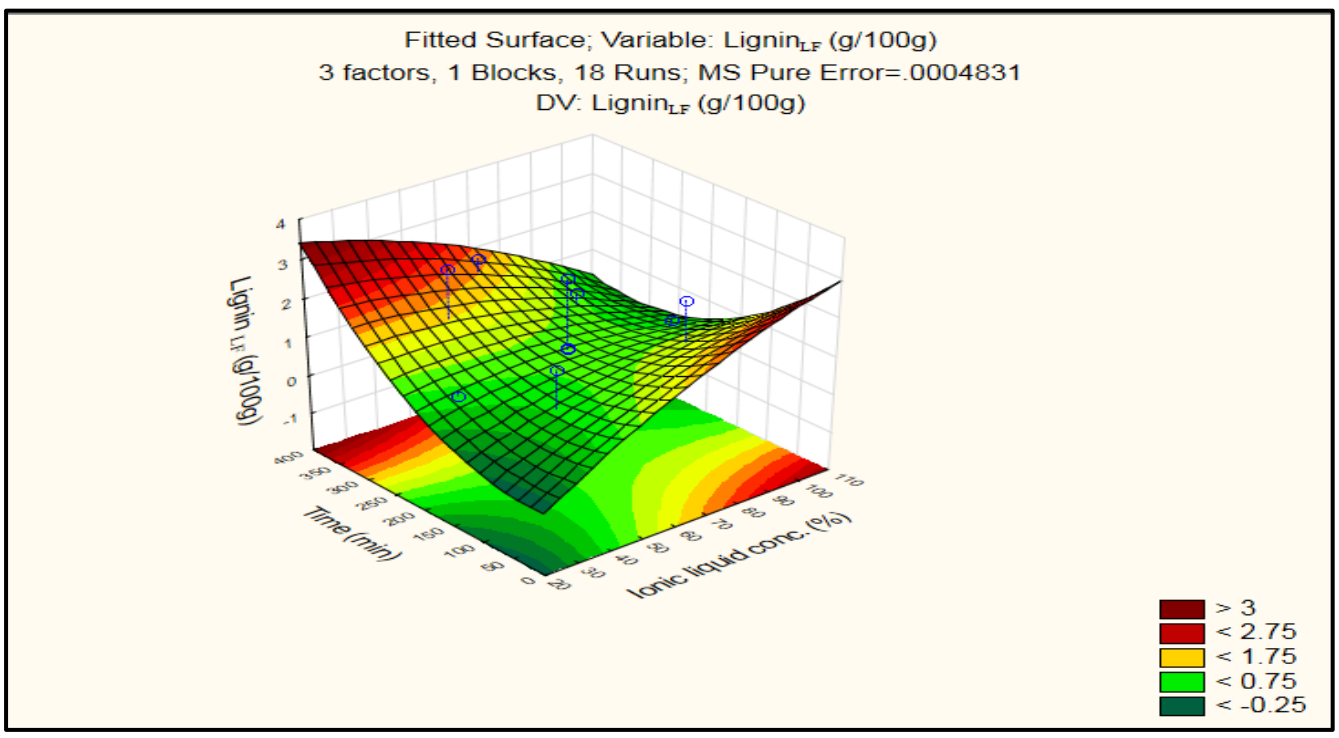
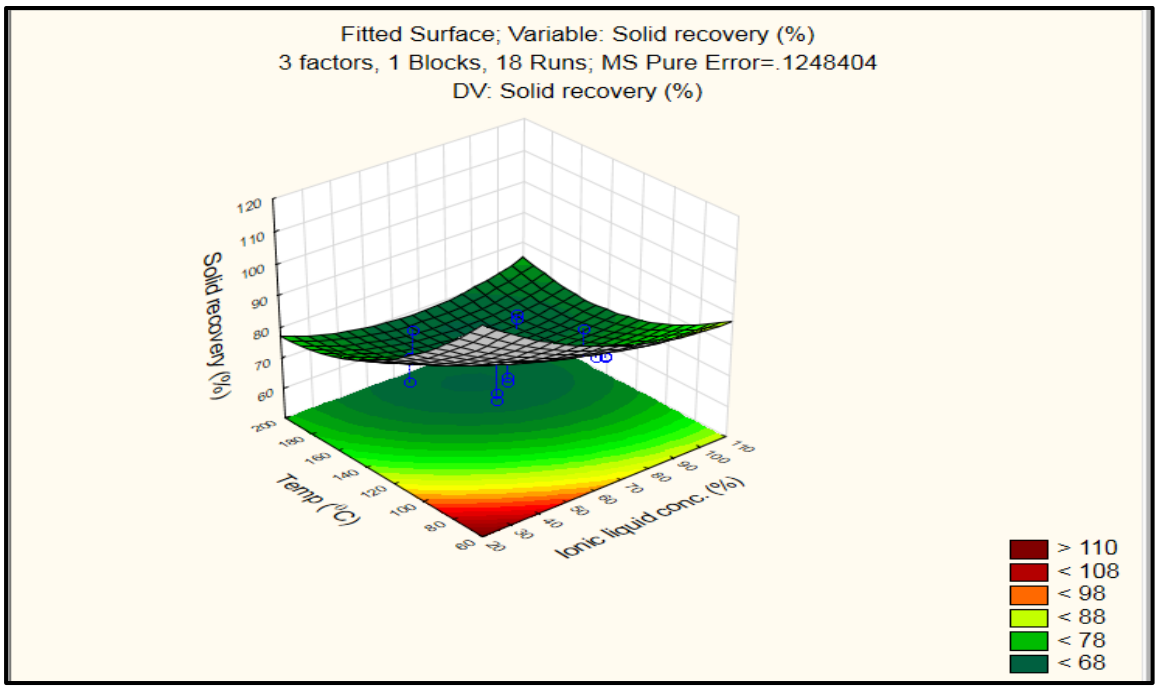
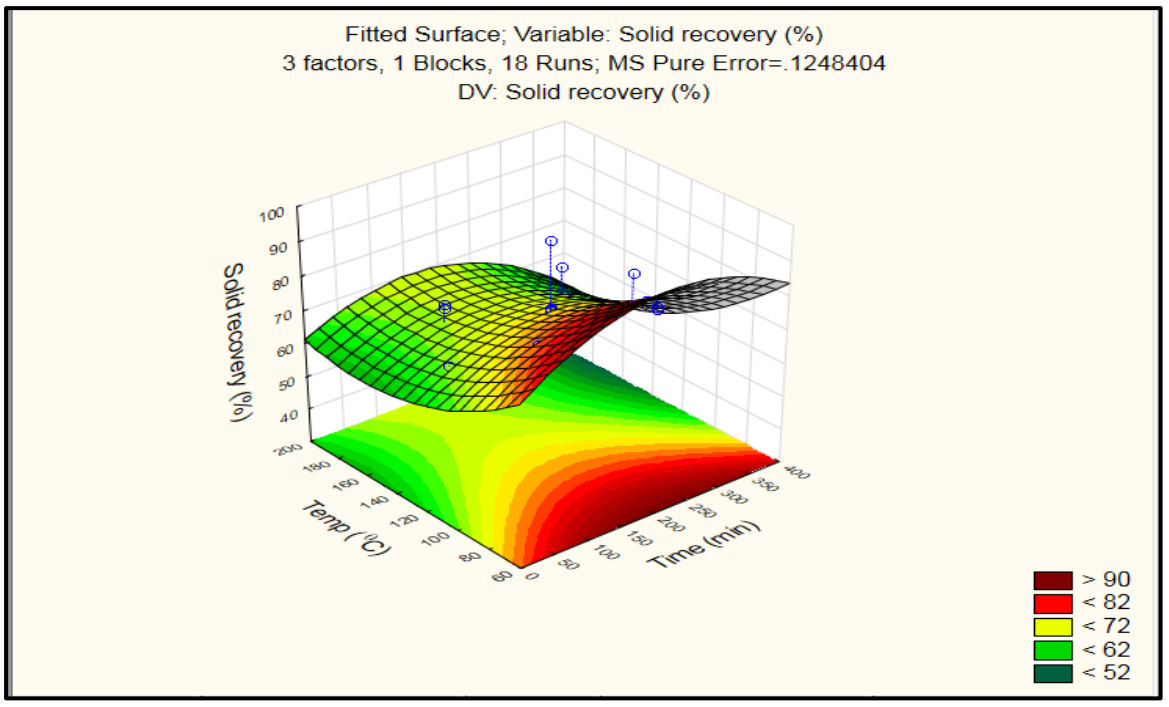


Figure D.0.17: Lignin dissolution (in pre-extracted bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

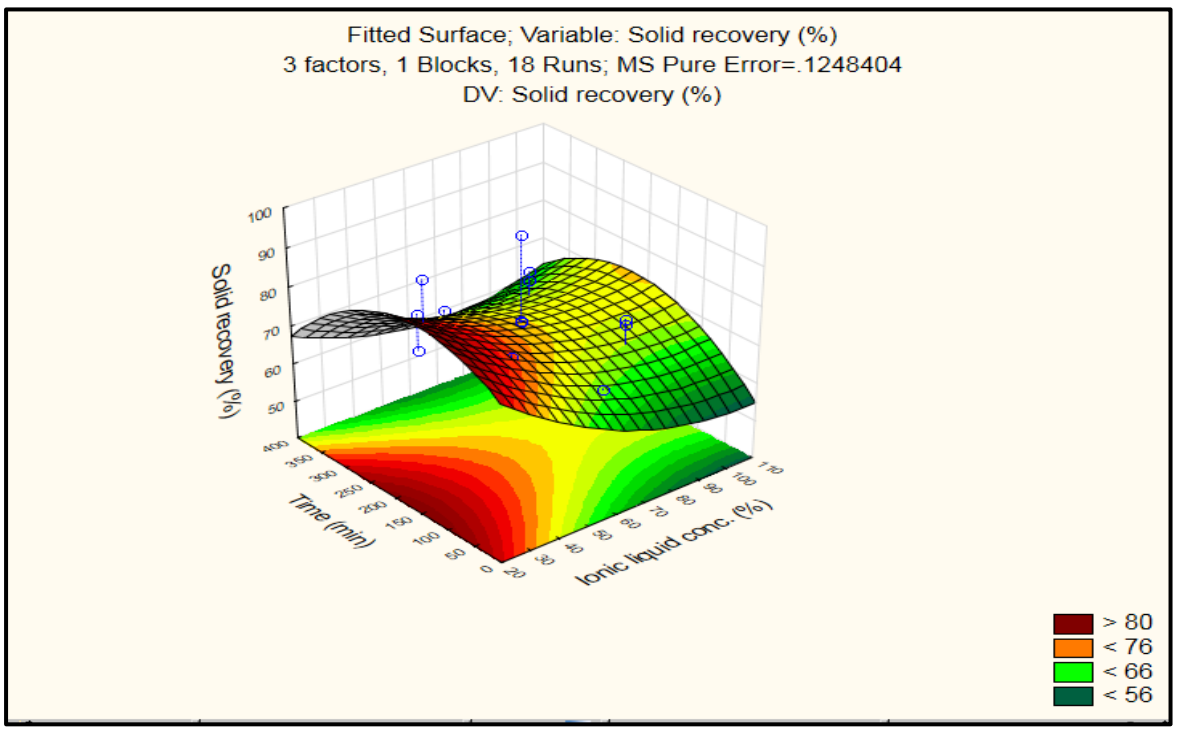
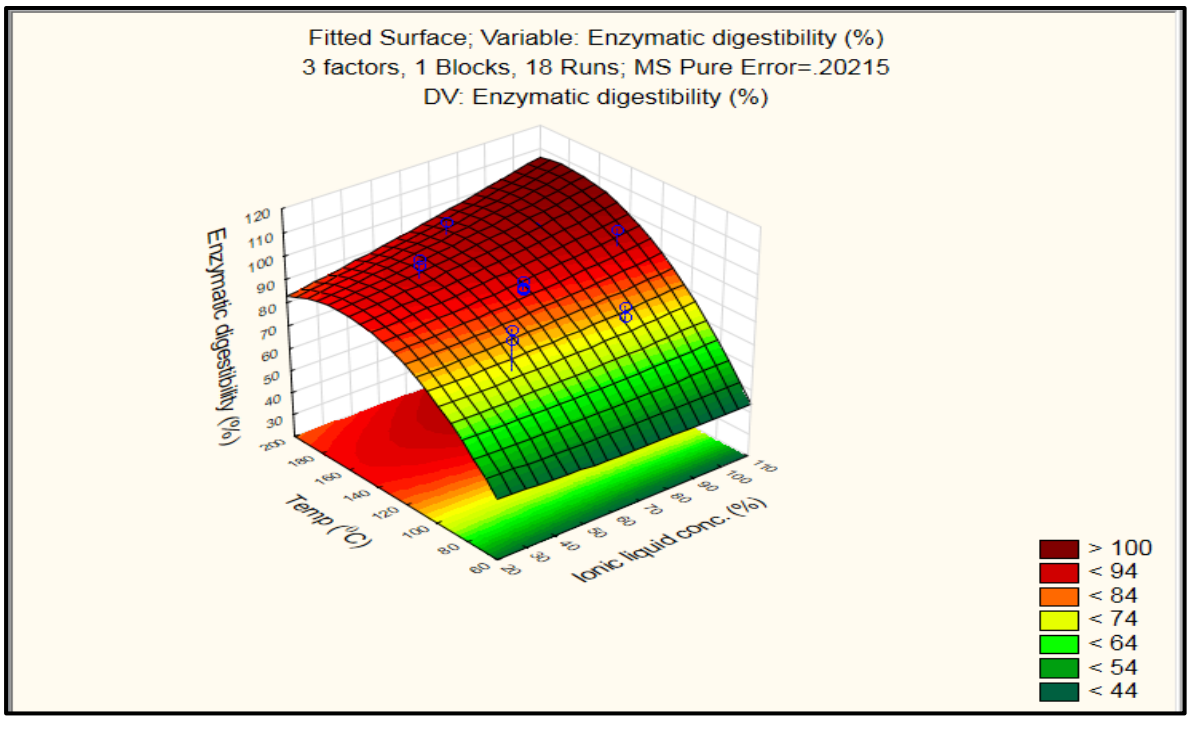
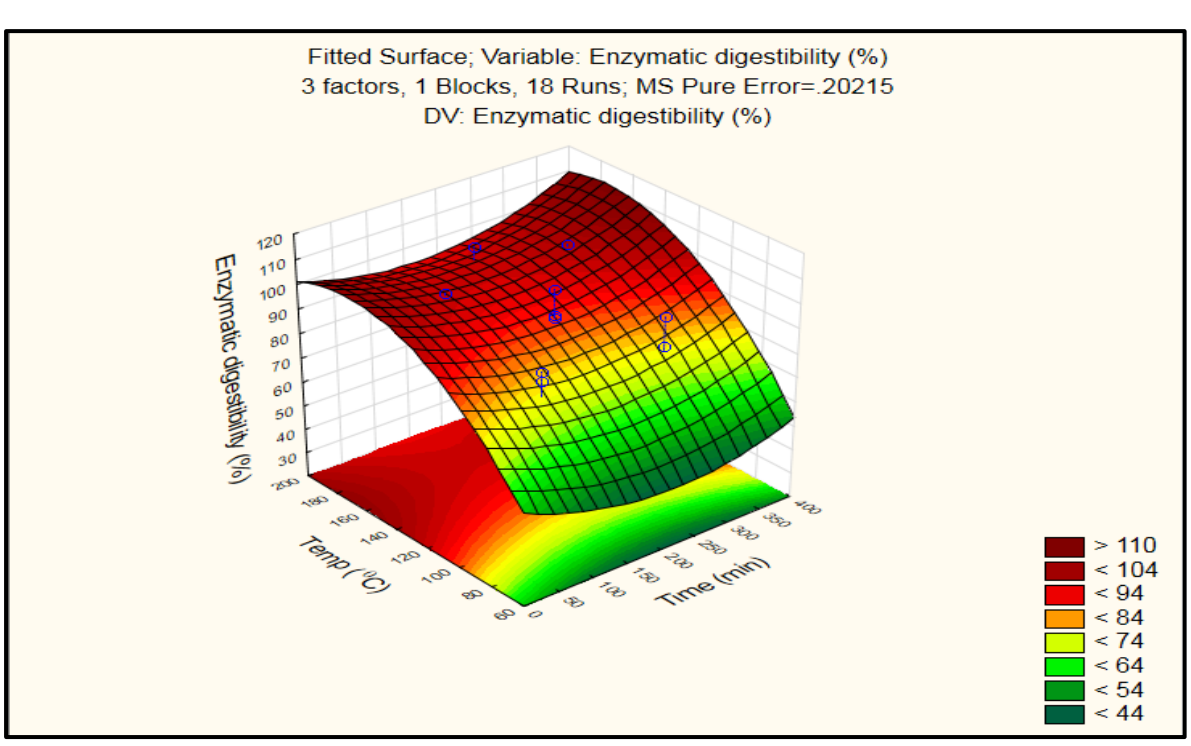


Figure D.0.18: Solid recovery (in pre-extracted bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

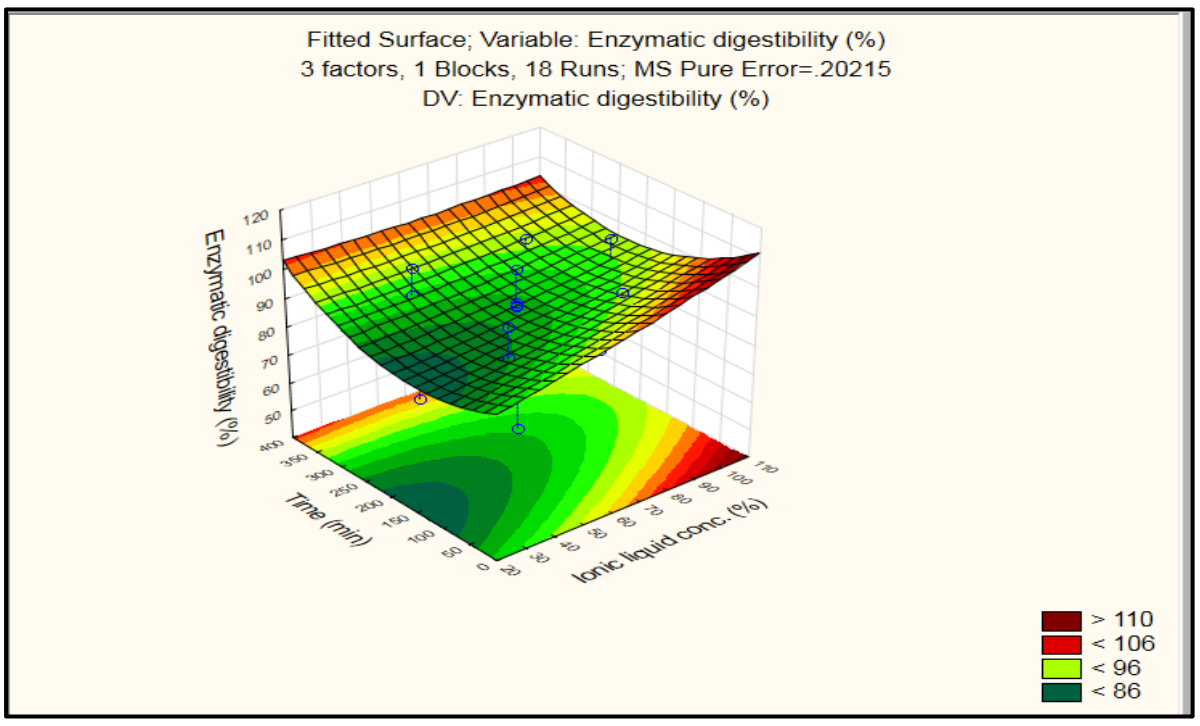
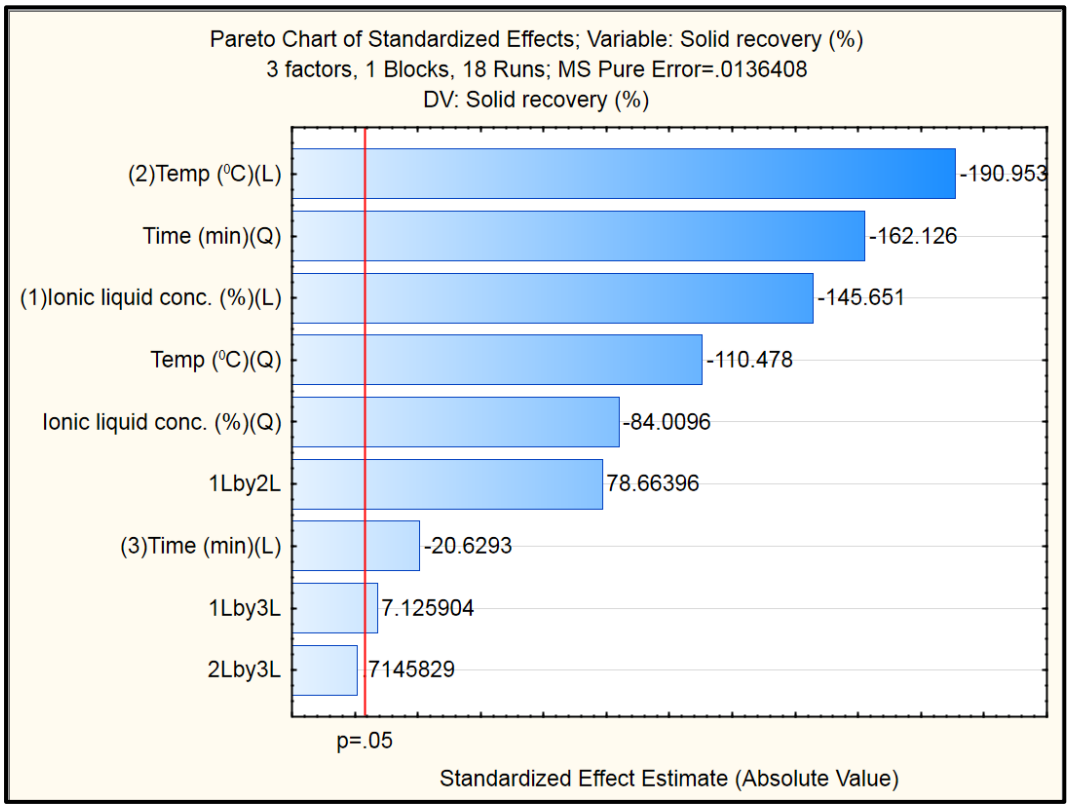


