ALKALINE POLYOL FRACTIONATION OF SUGARCANE BAGASSE AND EUCALYPTUS GRANDIS INTO FEEDSTOCK FOR VALUE ADDED CHEMICALS AND MATERIALS

by

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Abstract

The main components of lignocellulosic biomass cellulose, hemicellulose and lignin are feedstock for chemical and material manufacturing processes. Integrated biorefinery processes incorporate the production of these valuable components from lignocellulose feedstock in good yield and quality. The nature and complexity of lignocellulose materials and its components require a well-designed process to fractionate these components into individual streams, while special attention is paid to the easily hydrolysed component, hemicelluloses.

In the present study, a novel process for the fractionating sugarcane *(Saccharum officinarum)* bagasse (SCB) and *Eucalytpusgrandis* (EC) biomass into their main constituents (cellulose pulp, aqueous hemicellulose and lignin) is designed. Research focused on obtaining hemicelluloses in polymeric form or as biopolymers, while maintaining high yields and quality of cellulose and lignin polymers. This was achieved by following organosolv technique using high boiling point alcohols, xylitol and ethylene glycol as the fractionating solvents at concentrations between 20-30% (w/w) and 50-70% (v/v) respectively. The fractionation process' central composite design incorporated mild conditions, i.e. fractionation time between 2-4 hours, temperatures at 140-180 ºC catalysed by sodium hydroxide between 1-2 wt.% and also subsequently investigated the option of pre-extracting hemicelluloses from the feedstock at previously established conditions prior to further fractionation with ethylene glycol given its hemicellulose destructing nature from literature studies.

Results show hemicellulose alkaline pre-extraction to provide higher dissolutions and recoveries of hemicelluloses as compared to those extracted by direct fractionation with the two solvents. At optimum conditions xylitol fractionations achieved higher component recoveries as compared to ethylene glycol. However, ethylene glycol fractionations are more severe in dissolving not only hemicellulose and lignin from both materials but also cellulose. Ethylene glycol fractionations were also accompanied by a high degree of cellulose dissolutions, in some runs up to 39% of the initial, mostly at extreme conditions.

Hemicelluloses from all processes were recovered as biopolymers with weight-average molecular weight (*Mw*) evaluation revealing that alkaline pre-extracted hemicelluloses had highest weight-average molecular weights, 33 638 and 61 644 $gmol^{-1}$ for sugarcane bagasse and *Eucalytpus grandis* respectively, as compared to direct raw material fractionation processes which all gave below 23 000 $gmol^{-1}$ with xylitol processes giving higher molecular weights than ethylene glycol processes. Enzymatic hydrolysis of cellulose revealed ethylene glycol residues to be more digestible (≥60%) than xylitol derived residues (≤60%). Digestibility is further improved with fractionation of hemicellulose pre-extraction solids (≥80%). In terms of cellulose crystallinity, a general increase after fractionation was observed. Residual solids from ethylene glycol treatments displayed higher crystallinity (50.08% EC, 48.44% SCB) as compared to xylitol processes (32.44% EC, 43.98% SCB). Residual solids from the NaOH hemicellulose pre-extraction step also had high crystallinities (43.58% EC and 47.81% SCB) than the xylitol process but just lower than EG derived residual solids (≥48%). There is a major decline in the amount of syringyl and guaiacyl groups in the lignin residues after treatment for all processes supported by low intensity bands in Fourier Transform Infrared Resonance (FTIR). Minimal degradation of lignin fraction by both processes was observed with low fixed carbon content of lignin rich solids, below 20%.

In conclusion, xylitol fractionations overweighed ethylene glycol in hemicellulose, lignin and cellulose recoveries, and lignin and hemicellulose quality while ethylene glycol produced good quality cellulose. When compared to conventional organosolv fractionations (i.e. ethanol), these two polyols overweigh organosolv in aspects such as quality of cellulose, hemicellulose and lignin but comes short in terms of component recoveries particularly with ethylene glycol fractionations.

Opsomming

Die hoof-komponente van lignosellulose biomassa (sellulose, hemisellulose en lignien) dien as voer vir chemiese en material-vervaardigingsprosesse. Geïntigreerde bio-raffinadery prosesse sluit die produksie (teen goeie opbrengste en kwaliteit) van hierdie waardevolle lignosellulose komponente in. Die aard en kompleksiteit van lignosellulose materiale beteken dat die fraksionering daarvan in individuale komponente 'n goed-ontwerpte proses vereis, met spesiale aandag wat geskenk word aan die maklik gehidroliseerde komponent, hemisellulose.