Figure D.0.19: Glucan digestibility (in pre-extracted bagasse treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)

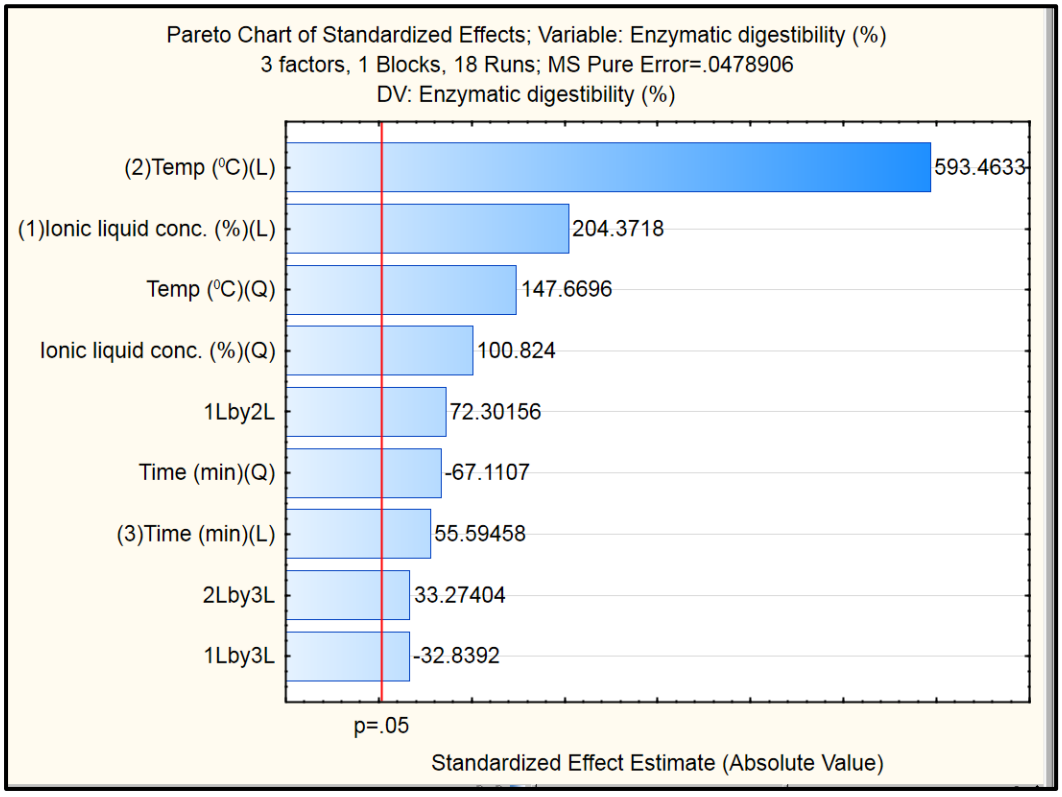
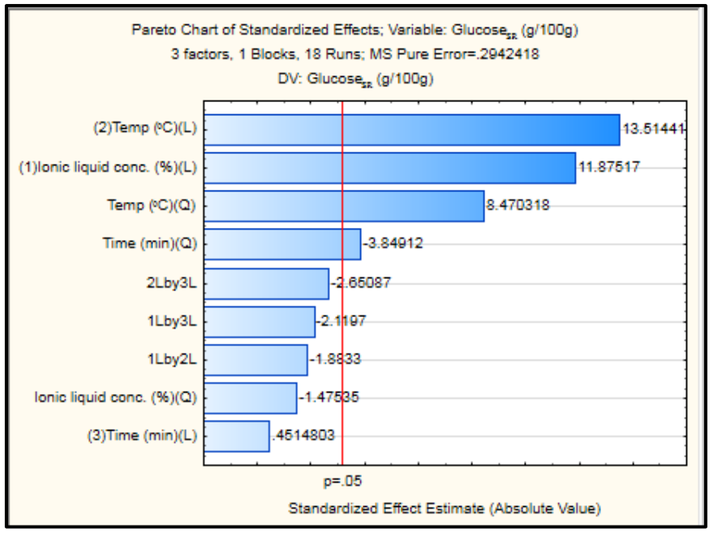
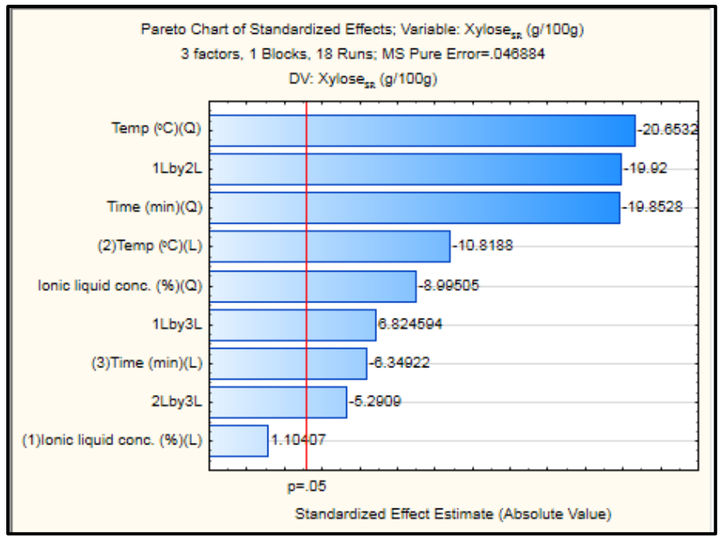
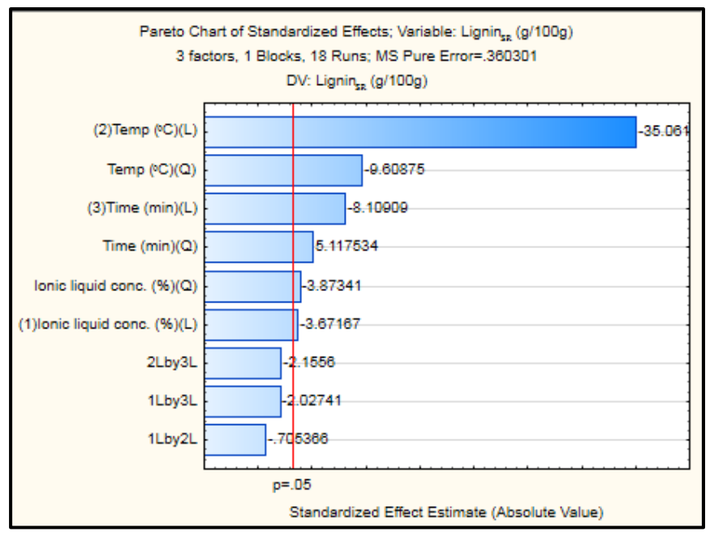


Figure D.0.20: The impact of ionic liquid treatment of virgin E. grandis, (a) solid recovery, and (b) glucan digestibility

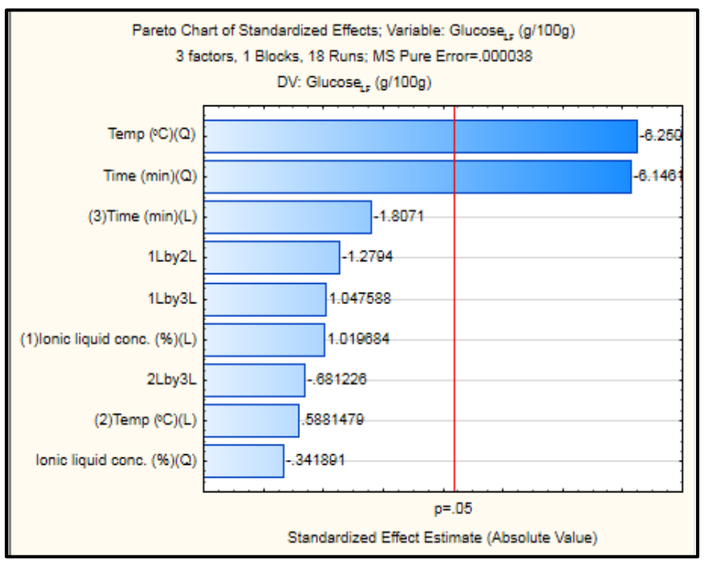
(a) (b) (c)

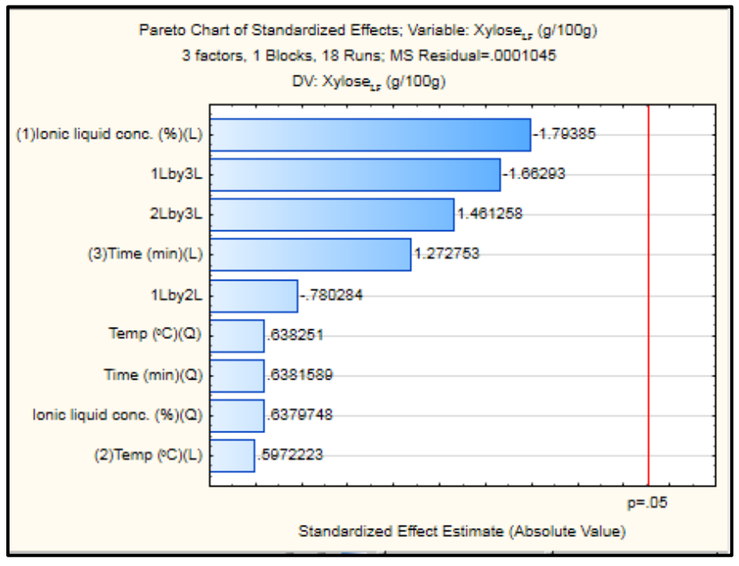






(d) (e) (f)





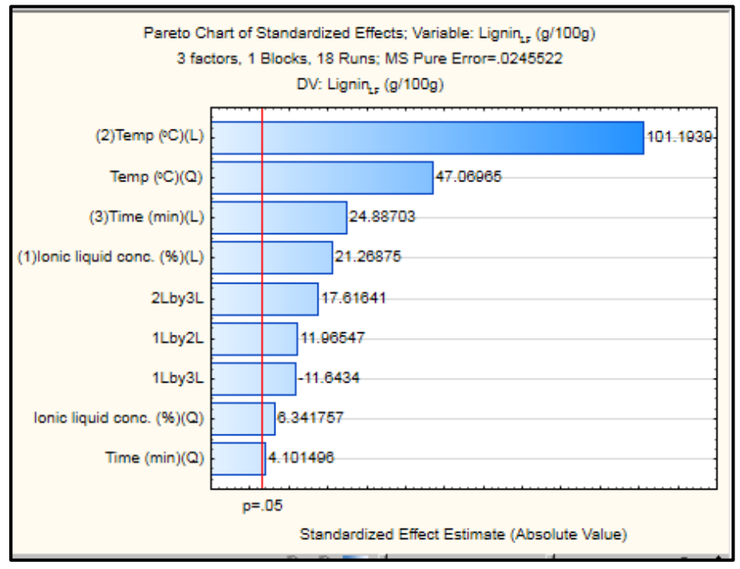
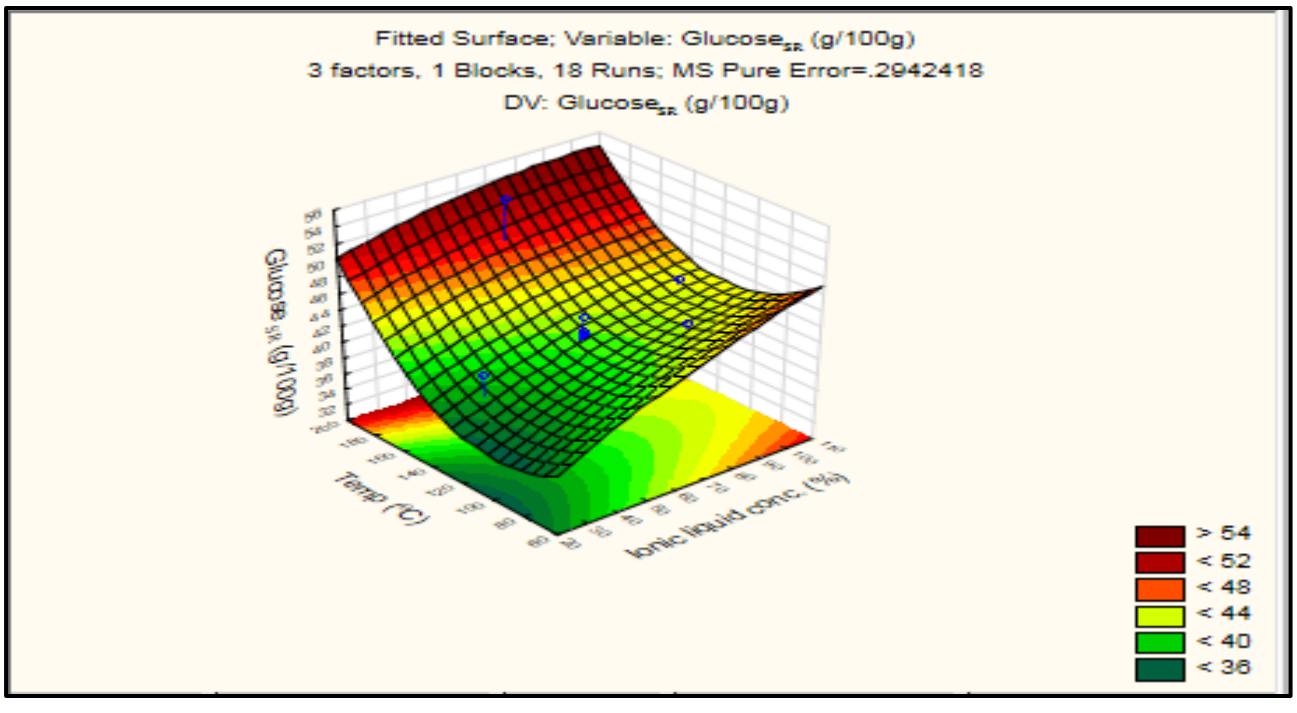
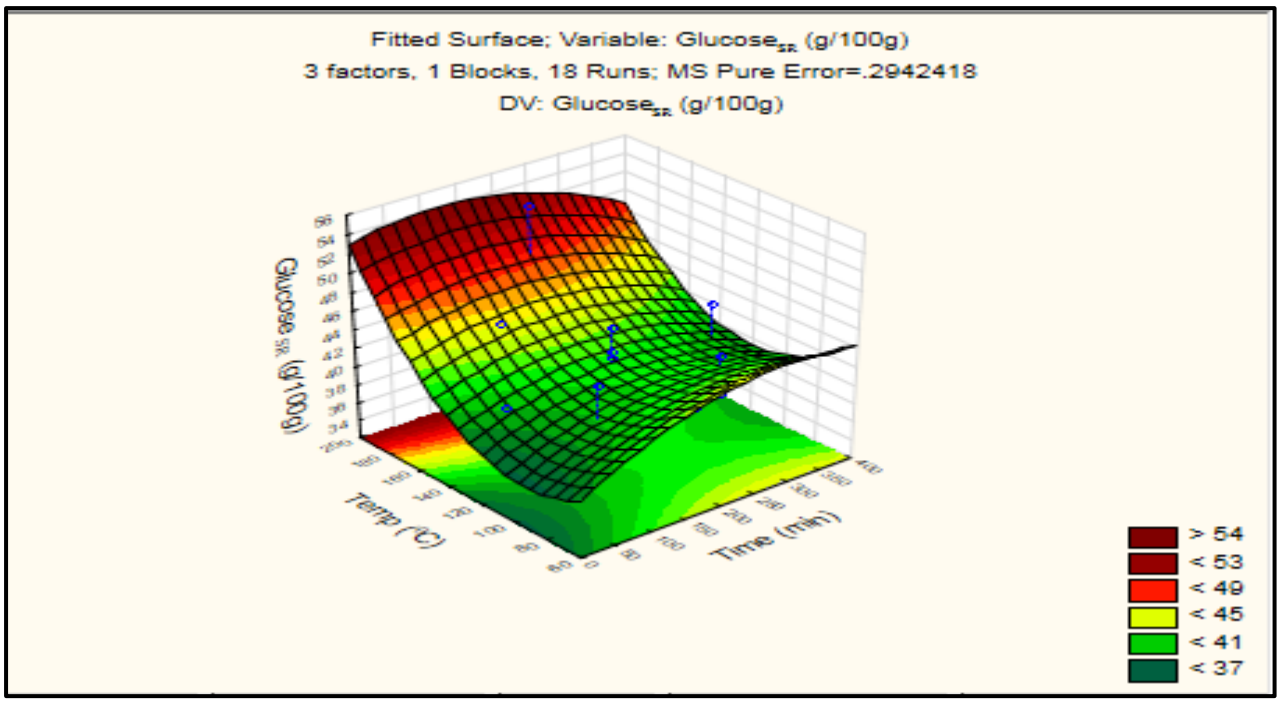


Figure D.0.21: The impact of ionic liquid treatment of virgin E. grandis, (a) glucan retained, (b) xylan retained, (c) lignin retained, (d) glucan dissolved, (e) xylan dissolved, and (f) lignin dissolved

(a)



(b)



(c)

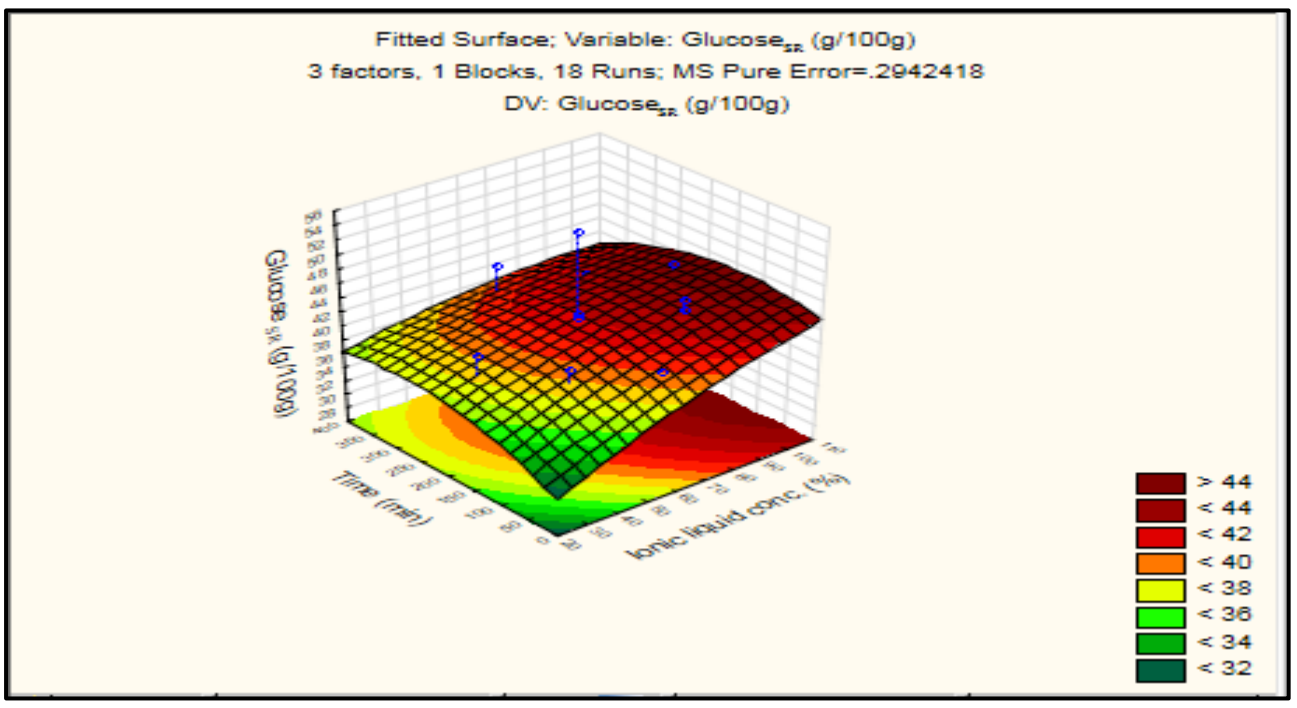
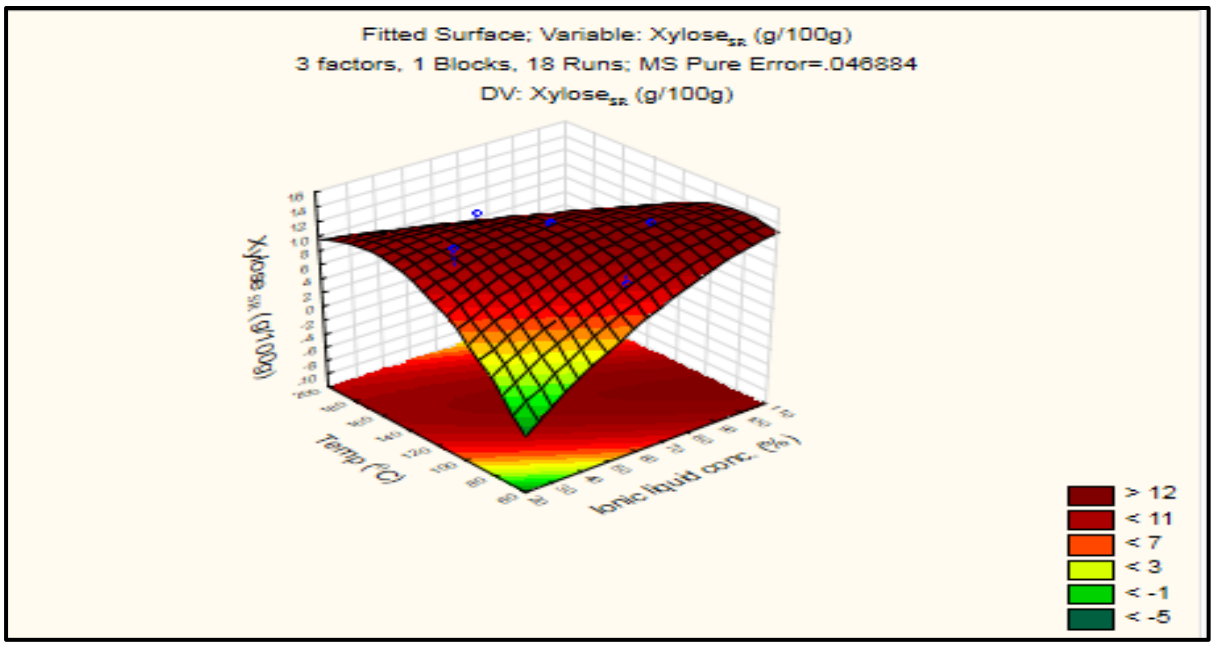
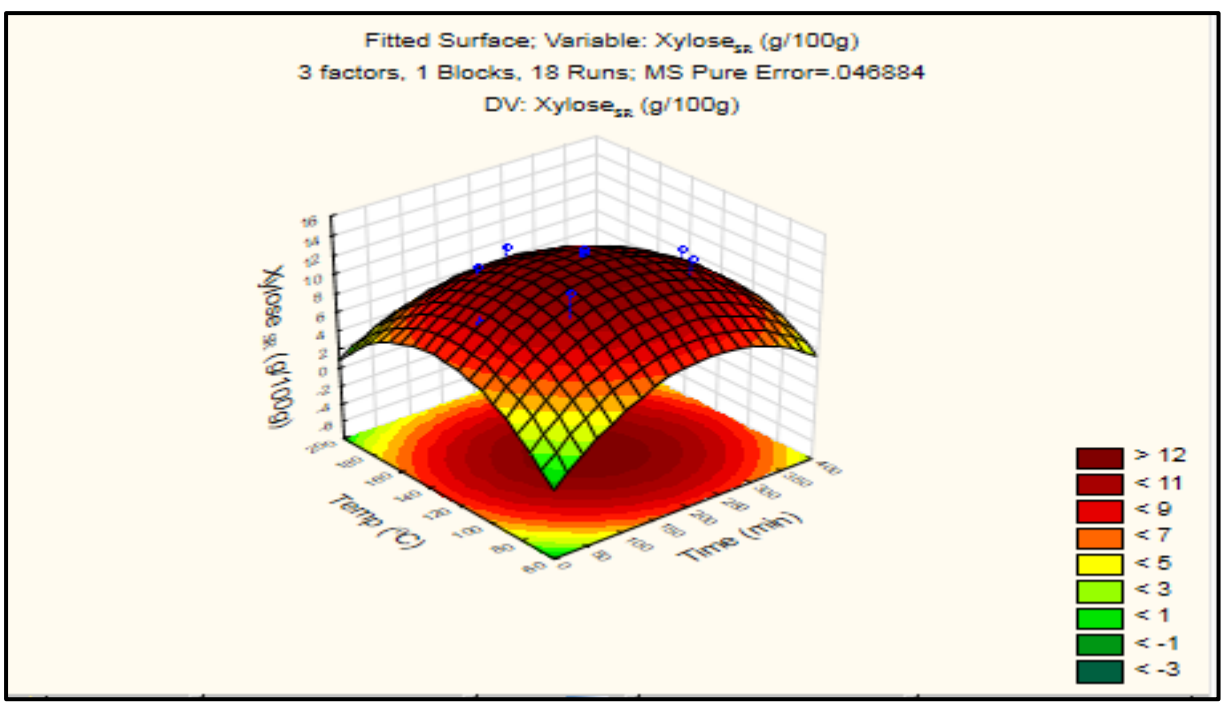


Figure D.0.22: Glucan retention (in virgin E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

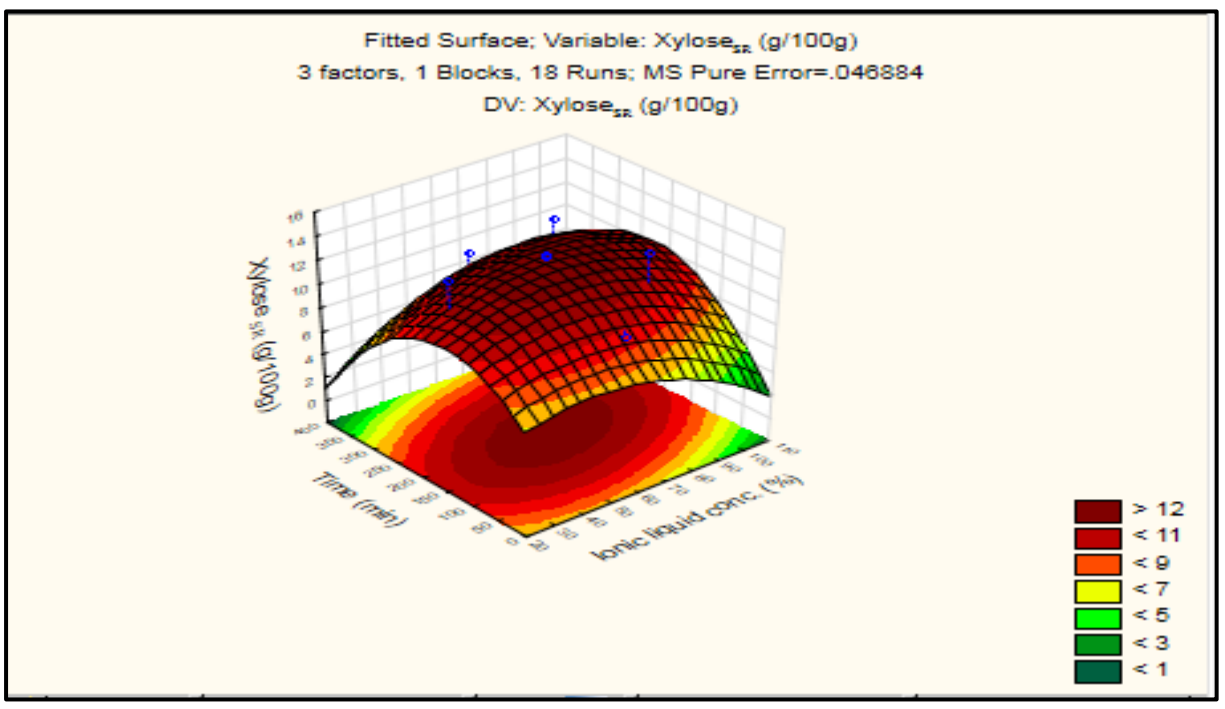
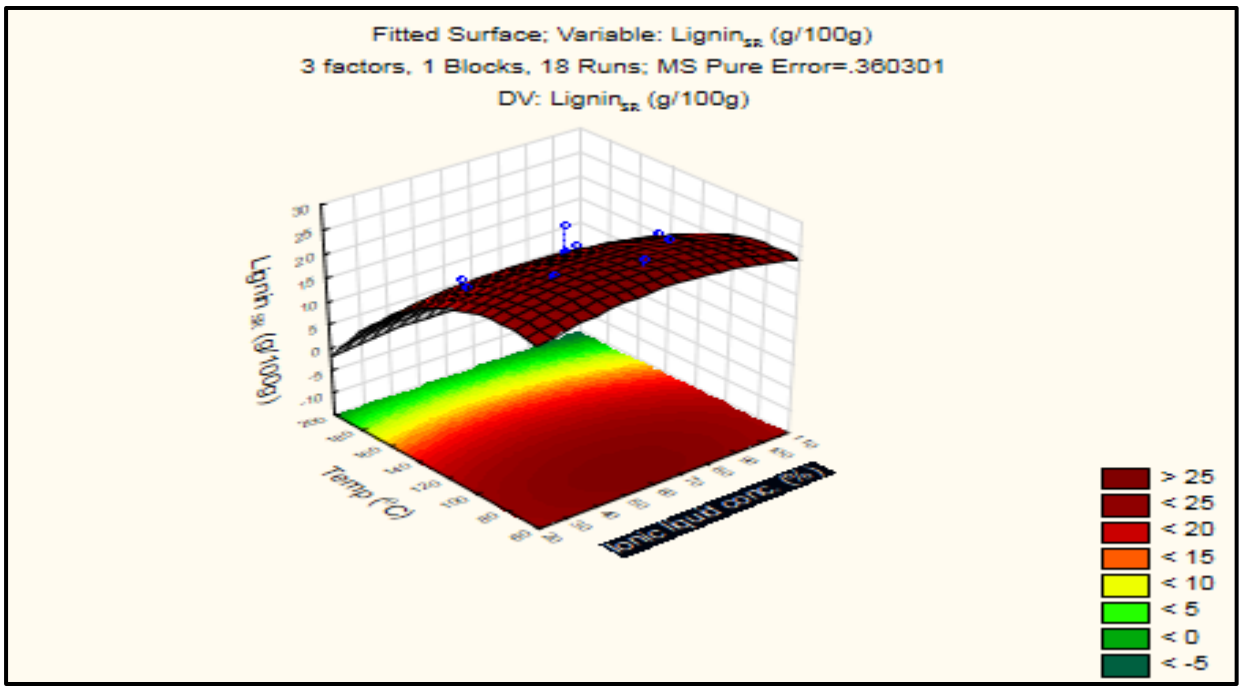
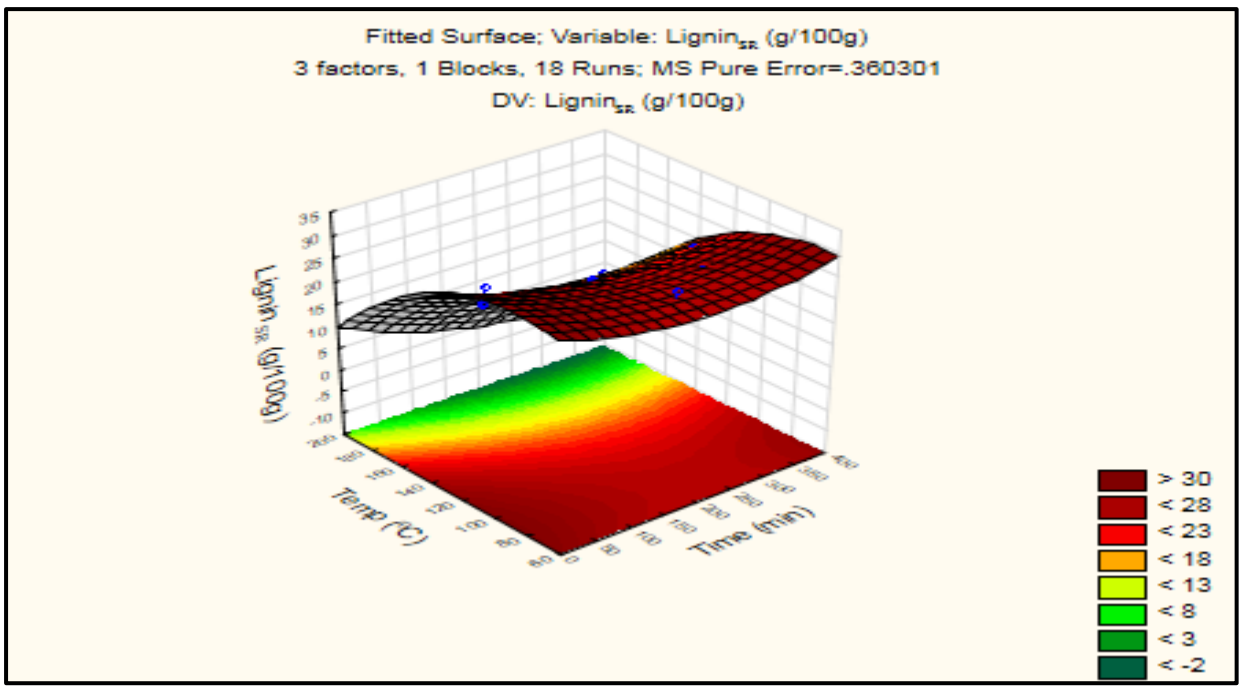


Figure D.0.23: Xylan retention (in virgin E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

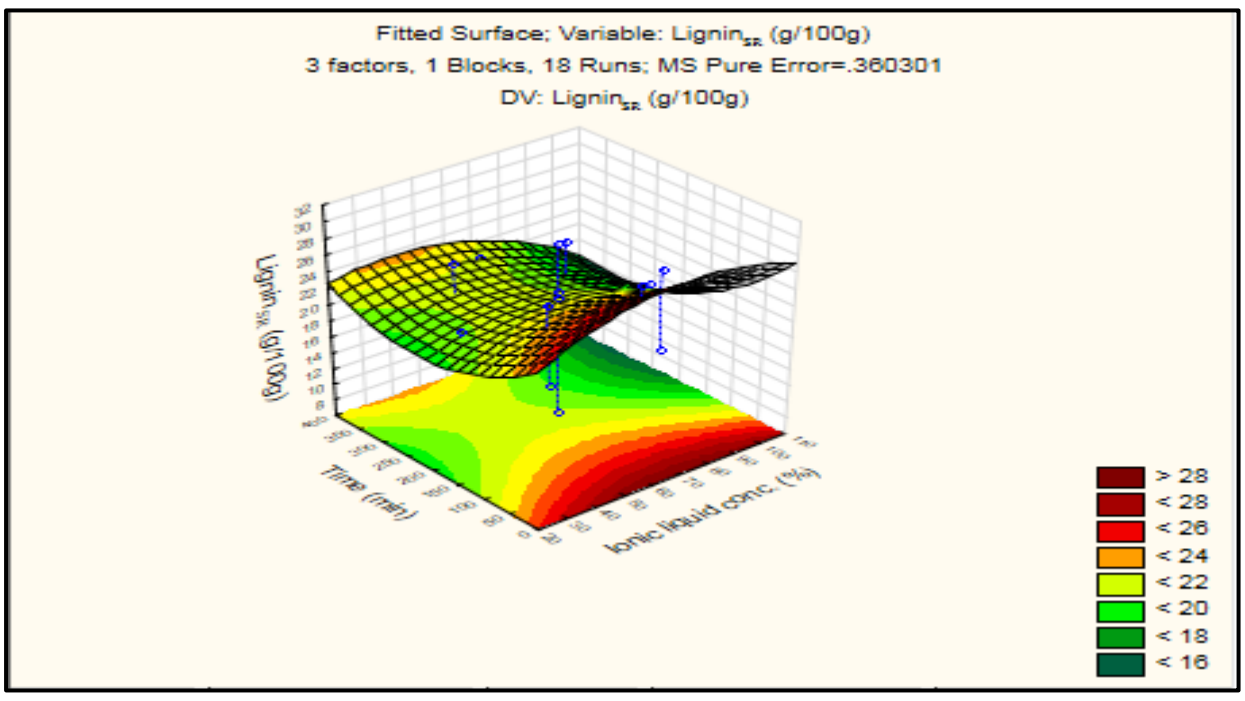
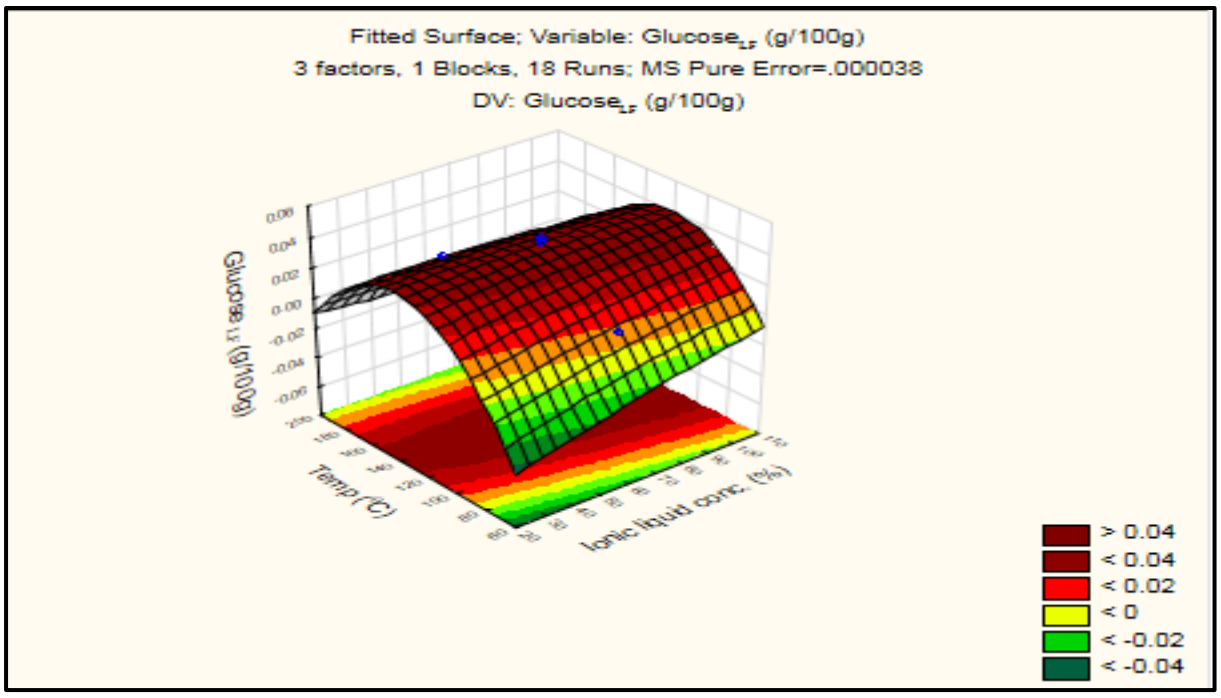
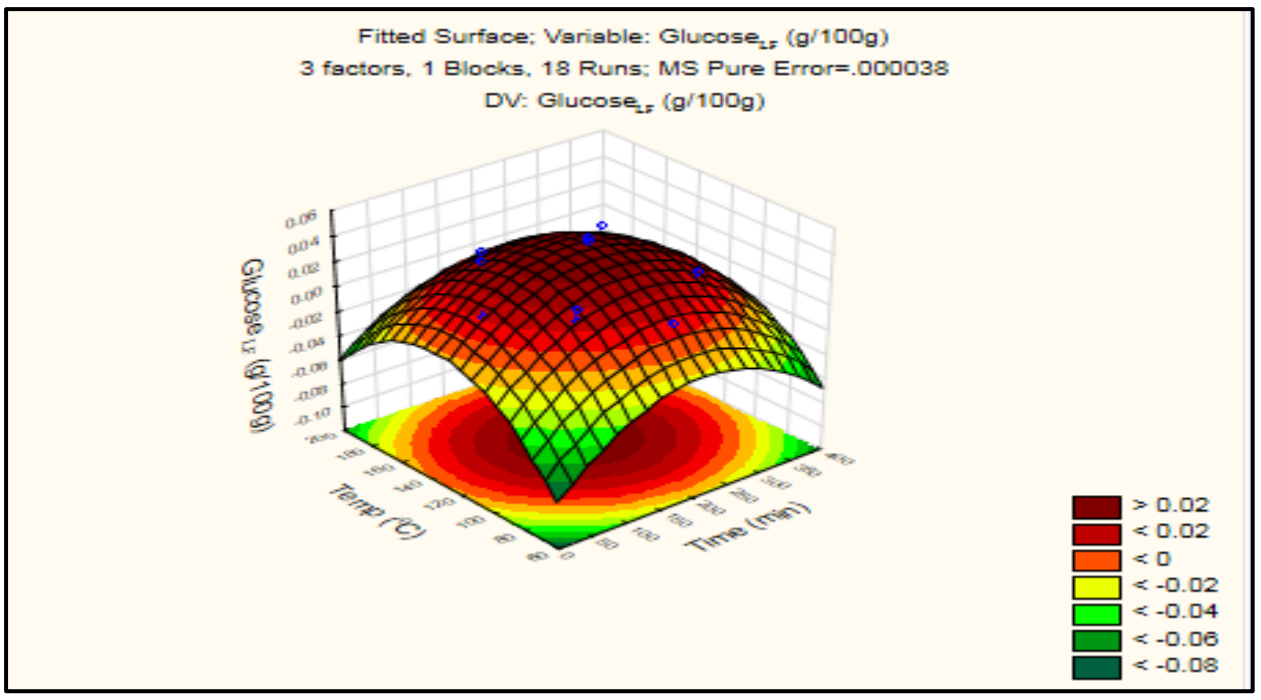


Figure D.0.24: Lignin retention (in virgin E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