In hierdie studie word 'n nuwe proses ontwerp vir die fraksionering van suikerriet (Saccharum officinarum) bagasse (SRB) en *Eucalytpus grandis* (EC) biomassa in hulle hoof-bestanddele (sellulose pulp, gehidreerde hemisellulose en lignien). Navorsing het gefokus op die verkryging van hemisellulose of in sy polimeriese vorm of as biopolimere, terwyl hoë opbrengste en kwaliteit van sellulose en lignien polimere gehandhaaf word. Dit is gedoen deur 'n orgasolv tegniek te volg, wat behels dat kookpunt alkohole, xylitol en etileen-glikol as die fraksioneringsoplosmiddels gebruik is, by konsentrasies tussen 20-30% (w/w) en 50-70% (v/v), onderskeidelik. Die fraksioneringsproses se sentrale saamgestelde ontwerp het gematigde toestande geïnkorporeer; d.w.s 'n fraksineringstyd tussen 2 en 4 ure, temperature tussen 140 en 180 ºC, en katalise deur natriumhidroksied tussen 1 en 2 massa%. Die opsie om die hemisellulose van die voer by voorheen vasgestelde toestande te ekstraheer, voor verdere fraksionering van etileenglikol, is ook ondersoek, as gevolg van die vernietigende aard daarvan (volgens literatuur).

Die resultate wys dat alkaliese hemisellulose pre-ekstraksie beter oplossing en hoër opbrengste van hemisellulose gee as wat dit met direkte fraksionering (met die twee oplosmiddels) die geval is. By optimale toestande het xylitol fraksionerings hoër komponent opbrengste bereik as etileenglikol. Etileenglikol fraksionerings los egter meer aggressief op, sodat nie net hemisellulose en lignien nie, maar ook sellulose oplos. Etileenglikol fraksionerings is ook vergesel deur 'n hoë mate van sellulose-verliese – in sommige lopies tot 39% van die aanvanklike hoeveelheid (meestal by ekstreme toestande).

Hemisellulose was in al die prosesse herwin as biopolimere, met 'n massa-gemiddelde molekulêre massa evaluering wat daarop dui dat alkaliese vooraf ge-ekstraheerde hemisellulose die hoogste molekulêre massas gehad het (onderskeidelik 33 638 en 61 644 $gmol^{-1}$ vir suikerriet bagasse en E. grandis). Hierteenoor het direkte roumateriaal fraksioneringsprosesse almal minder as 23 000 $gmol^{-1}$ gelewer, met xylitol prosesse wat hoër molekulêre massas gelewer het as etileenglikol prosesse. Ensemiese hidroliese van sellulose het daarop gedui dat etileenglikol reste meer verteerbaar (≥60%) as xylitol afgeleide reste (≤60%) is. In terme van sellulose kristalliniteit was 'n toename na fraksionering in die algemeen gevind. Vastestof reste, van etileenglikol behandelings, het hoër kristalliniteit (50.08% EC, 48.44% SCB) getoon as xylitol prosesse (32.44% EC, 43.98% SCB). Vastestof reste van die NaOH hemisellulose pre-ekstraksie stap het ook hoër kristalliniteite (43.58% EC en 47.81% SCB) tot gevolg gehad as die xylitol proses, maar net laer as EG afgeleide vastestof reste (≥48%). Daar is 'n groot afname in die heoveelheid syringyl en guaiacyl groepe in die lignien-reste na behandeling vir alle prossesse, ondersteun deur lae-intensiteit bande in Fourier Transform Infrarooi Resonansie (FTIR). Minimale degradering van lignien is ge-observeer vir beide prosesse, met 'n lae vaste-koolstof inhoud van die lignien-ryke vastestof (minder as 20%).

Ten slotte het xylitol fraksionerings beter as etileenglikol in terme van die totale herwinning van hemisellulose, lignien en sellulose en die kwaliteit van hemisellulose. Hierteenoor het etileenglikol sellulose van 'n goeie kwaliteit geproduseer. Wanneer hierdie twee poliole met konvensionele organosolv fraksionerings (d.w.s. etanol) vergelyk word, doen eersgenoemde beter in terme van sellulose, hemisellulose en lignien kwaliteit. Dit skiet egter tekort in terme van die komponent opbrengste – veral met etileenglikol fraksionerings.