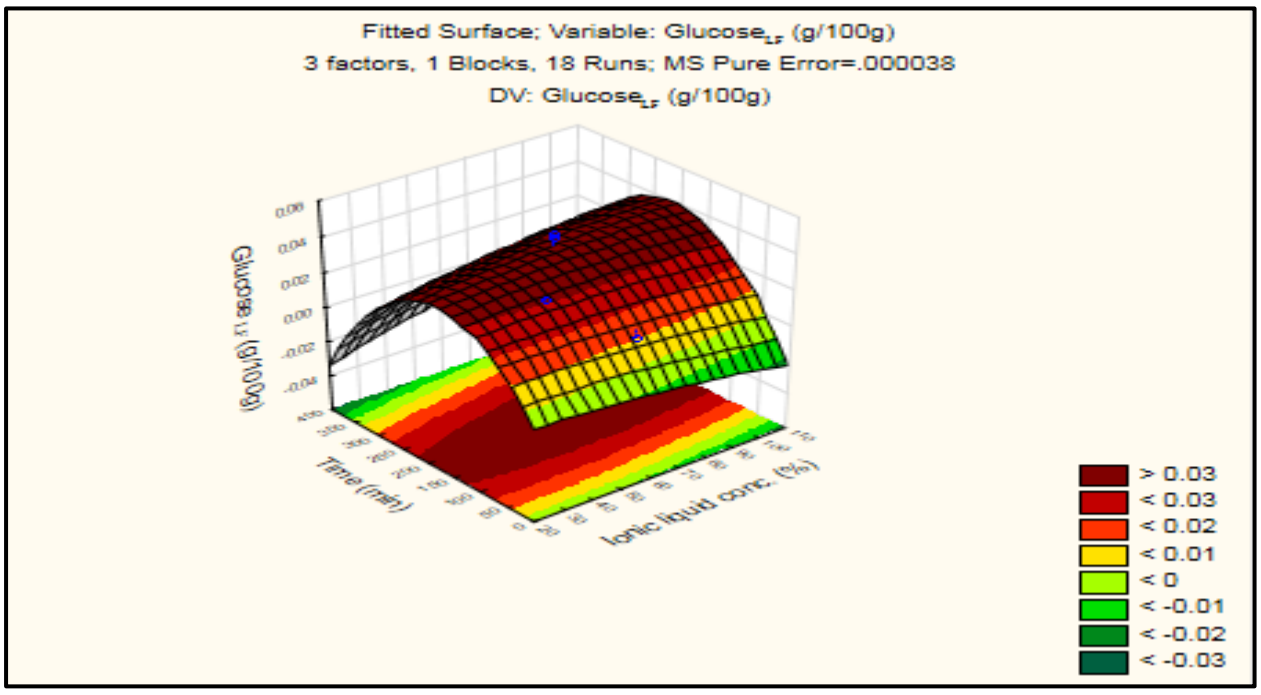
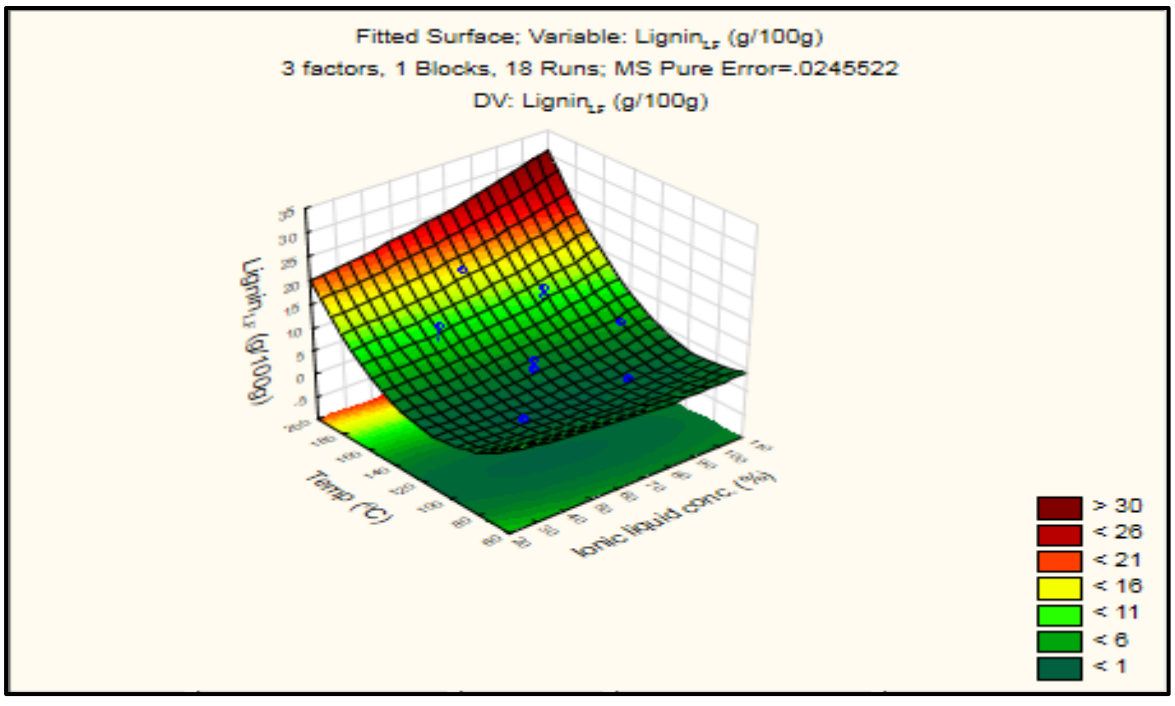
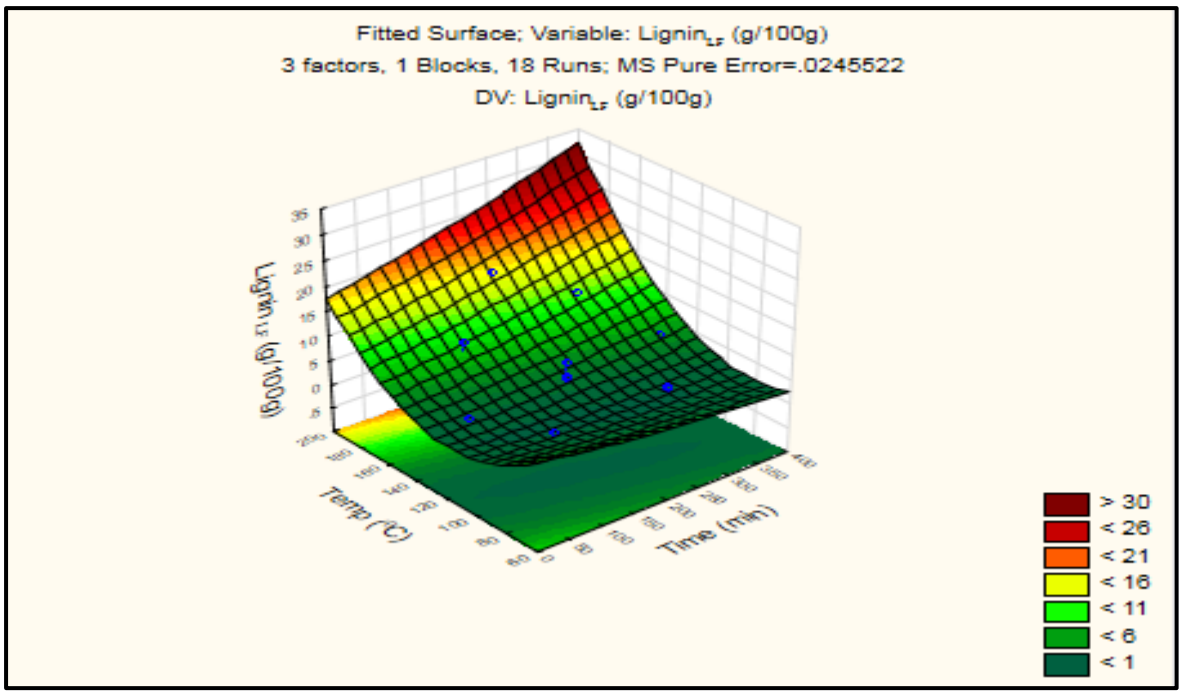


Figure D.0.25: Glucan dissolution (in virgin E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

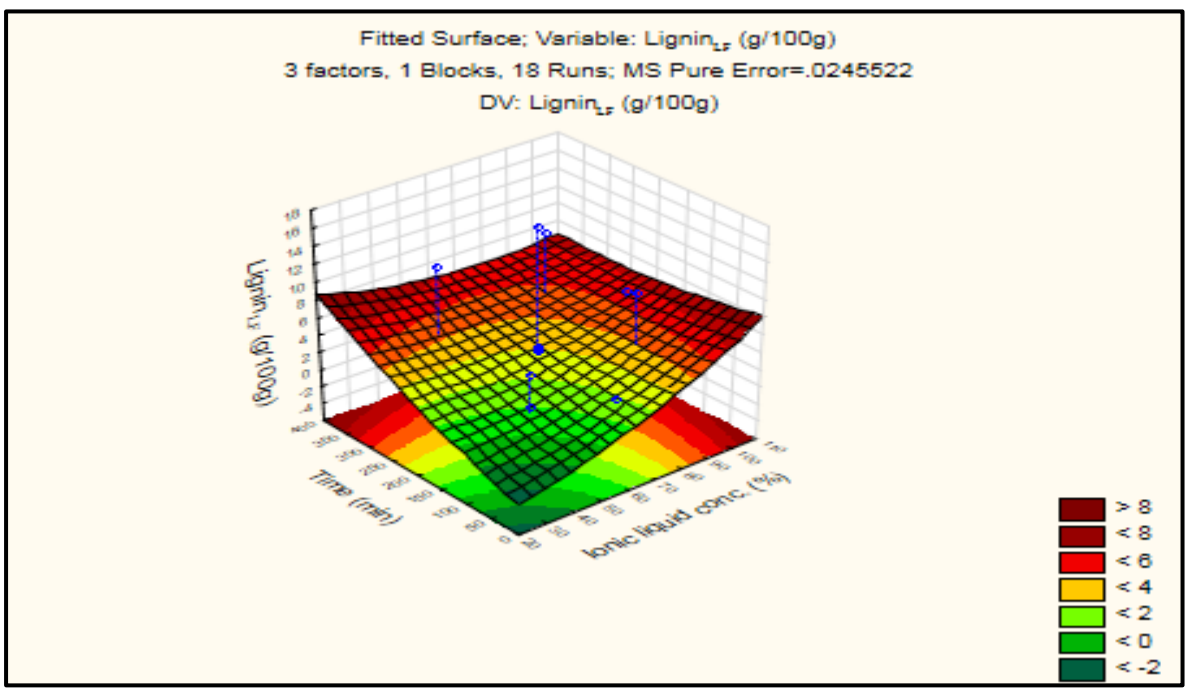
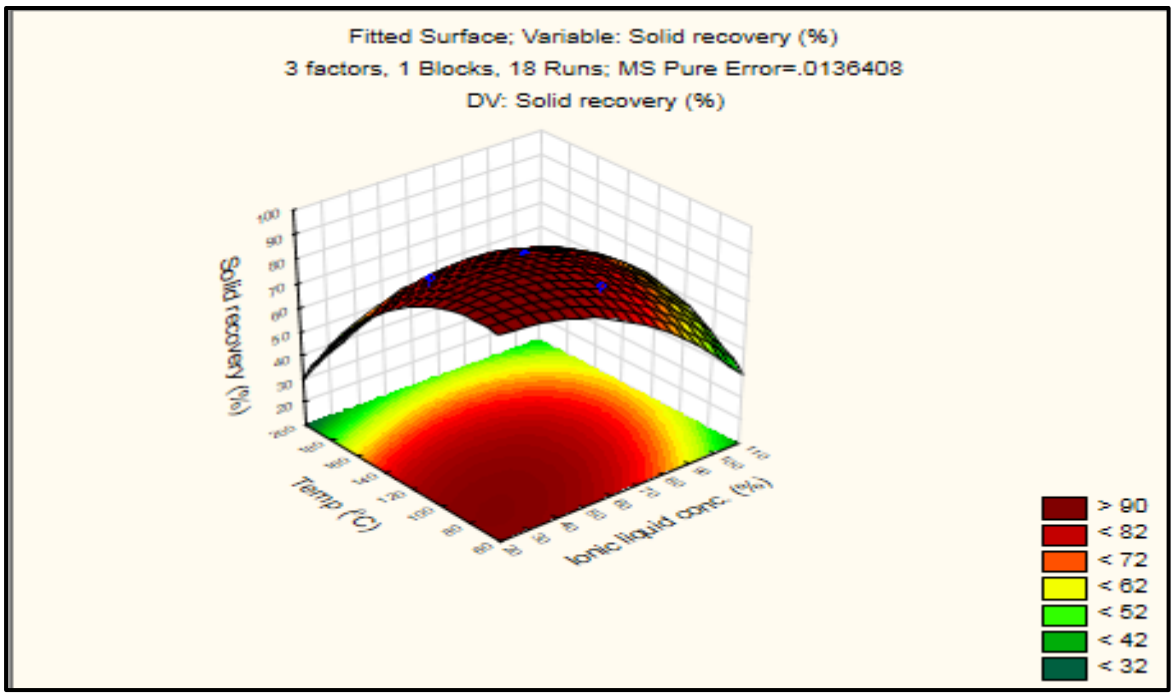
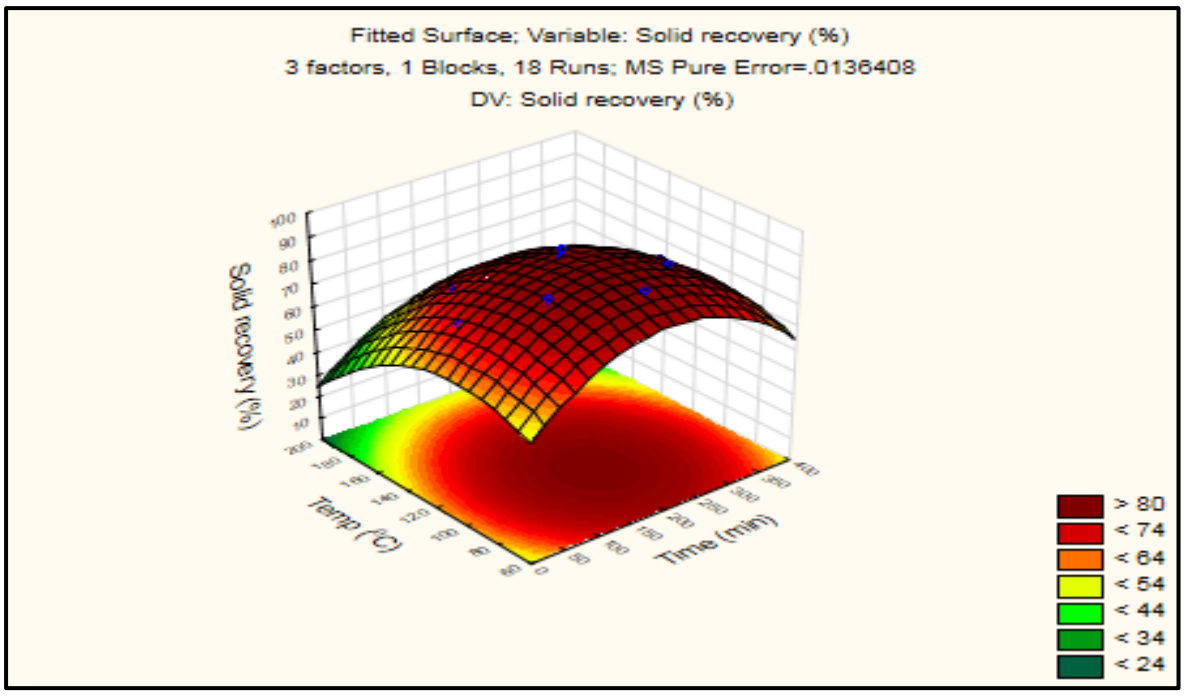


Figure D.0.26: Lignin dissolution (in virgin E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

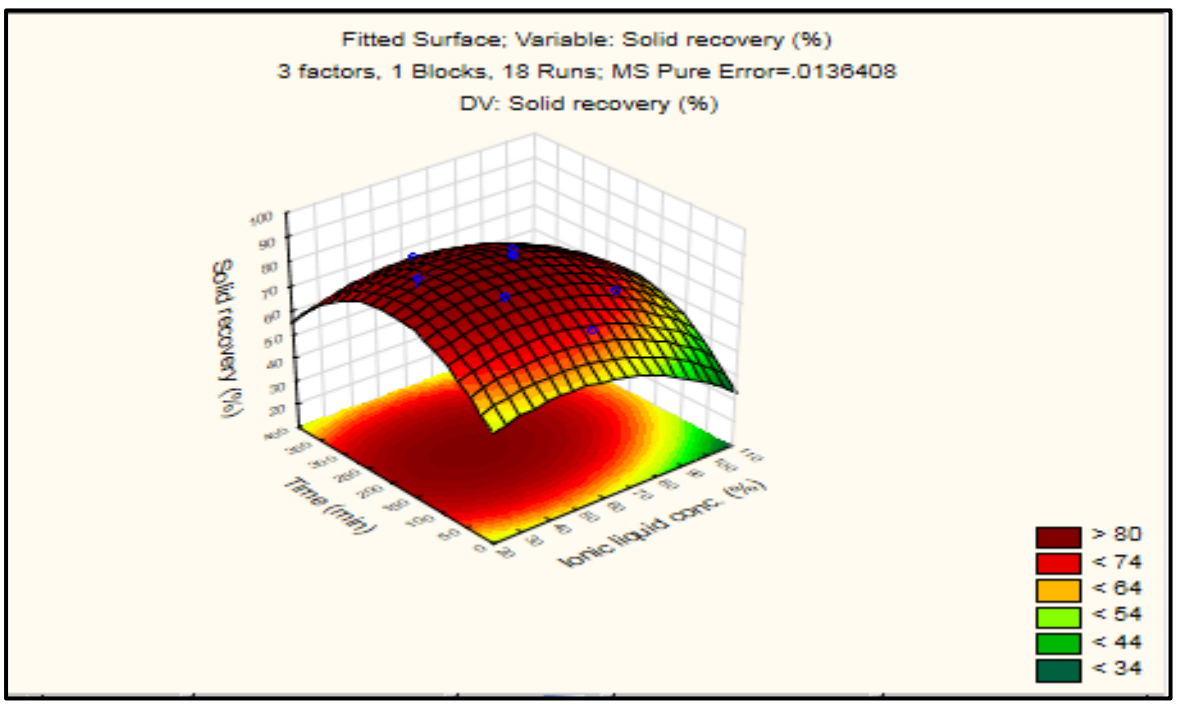
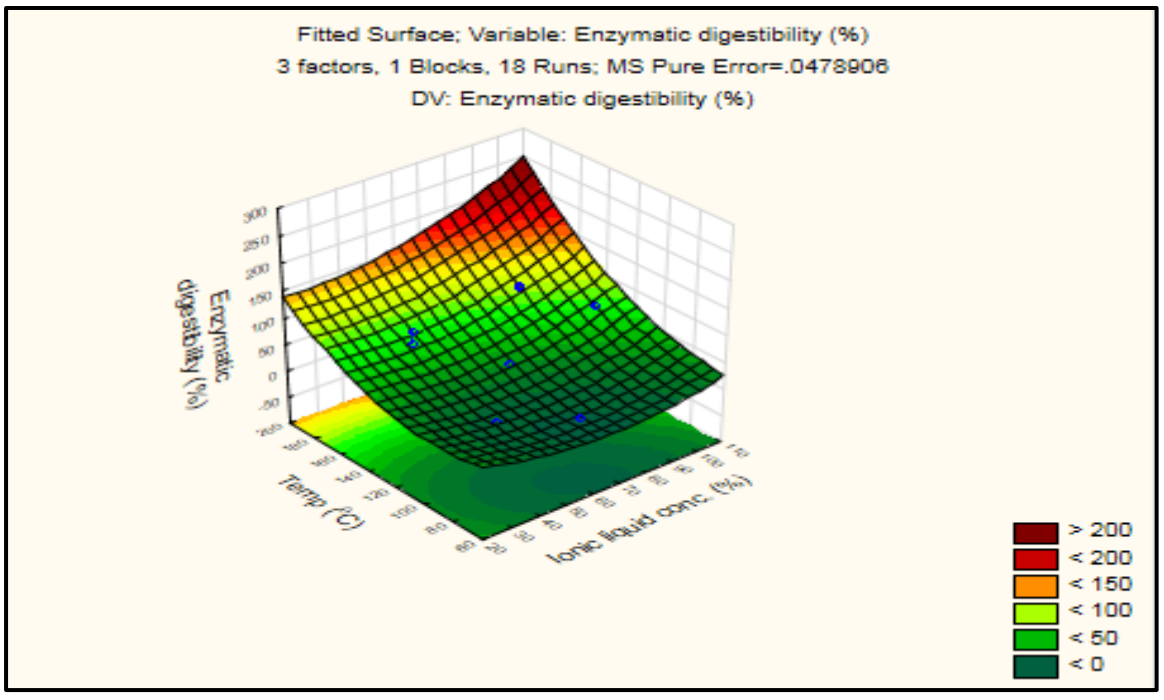
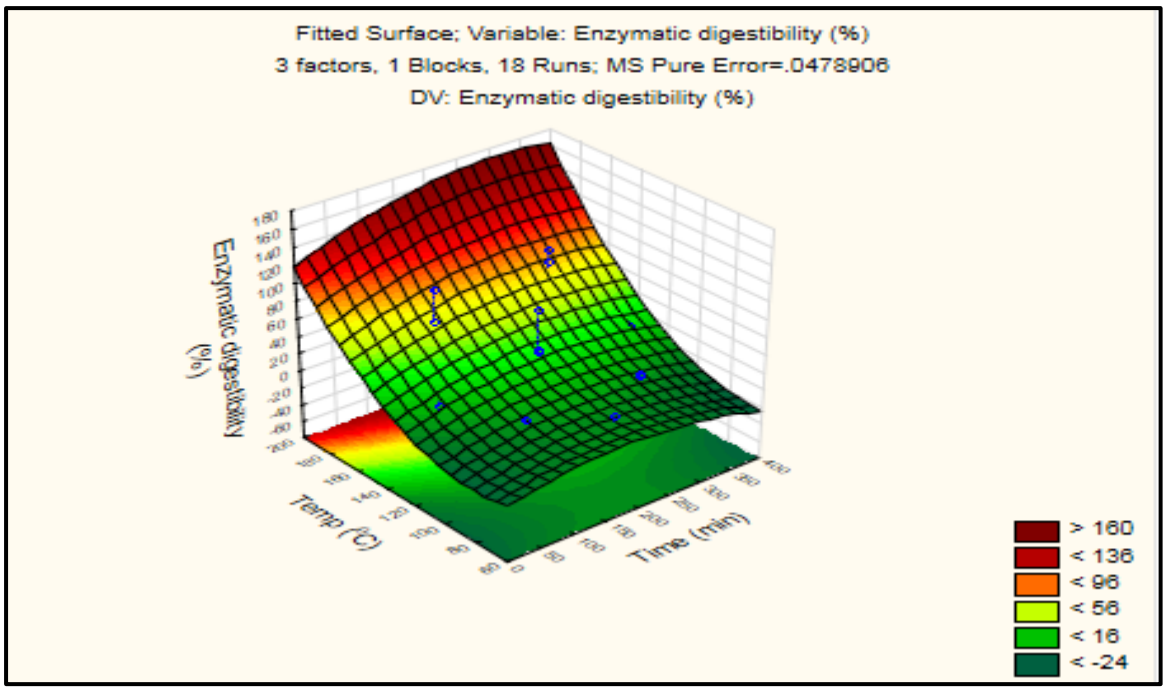


Figure D.0.27: Solid recovery (in virgin E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

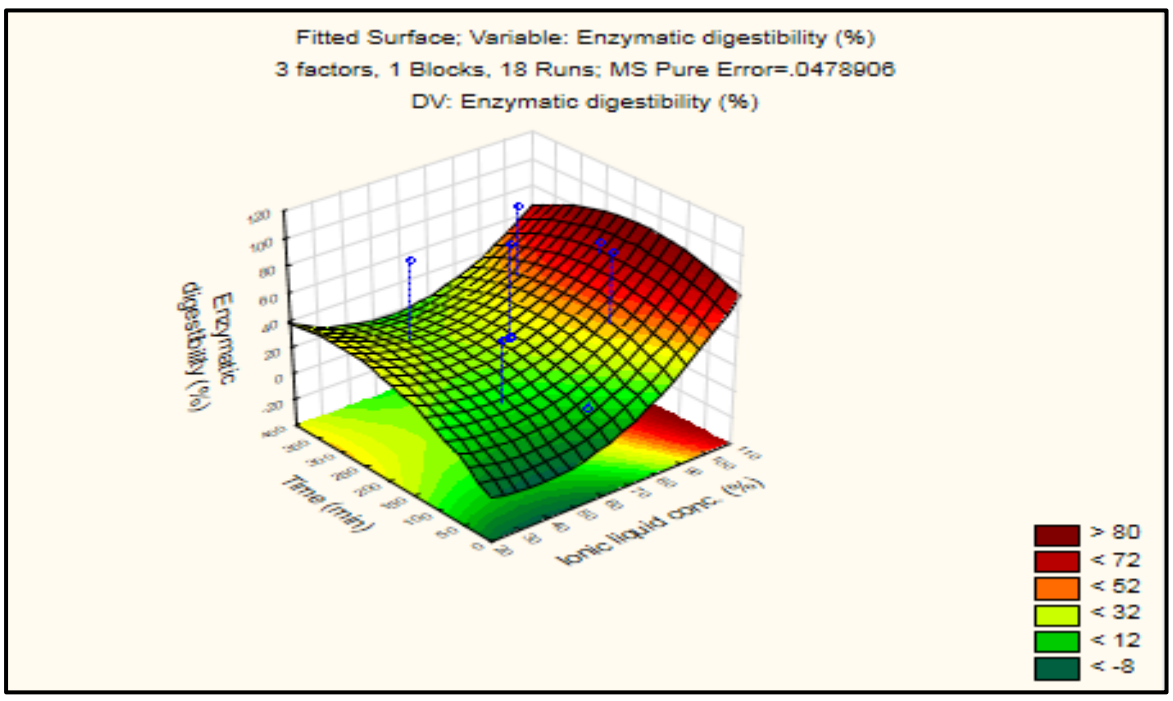
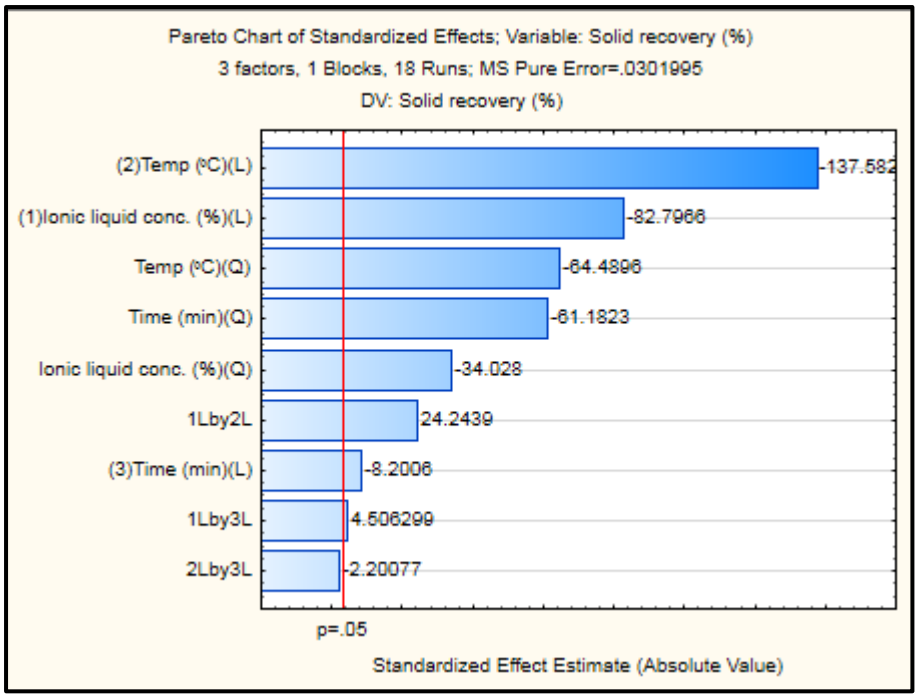


Figure D.0.28: Glucan digestibility (in virgin E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)

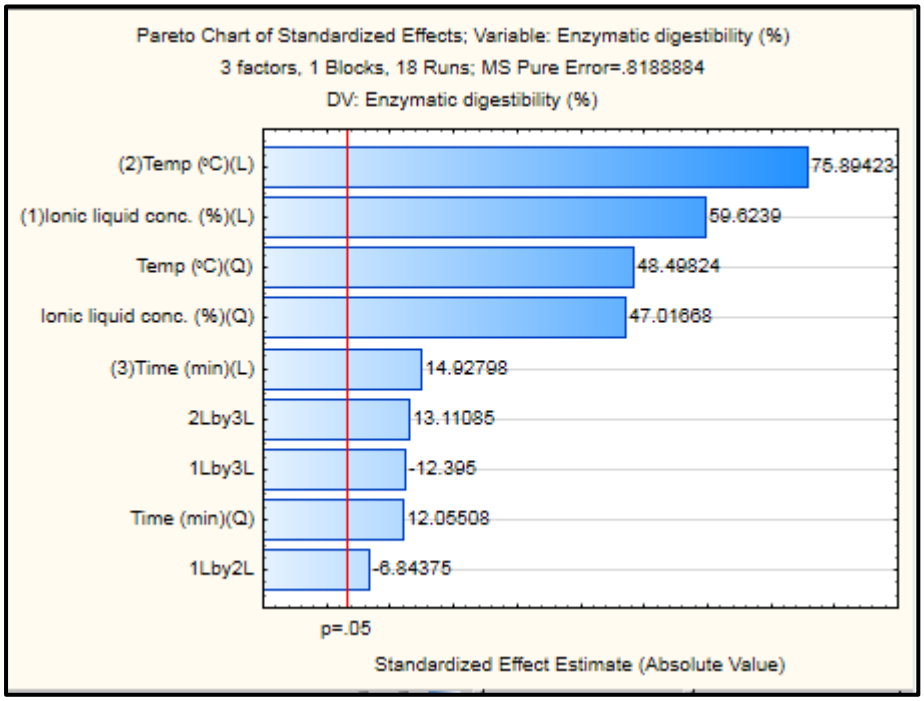
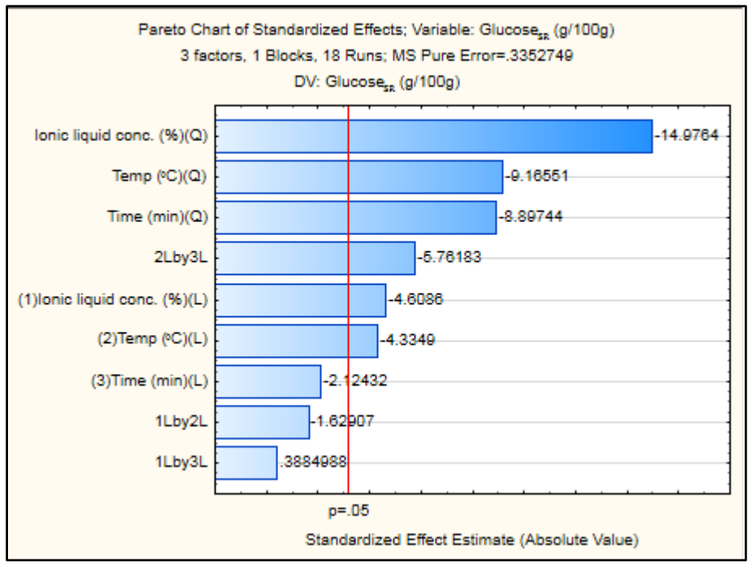
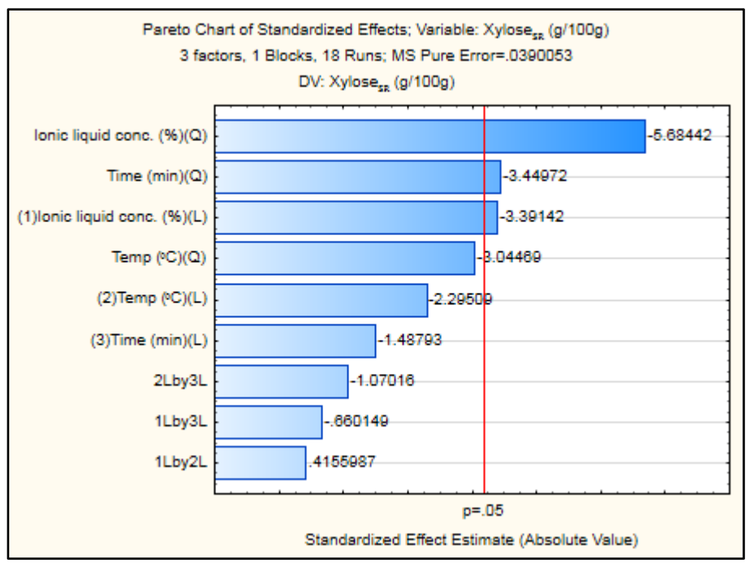
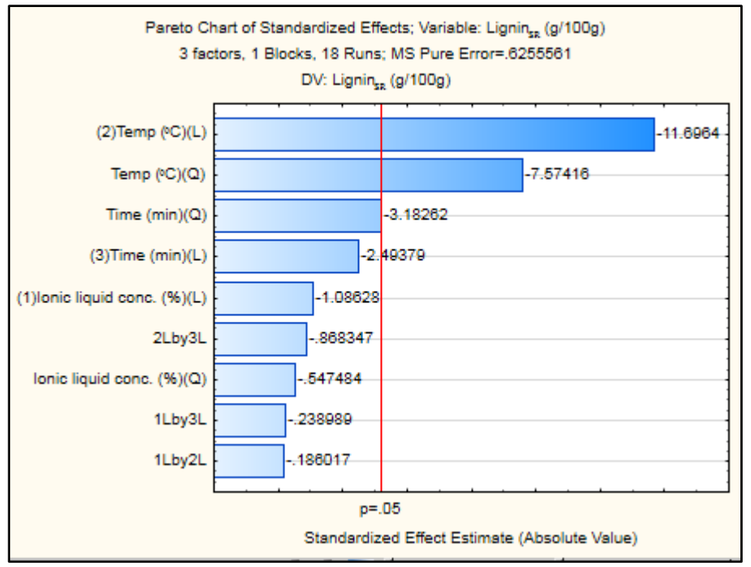


Figure D.0.29: The impact of ionic liquid treatment of virgin E. grandis, (a) solid recovery, and (b) glucan digestibility

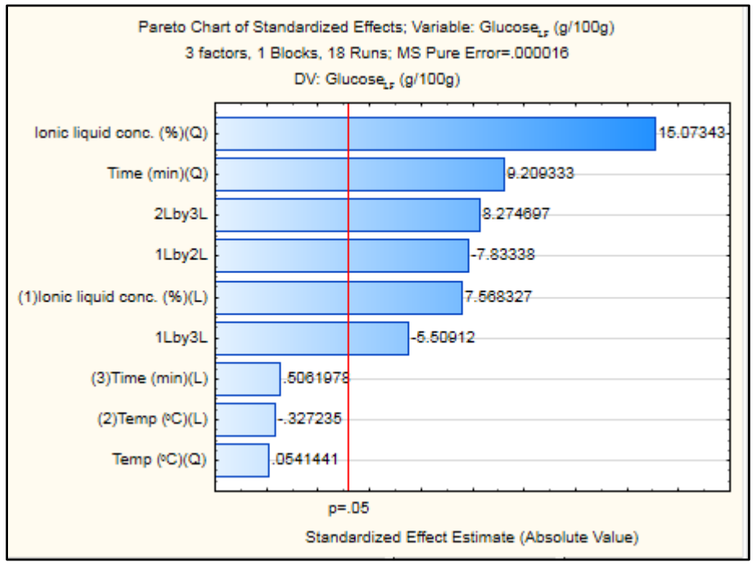
(a) (b) (c)

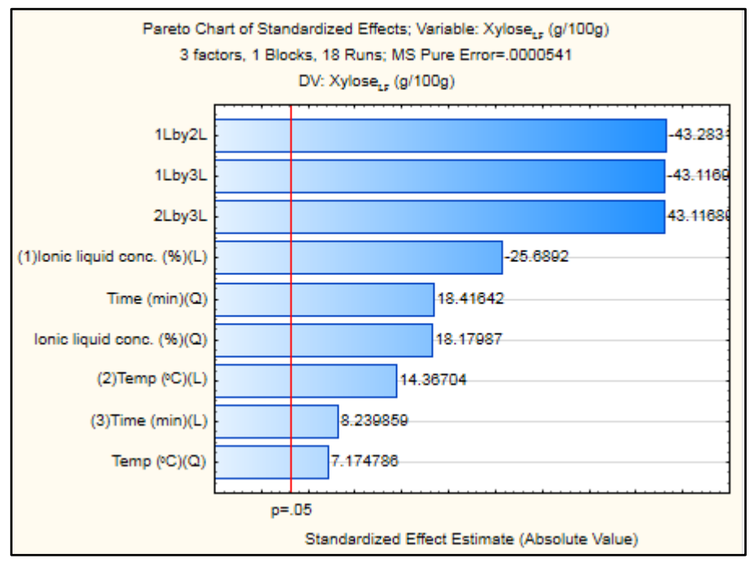






(d) (e) (f)





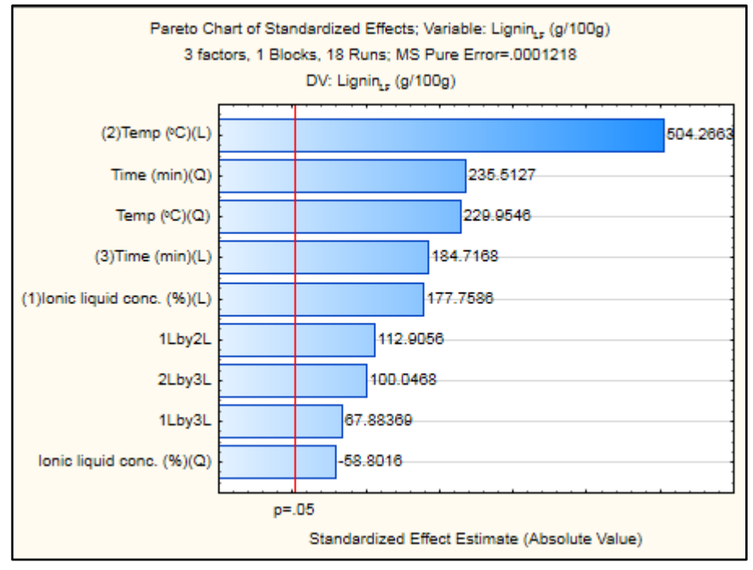
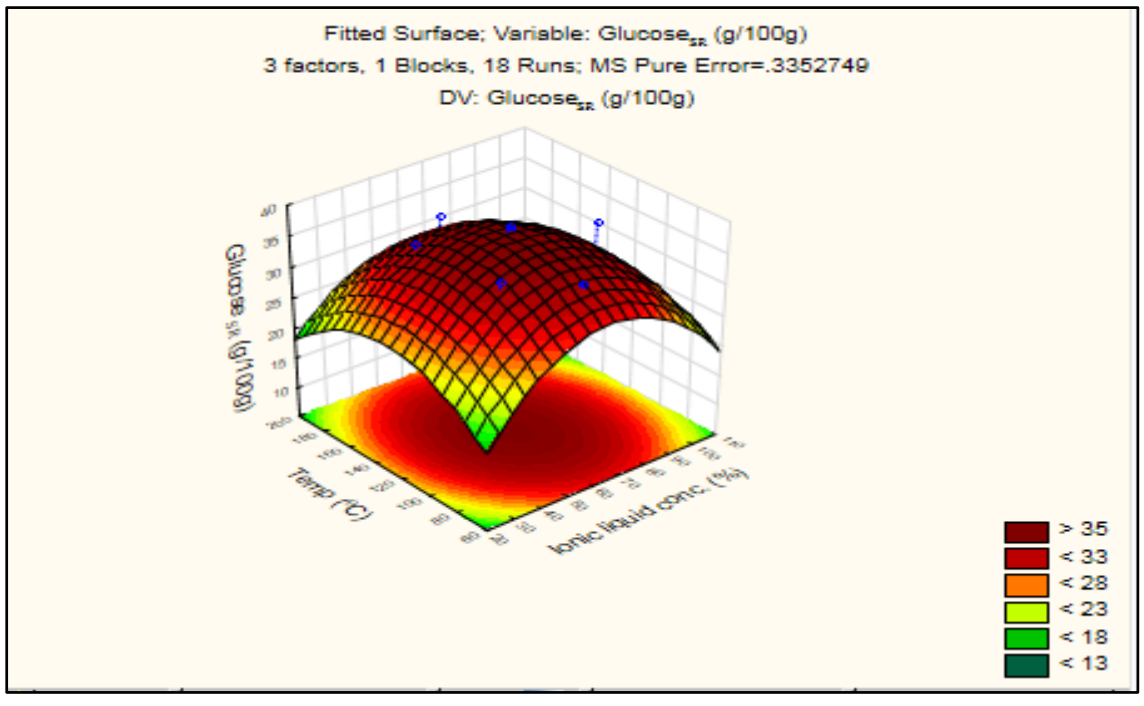
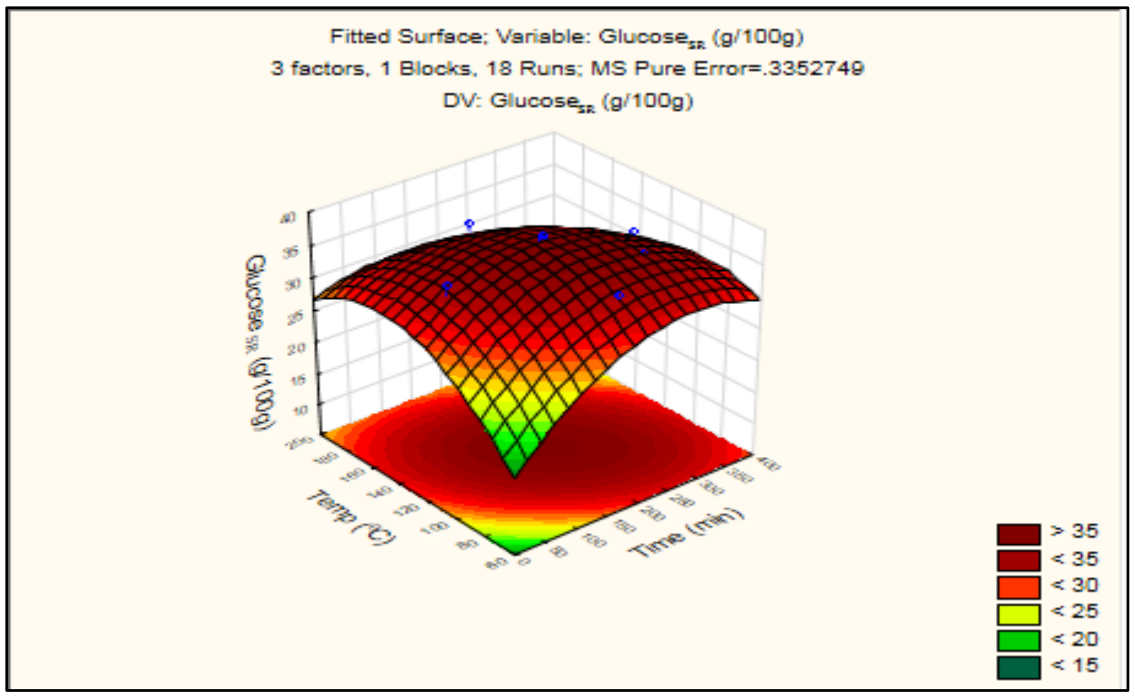