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This has been one of my most challenging endeavours. It has finally brought me here, with new knowledge and a promising future, I so look forward to.

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In the memory of my beloved grandmother, Meekulu Mukwaanime waKadedu.

For my siblings, Adam, Eben-Ezer, Eden, Immanuel, Nestory, Omega and Onesmus. Despite your hurdles, you have always had the determination to attend school and get education. I dream of the day you all graduate, there can be no greater joy.

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Abbreviates

Abbreviation	Abbreviated word
EG	Ethylene glycol
SCB	Sugarcane bagasse
EC or E. grandis	*Eucalyptus grandis*
HPLC	High Performance Liquid Chromatography
FTIR	Fourier Transform Infrared Resonance
SEC	Size Exclusion Chromatography
EH	Enzymatic hydrolysis
S/G	Syringyl-to-guaiacyl ratio
CI	Crystallinity Index
NREL	National Renewable Energy Laboratory
GC-MS	Gas Chromatograph Mass Spectrometer
CCD	Central Composite Design

Keywords and General definition

Lignocellulose	A group of fibrous dry biomass materials predominantly composed of carbohydrates and lignin polymers.
Cellulose	Polysaccharide of covalently bonded glucose molecules
Hemicellulose	A group of non-cellulose or pectin polysaccharides
Lignin	Polymer of extensive interconnected phenyl propane units
Polymer	A long and complex chain of monomers and oligomeric molecules
Oligomer	A molecule complex build of a limited monomeric units
Monomer	A molecule that can be bonded to similar molecules forming an oligomer or polymer
Biorefinery	Mass integrated chemical and materials production systems using biomass as primary raw material
Lignocellulose Fractionation	Chemical process combined with engineering designs that disintegrate lignocellulose into its main constituents, cellulose, lignin and hemicelluloses.
Polyol	Polyhydric alcohols characterised by having more than one hydroxyl group and associated with high boiling points.

Chapter One

1 INTRODUCTION

1.1 Background and motivation

A number of studies have been carried out on lignocellulose biomass pre-treatments. These investigations into lignocellulose biomass pre-treatments are predominantly owing to the increasing demand for fuels such as ethanol, butanol and methanol and also partly due to the demand for greener fuels and clean production systems. Biomass generated fuels are used as is or blended with conventional fuels such as petrol or diesel (Hendriks & Zeeman, 2009; Leibbrandt, 2010; Xuan Li, 2010; Pérez, Muñoz-Dorado, de la Rubia, & Martínez, 2002). Lignocellulose pre-treatment methods towards the production of these essential fuels are well established, with continuous research into processes that will work towards better efficiency, reduced production costs, high yields, less energy input and ultimately the conversion of all fuel yielding biomass components into fuel, for instance 100% conversion of carbohydrates into biofuels.

Lignocellulose pre-treatment research focus has persistently been on biofuels. Therefore the demand to produce biofuels from lignocellulose comes at the expense of other components of lignocellulose. Most pre-treatment methods are aimed at maximizing digestibility and recovery of glucose from the cellulose rich solid that remains after the pretreatment process. A similar concept is used in the paper making industry, the pulping process aims at removing as much lignin from the lignocellulose structure to enhance the cellulose. In the process, hemicelluloses are either destroyed or dissolved in the spent liquor which is considered waste. These approaches do not give as much regard to other constituents of lignocellulosic biomass, i.e. hemicellulose, lignin and extractive components. In essence, the pre-treatment approach aims at value addition of only a proportion of lignocellulose. For most lignocellulose materials, cellulose represents an average of 30-45% of the overall material; it would be interesting to explore potential uses of the other proportion.

Derivatization of lignocellulosic materials into chemicals, materials and value added products other than fuels have enabled lignocellulose fractionation as a better approach which considers all elements of the lignocellulosic structure as useful in a biorefinery. Lignocellulosic materials are fractionated to isolate its major components; lignin, hemicellulose and cellulose into their individual streams. This is achieved

through a well-designed combination of solvent(s), temperature, pressure and residence times. Some studies have employed catalysts to improve their fractionations (Area, Felissia, & Vallejos, 2009). Ultimately the goal is to separate these components into individual streams to get good yield and quality of each (Diedericks, van Rensburg, & Görgens, 2012). The streams can then be further processed into value added products of choice, for instance cellulose can be hydrolyzed with enzymes into fermentable sugars (saccharification) for conversion to alcohols, organic acids and hydrocarbons. Hemicellulose can be converted into hemicellulose polymer derivatives such as composites, packaging and paper additives and lignin into polymers and carboxylic acids.

Various fractionation and treatment methods have been applied to lignocellulose such as organosolv, alkalis, acid and ionic liquids (Diedericks, 2013; Moghaddam et al., 2014; Peng, Peng, Xu, & Sun, 2012). Alkaline solvents utilizes lower pressures and temperatures to fractionate lignocellulose materials (Kumar, Barrett, Delwiche, & Stroeve, 2009a), however at longer fractionation times in the order of hours or days (Mohammed, 2012; Mosier et al., 2005). Alkaline solvents works on the basis of delignification and deacetylation to effect fractionation (Kumar, Barrett, Delwiche, & Stroeve, 2009b). When compared with acidic solvents in a fractionation process, alkaline solvents are known to cause less degradation of sugars (Kumar et al., 2009b; Mohammed, 2012; Mosier et al., 2005). Furthermore, the salts can be regenerated or recovered from the fractionation liquor. Some of commonly used basic salts are sodium, calcium, potassium and ammonium hydroxides (Kumar et al., 2009b).

Acids have also been explored for fractionation and pretreatment processes based on their effect of breaking down hydronium ions, intermolecular and intramolecular bonds of cellulose, hemicellulose and lignin (Guo, Fang, Xu, & Smith, 2012). Concentrated or diluted acid can be used in the process with minimum dissolution of cellulose. Commonly used acids are sulfuric acid, phosphoric acid and hydrochloric acid, while organic acids such as maleic acid and fumaric acid have also been explored (Katahira, Sluiter, Schell, & Davis, 2013; Y. Kim, Kreke, & Ladisch, 2013). Drawbacks in using acidic solvents lay in their degradation effect acids have on lignocellulose components. The process is however accompanied by degradation of monomers, challenges of recovering and reusing the acids and corrosion of equipment (Kumar et al., 2009b; Leibbrandt, 2010; Xiu, Zhang, & Shahbazi, 2010).

Ionic liquid fractionations of lignocellulose components have been utilized recently successfully (da Costa Lopes, João, Morais, Bogel-Łukasik, 2013; Fort et al., 2007; Isik, Sardon, & Mecerreyes, 2014; Leskinen, King, Kilpelainen, & Argyropoulos, 2011). Ionic liquid are organic salts with melting temperatures below 100˚C and can be used both as solvents and catalytic reagents in fractionation systems (Guo et al., 2012). Ionic liquids are effective solvents for dissolving cellulose for their effective hydrolysis of hydrogen bonds. Commonly used ionic liquids include 1-butyl-3-methylimidazolium chloride {[BMIM][Cl]} and

3-allyl-1- methylimidazolium chloride {[AMIM][Cl]} (Brandt, Gräsvik, Hallett, & Welton, 2013; Leskinen, Kelley, & Argyropoulos, 2015). Drawbacks of using ionic liquids is their reported toxicity and biodegradability (Gírio et al., 2010).