Figure D.0.30. The impact of ionic liquid treatment of pre-extracted E. grandis, (a) glucan retained, (b) xylan retained, (c) lignin retained, (d) glucan dissolved, (e) xylan dissolved, and (f) lignin dissolved

(a)



(b)



(c)

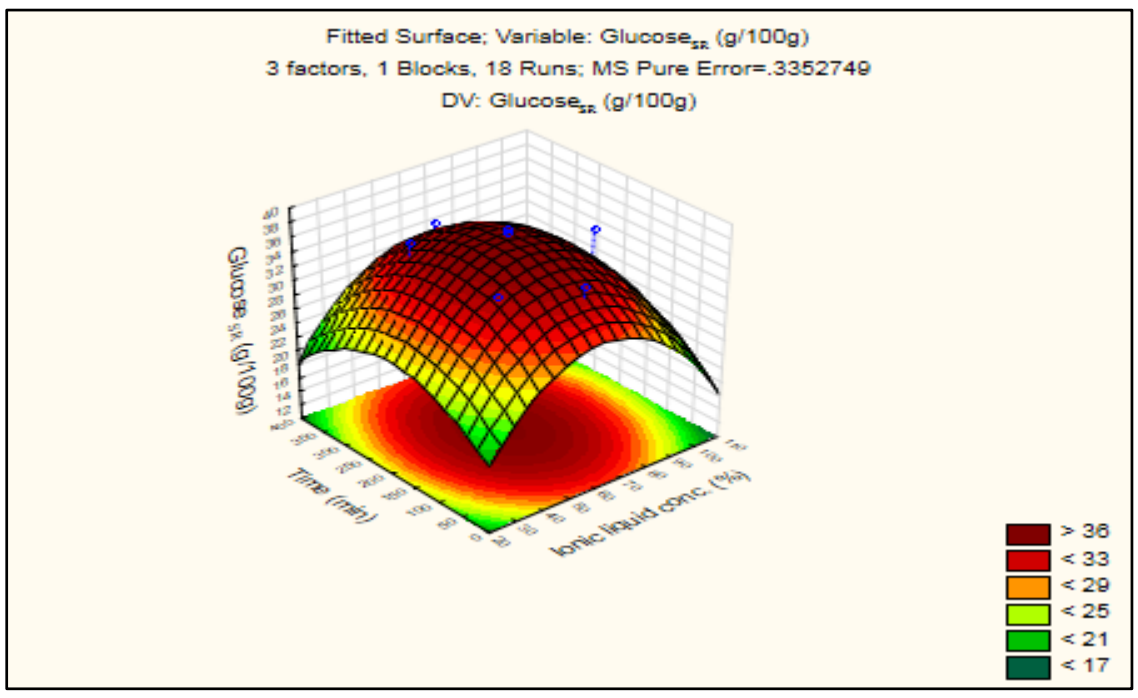
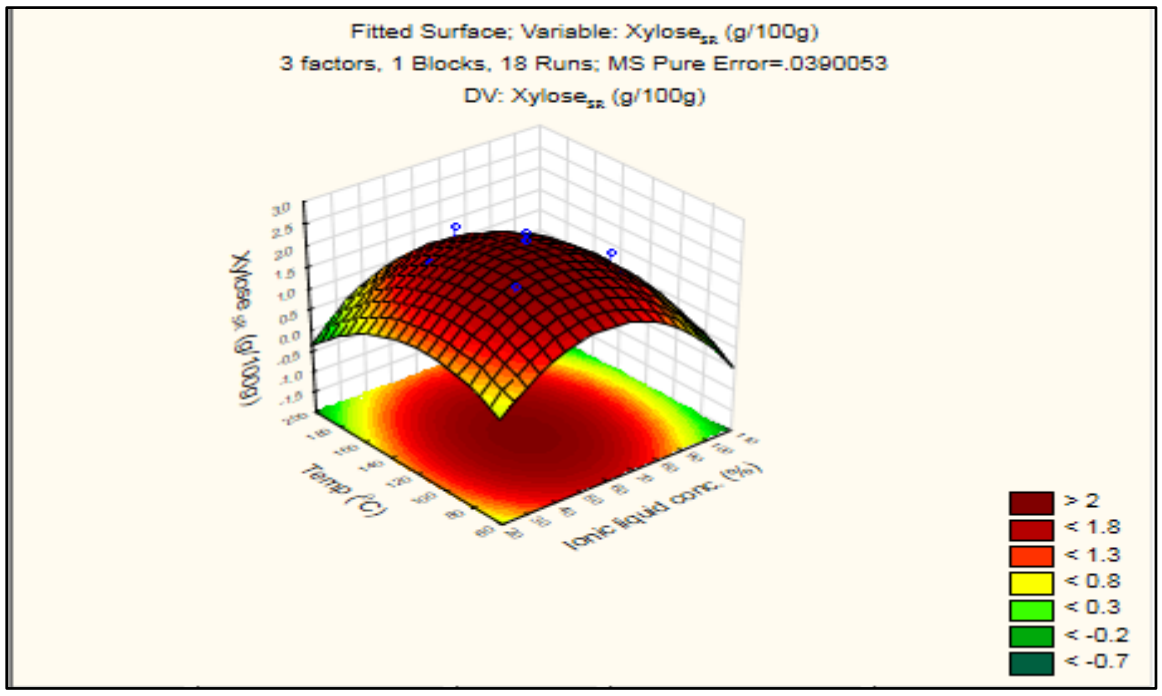
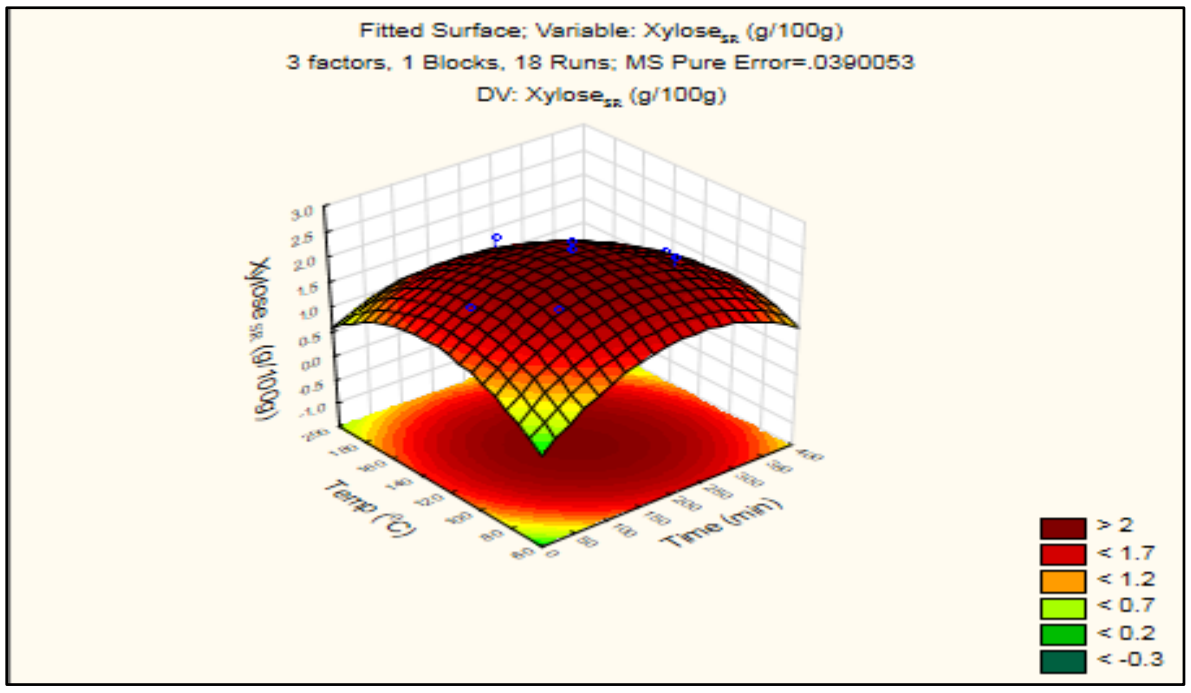


Figure D.0.31: Glucan retention (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

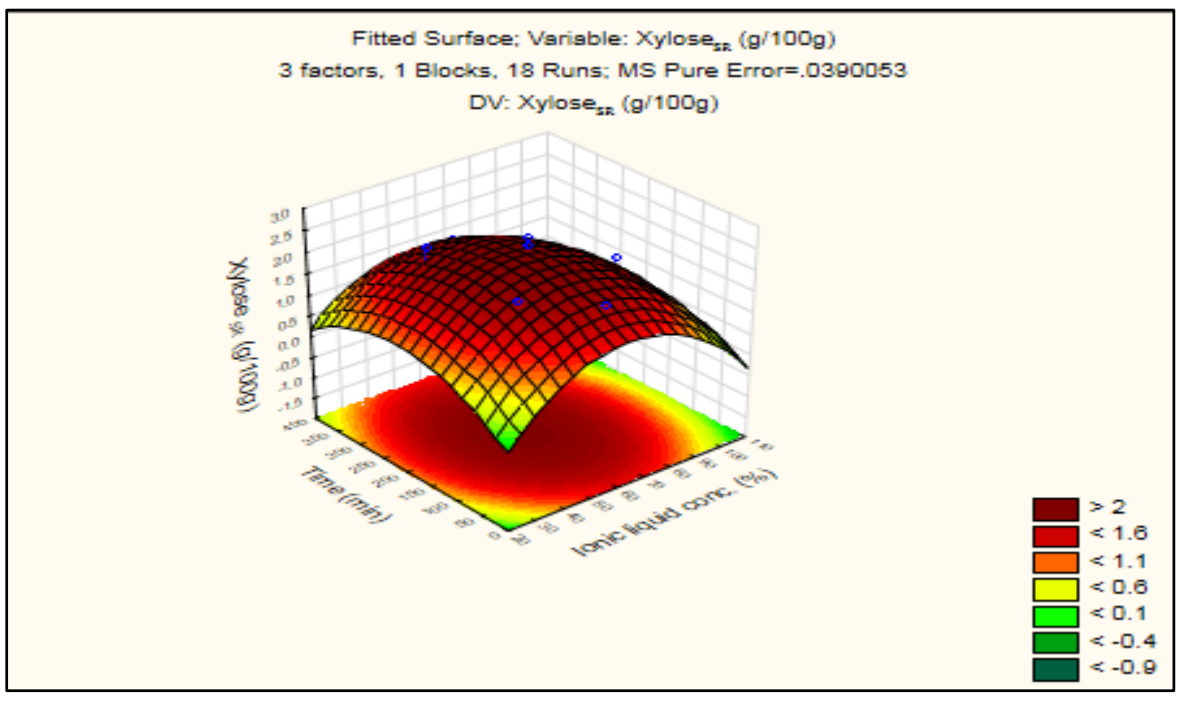
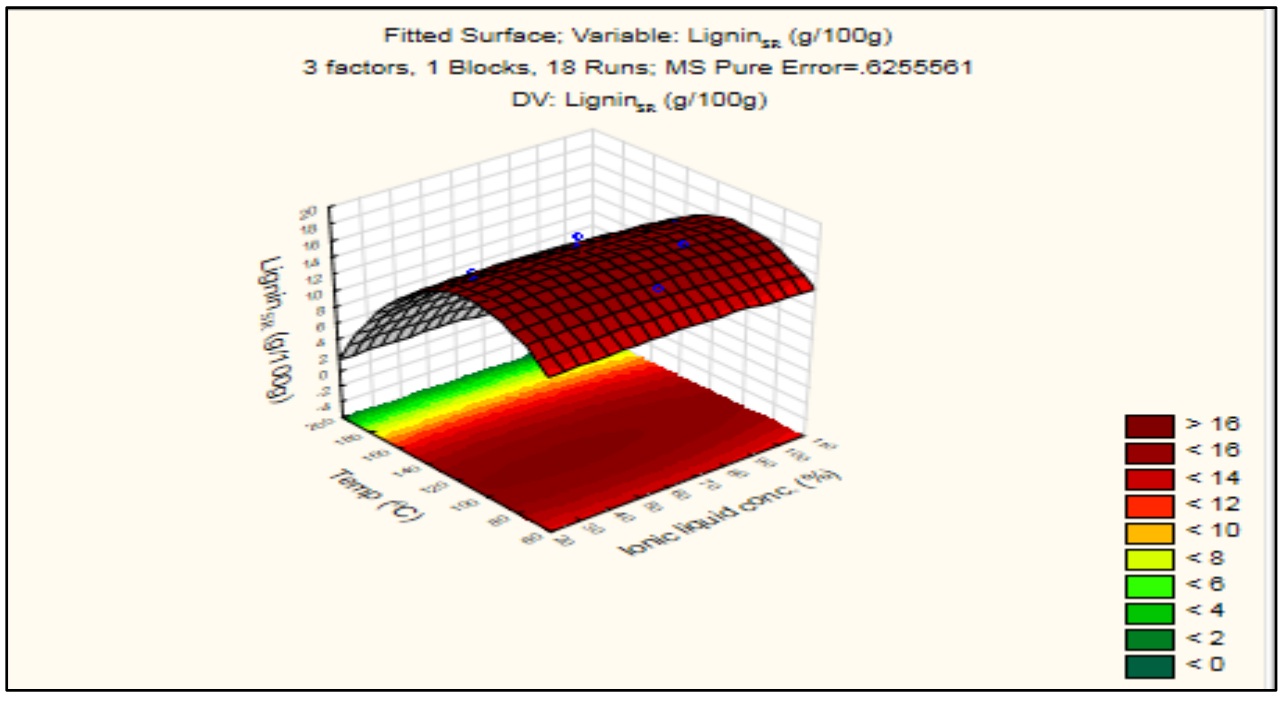
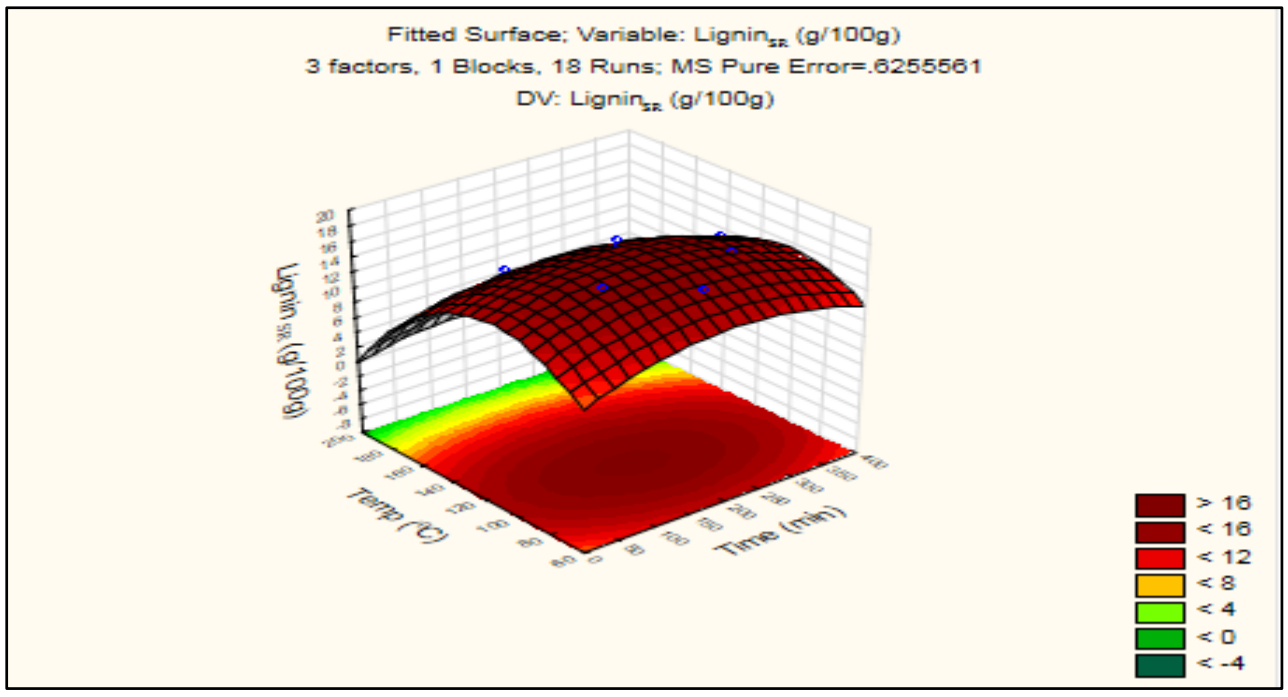


Figure D.0.32: Xylan retention (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

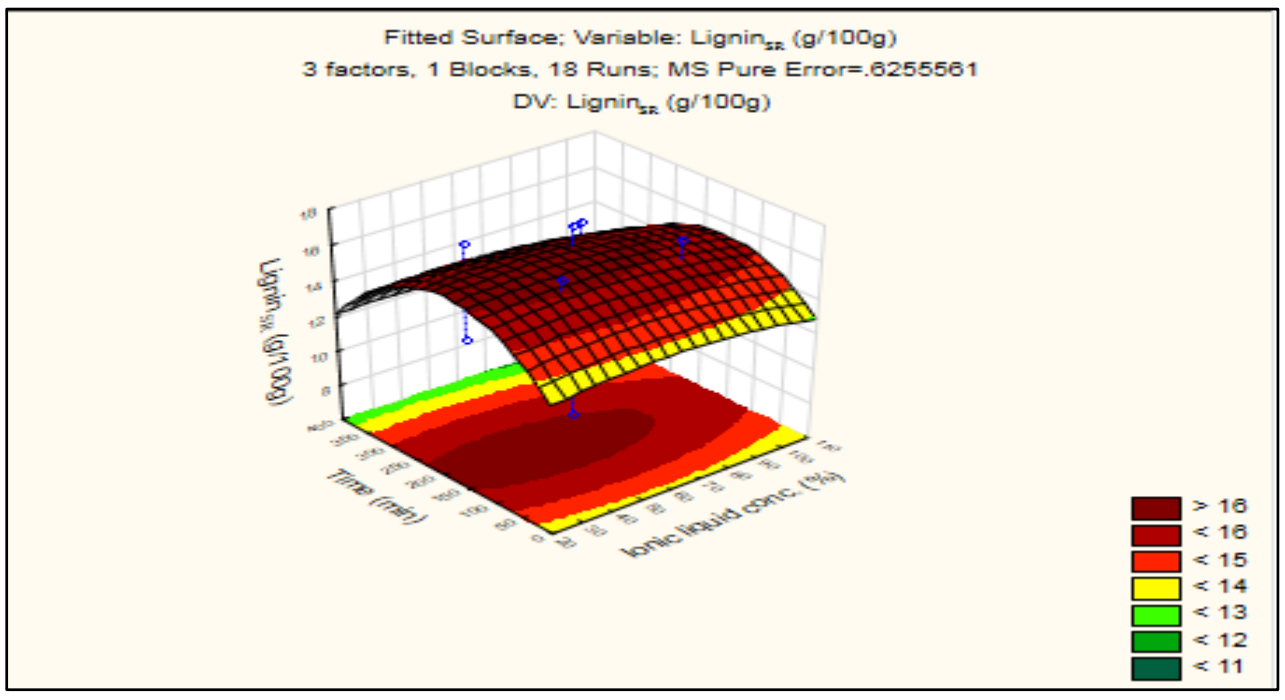
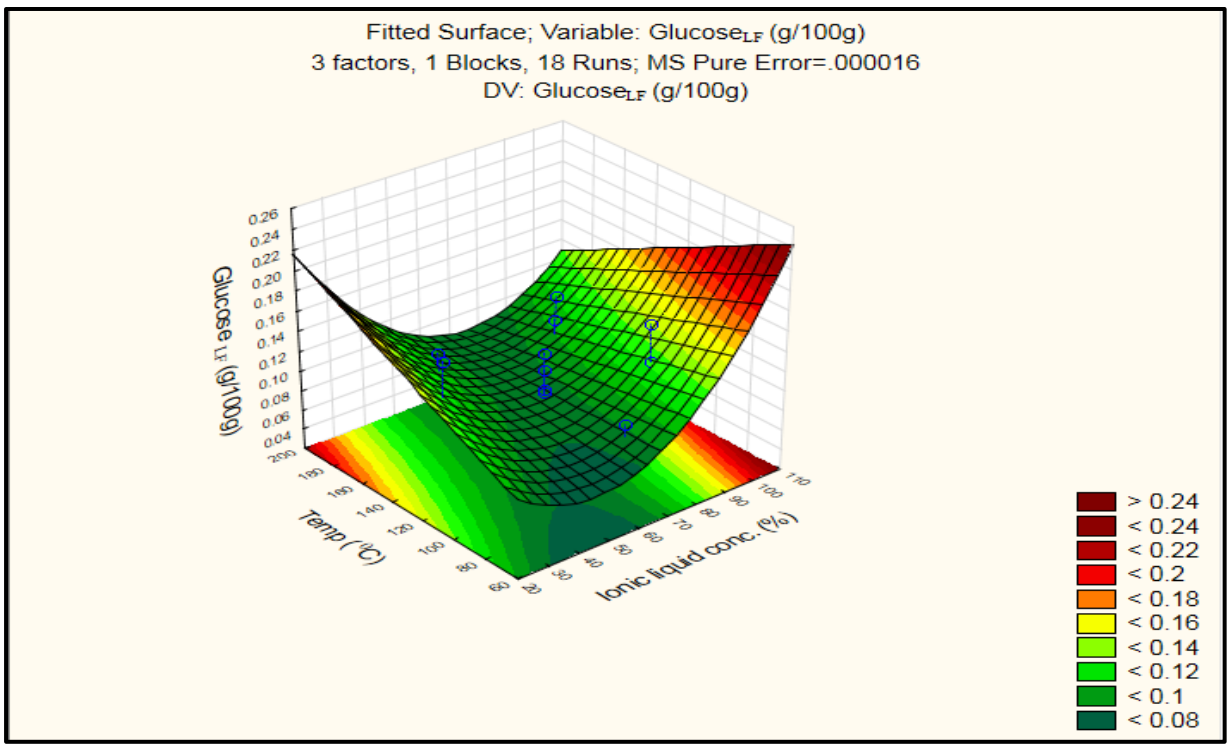
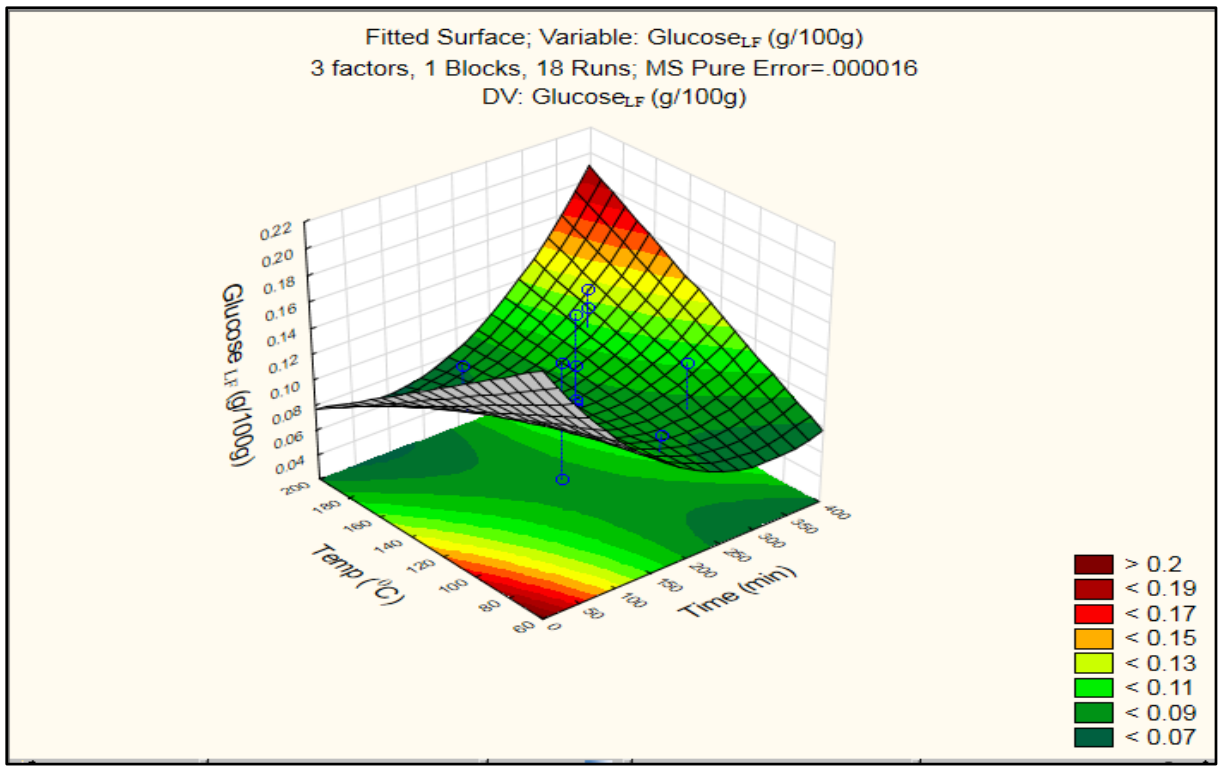


Figure D.0.33: Lignin retention (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

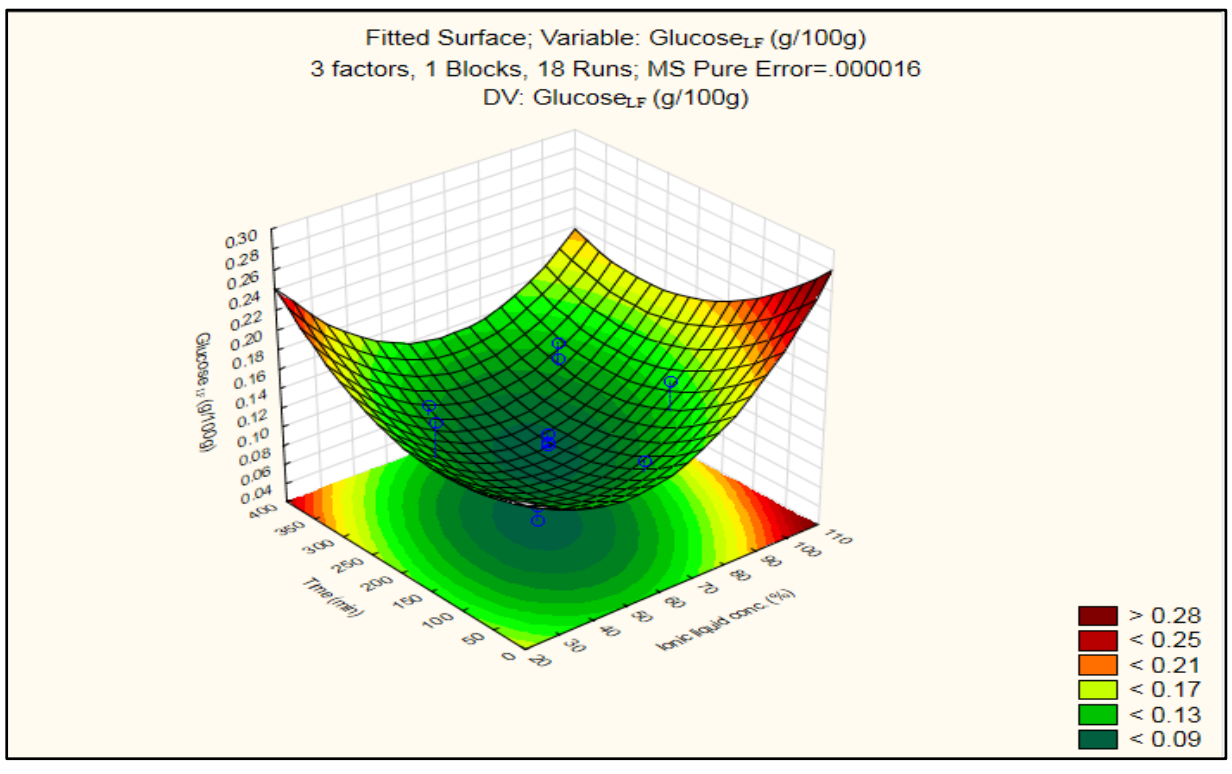
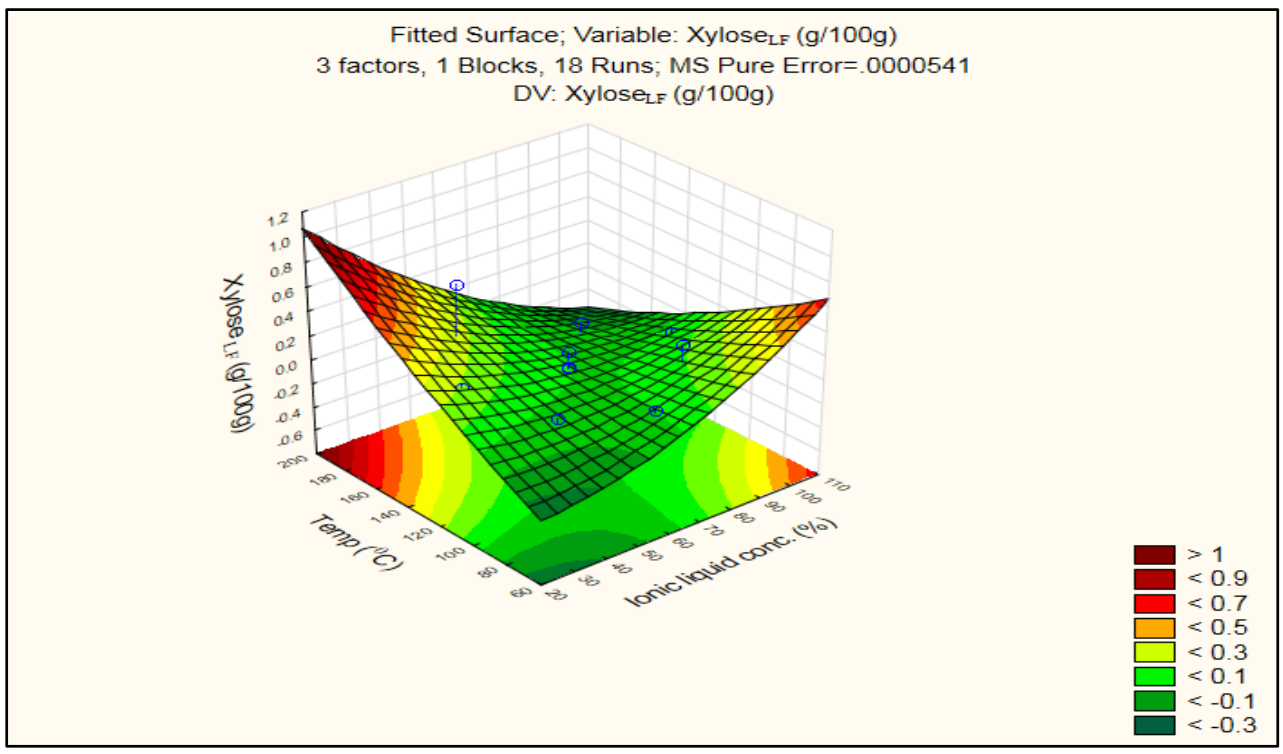
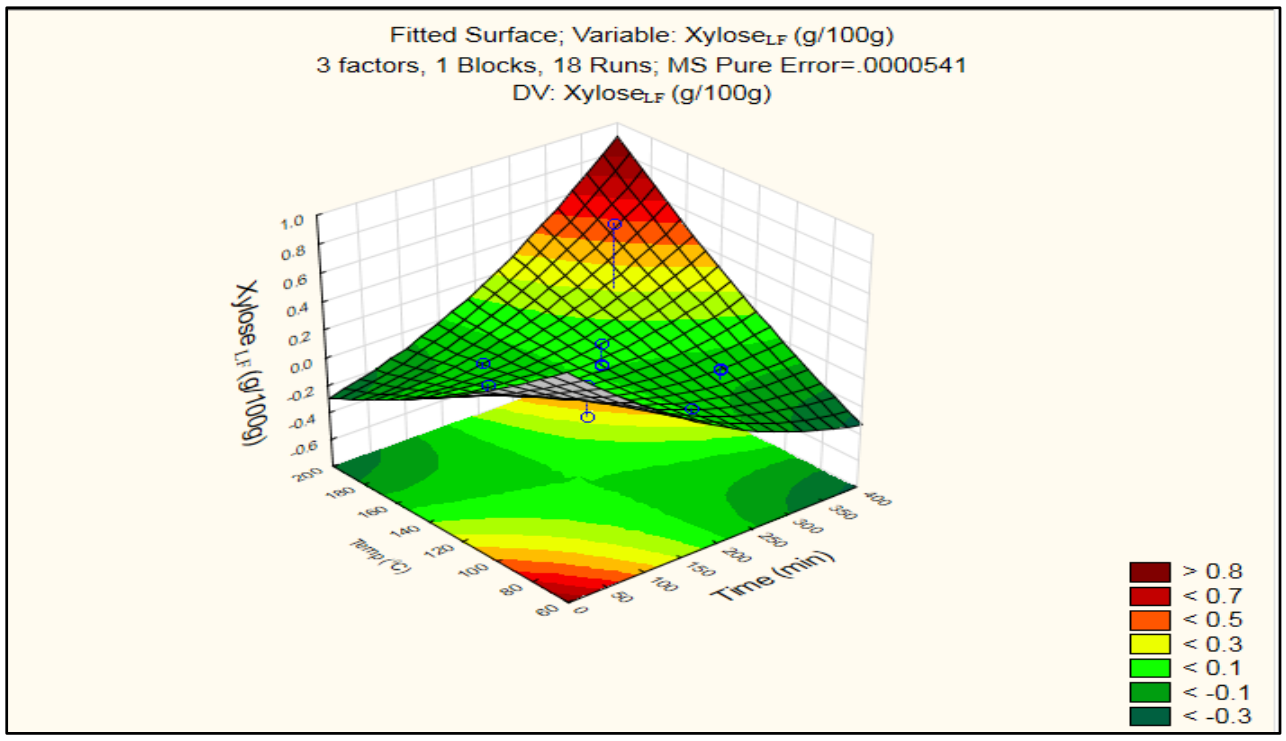


Figure D.0.34: Glucan dissolution (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

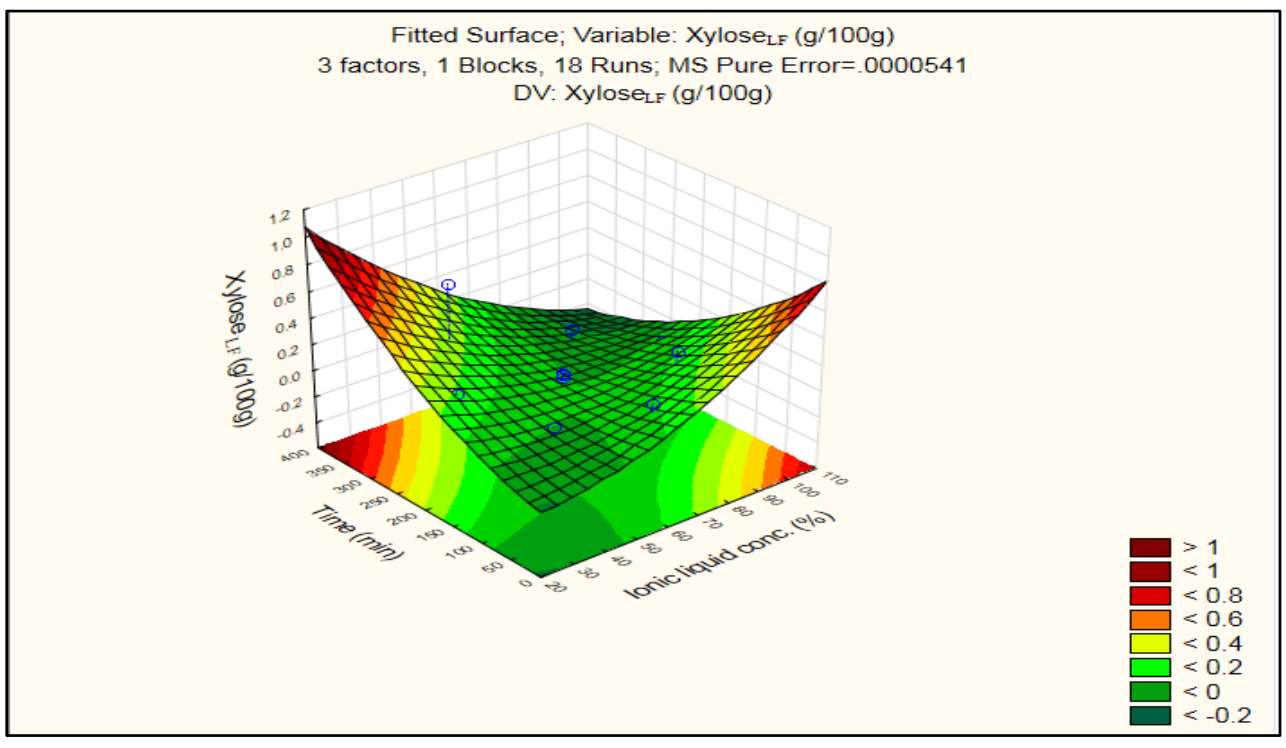
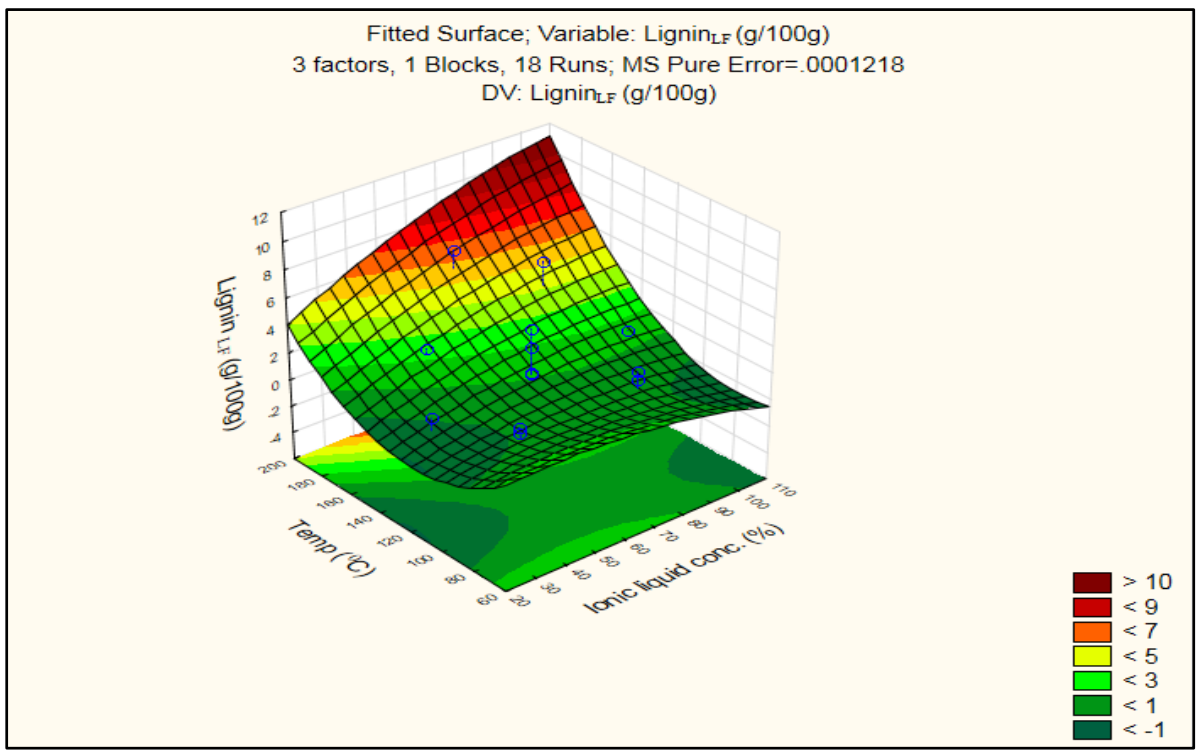
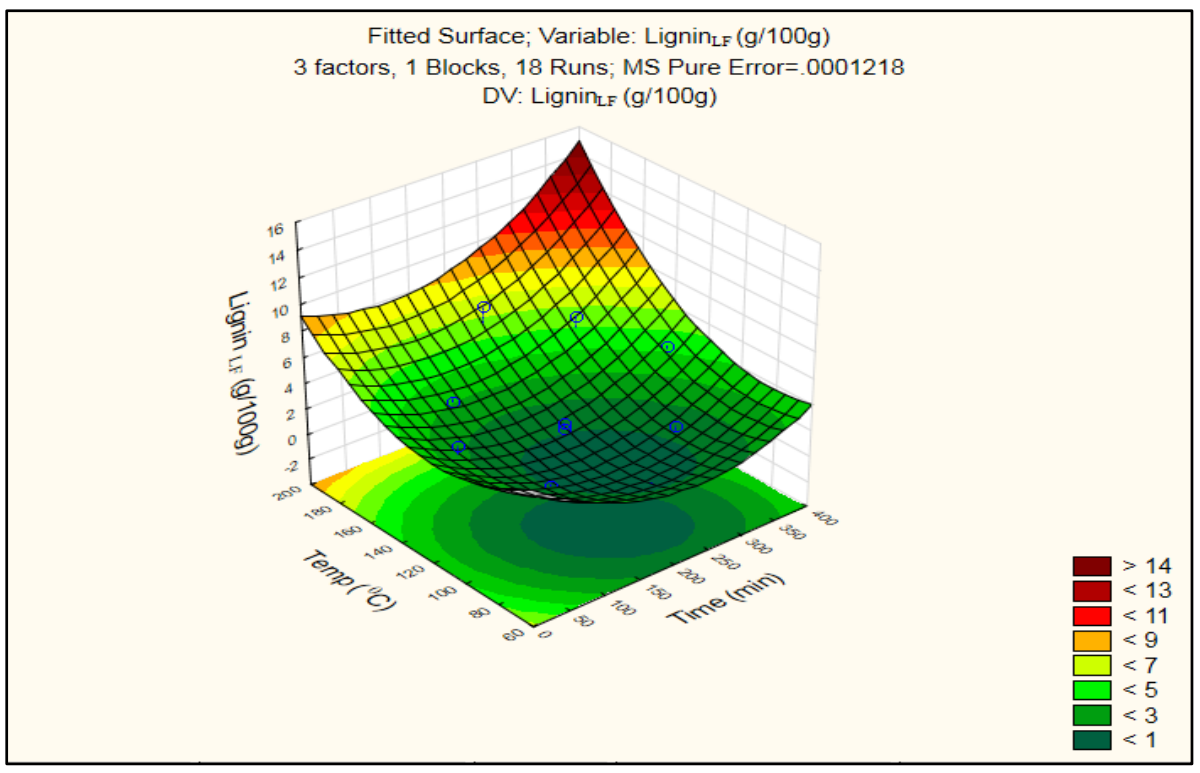


Figure D.0.35: Xylan dissolution (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

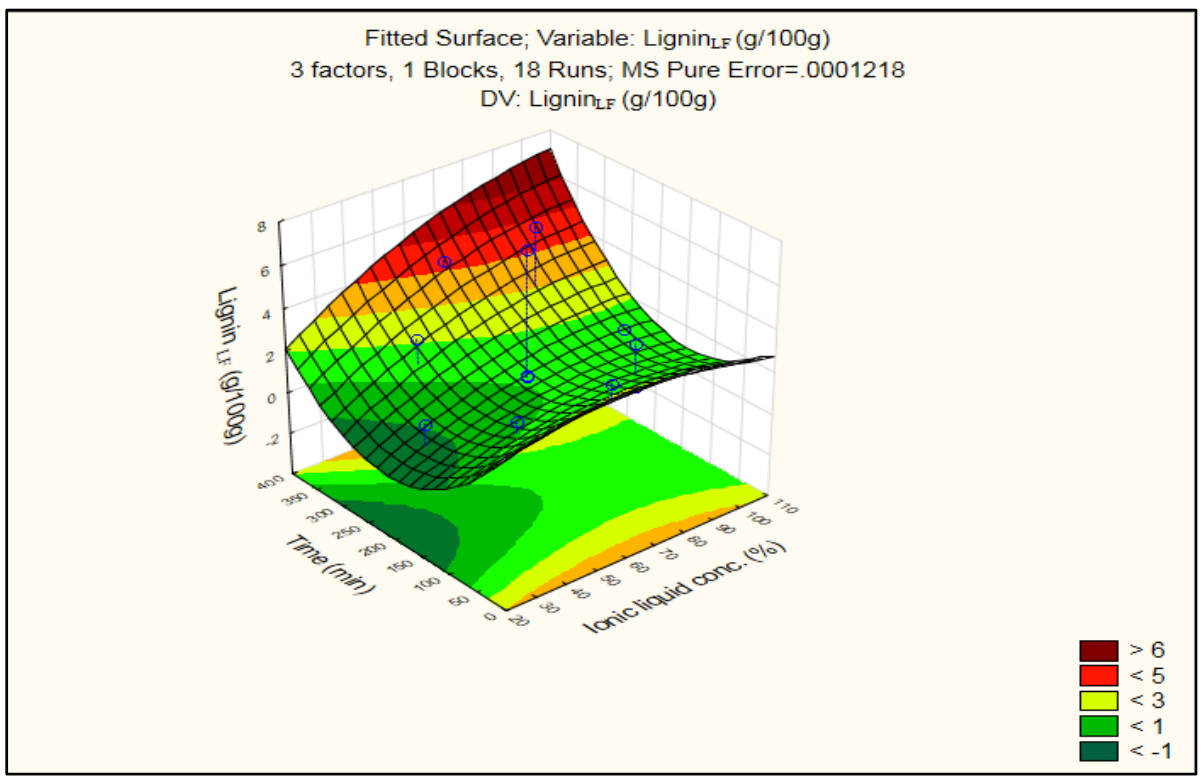
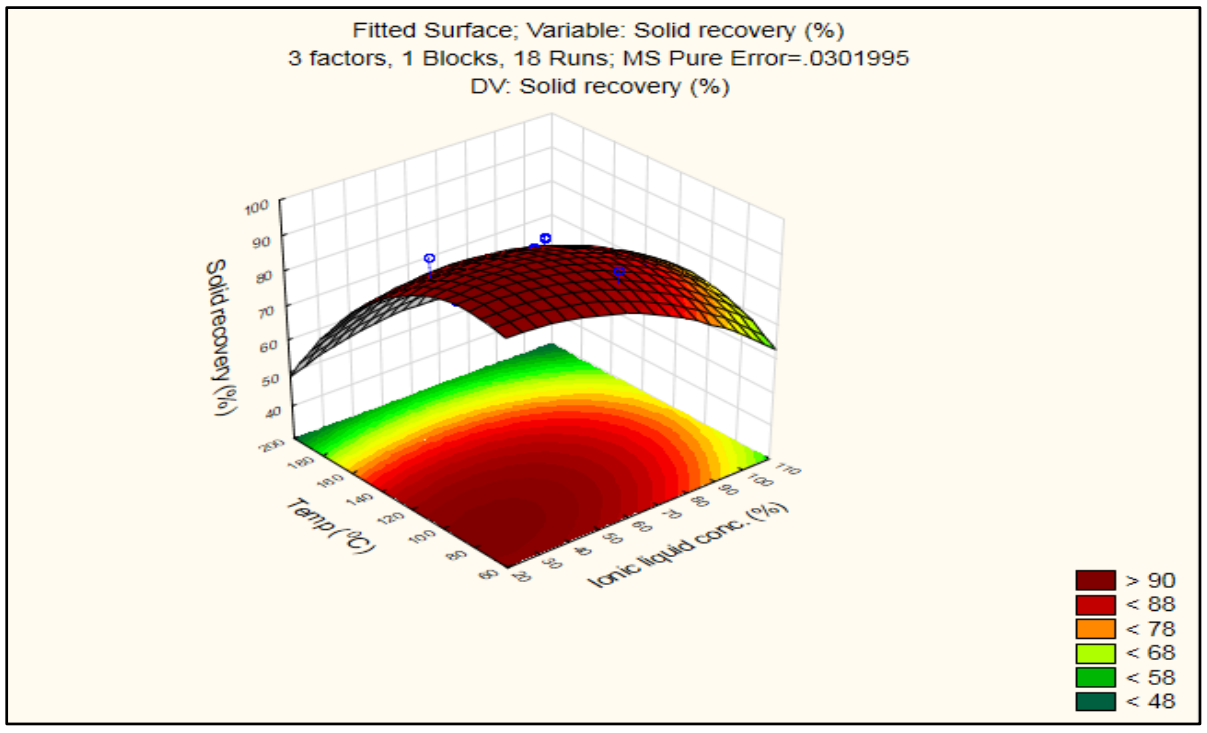
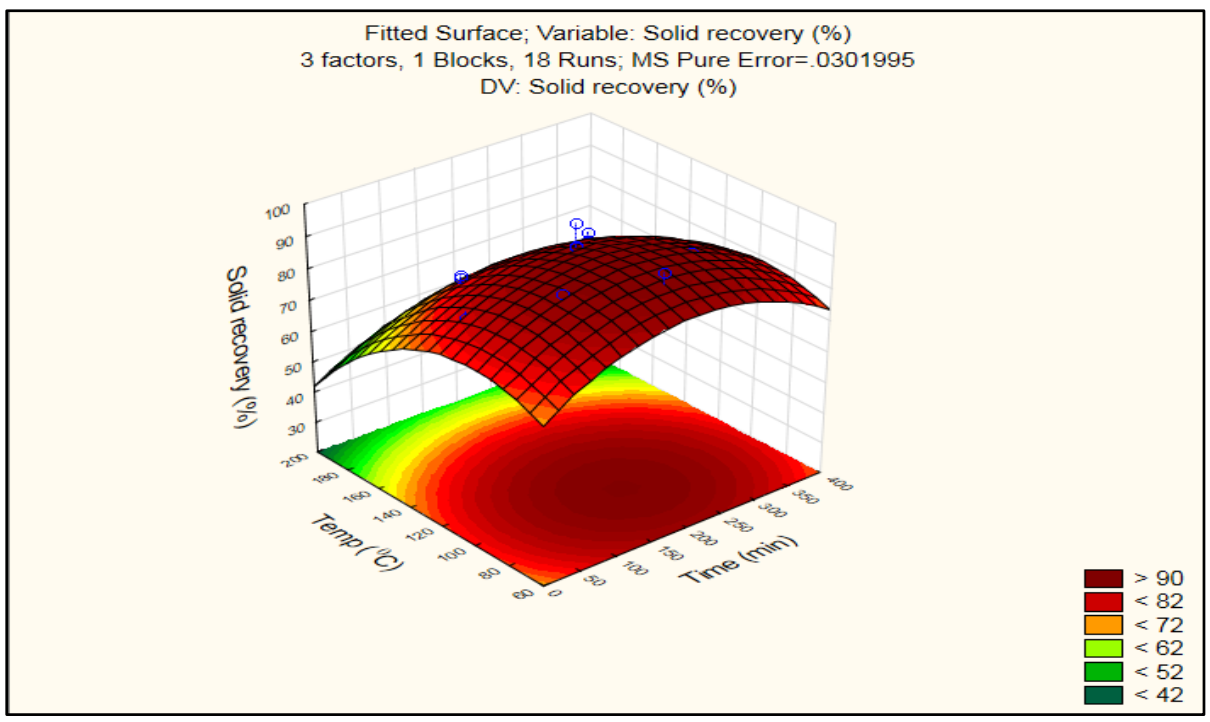


Figure D.0.36: Lignin dissolution (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

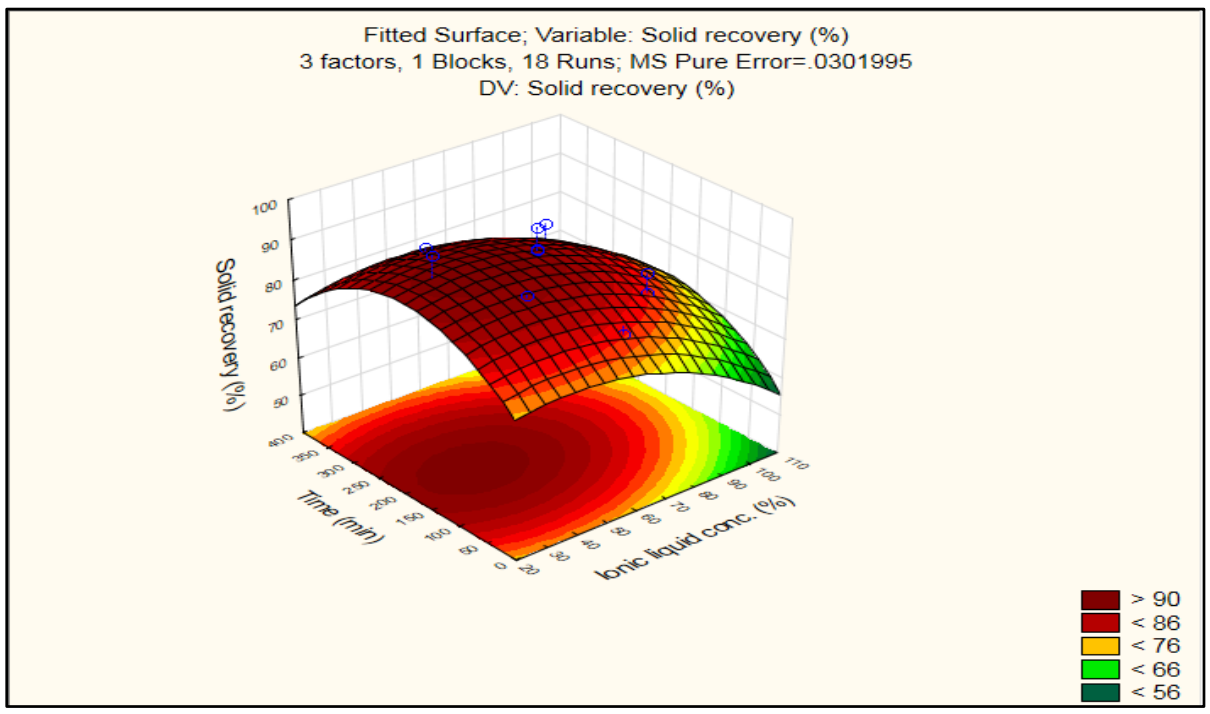
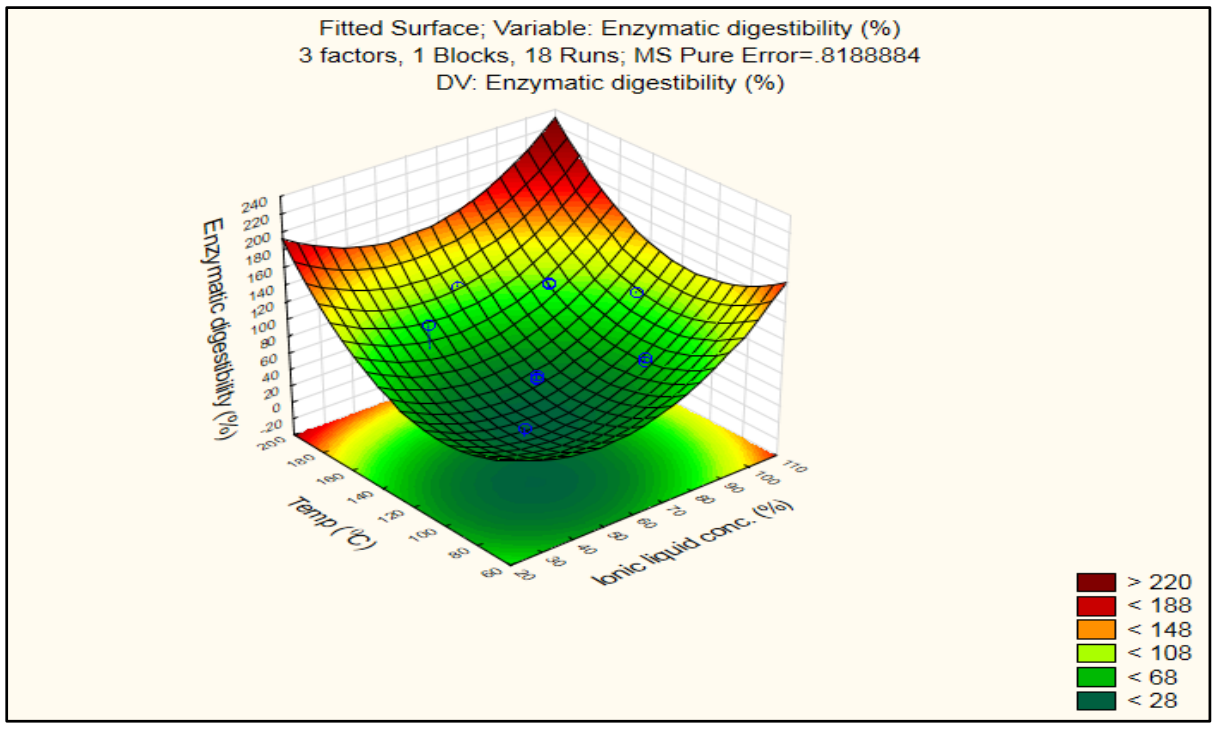
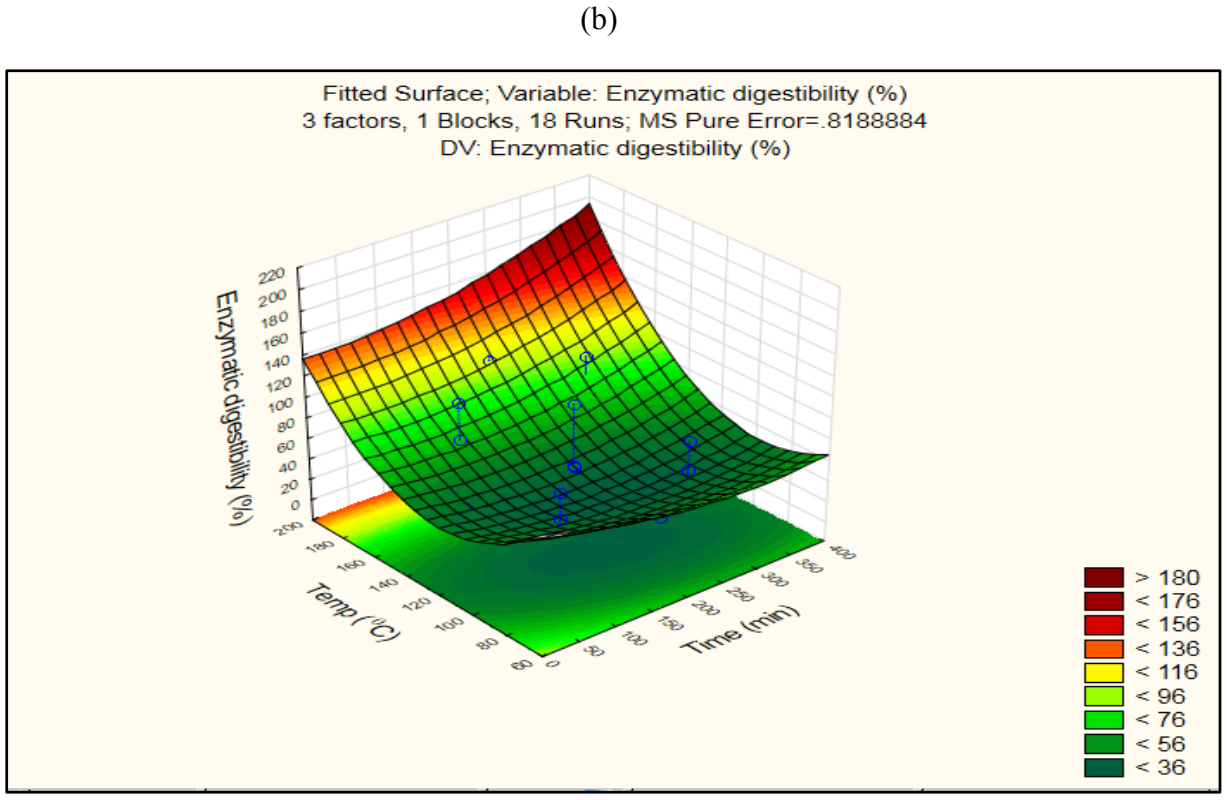


Figure D.0.37: Solid recovery (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration

(a)



(b)



(c)

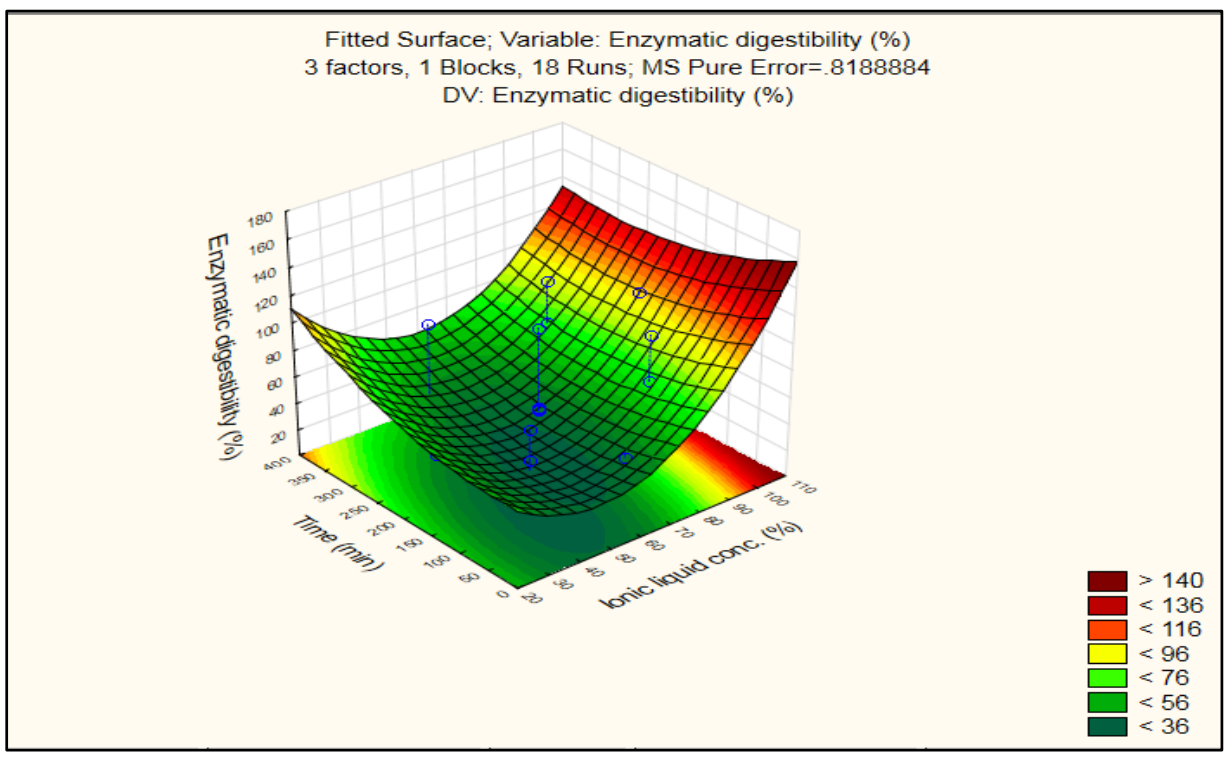


Figure D.0.38: Glucan digestibility (in pre-extracted E. grandis treatment) as a function of (a) temperature and ionic liquid concentration, (b) temperature and time, and (c) time and ionic liquid concentration