Organosolv fractionations involves the use of aqueous-organic solvents or pure organic solvents such as ethanol and methanol (Peng et al., 2012) sometimes assisted with a catalyst (Kumar et al., 2009b). The technique yields a cellulose rich pulp and a liquor concentrate of hemicelluloses, lignin and smaller proportions of cellulose. The reaction route of the procedure is based on hydrolysis of hemicelluloses and delignification (Moghaddam et al., 2014). Organosolv is known to produce hemicellulose biopolymers, cellulose with low lignin contamination and highly branched lignin (Romani, Ruiz, Pereira, & Teixeira, 2013). Additionally, organic solvent recyclability helps to reduce process costs (Romani et al., 2013; vom Stein et al., 2011). Alkaline solvents are also used to fractionate lignocellulose based on hydrolysis (saponification) of intermolecular ester-lignin bonds, releasing lignin into the hydrolysate while a portion of hemicelluloses is also dissolved along (Rabetafika, Bchir, Blecker, Paquot, & Wathelet, 2014). The conditions of hydrolysis such as temperature, time, solvent type and concentration determine the amount of hemicellulose or lignin dissolved (Harmsen, Huijgen, López, & Bakker, 2010). Fractionation with alkaline solvents sodium carbonate, sodium hydroxide and ammonia has been reported (Harmsen, Huijgen, López, & Bakker, 2010). Acids have also been widely used in fractionation of lignocellulose materials. Common acids utilized are sulfuric acid and phosphoric acid (Lavarack, Griffin, & Rodman, 2002; Vilcocq, Castilho, Carvalheiro, & Duarte, 2014). Acid fractionations (dilute or concentrated) are associated with high dissolution of hemicelluloses (up to >80%) which are mostly recovered as monomers (Diedericks et al., 2012). Other fractionation approaches have included mechanical treatment to simplify the subsequent treatment with solvents by increasing reaction surface area of lignocellulose materials (Inoue, Yano, Endo, Sakaki, & Sawayama, 2008; Moxley, Zhu, & Zhang, 2008; Sun, 2009).

Fractionation treatments do not always fractionate lignocellulose components in the required yields or quality. Fractionation treatments differ in the nature of their application, the type of lignocellulosic biomass treated and the desired end product. Furthermore, a desirable fractionation method would be one that recovers all components in good yields and quality. Hemicelluloses in polymeric and oligomeric form are highly sought after materials in the pharmaceutical industry and for various other applications (Brienzo, Siqueira, & Milagres, 2009; Peng et al., 2012); therefore ideal fractionation processes should preserve hemicellulose polymers. In order to preserve and recover hemicelluloses in polymeric form the choice of fractionation method is thus dictated partly by reactivity of the solvent with hemicelluloses in the material. This is because hemicelluloses are more thermally unstable than cellulose and lignin (Hendriks & Zeeman, 2009). Its biopolymers and oligomeric sugars are easily reduced to monomers in the presence of particularly acidic conditions (Jacobsen & Wyman, 2000; Vilcocq, Castilho, Carvalheiro, & Duarte, 2014), even at dilute acid concentrations (Dussán, Silva, Moraes, Priscila, & Felipe, 2014) or at

temperatures as low as 80 °C, hemicellulose is still recovered mainly as monomers (vom Stein et al., 2011). Degradation of all three components is even more evident with longer fractionation times, for instance longer than 4 hours (Carà et al., 2013; Ma et al., 2014). Target applications for cellulose as mentioned earlier, i.e. conversion to alcohols, can only be achieved if cellulose is also fractionated in good yield, i.e. recovery above 80% of original raw material cellulose and good quality, i.e. cellulose enzymatic digestibilities above 80%. Cellulose degradation is also eminent at high temperatures (Area et al., 2009; Deng, Zhang, & Wang, 2014; Yoon, Han, & Shin, 2014). Similarly, lignin polymers ideally should not be too degraded or defragmented after the fractionation process for its industrial applications.

Hemicellulose polymers, high yields (>80% of original) and good quality of cellulose and less degraded lignin can be produced from lignocellulose materials through a combination of hemicellulose pre-extraction using alkaline solvents (Peng et al., 2012) and further fractionation of cellulose and lignin with organic solvents. In this study, the biorefinery concept is applied through fractionation of two local feedstock, sugarcane bagasse and *Eucalyptus grandis* using polyol solvents at alkaline conditions. Biorefinery concept is defined in the context of an integrated bioenergy (multi-biofuel, heat, power) and bio-based products (food, feed, materials and value added chemicals) production systems that utilize renewable bio-based sources or feedstock to produce these end products (Xiu et al., 2010). A biorefinery maximizes utilization of all components from the feedstock including their intermediaries to ensure little or no waste is generated from the production systems for efficient and sustainable production. In South Africa, sugarcane bagasse and *E. grandis* chips are some of the streams produced from sugar, paper and pulp mills. Sugarcane bagasse is therefore burned to heat boilers and generate supplementary electricity (M. Kim & Day, 2011). Eucalyptus wood chips from the pulping and timber industries are also treated similarly, burnt to produce heat or electricity (Romani et al., 2013) and because these materials are produced in large volumes, they present an opportunity for alternative use such as conversion into value added products and materials. The study was done at moderate conditions and optimized to achieve the best yield and good quality cellulose, hemicellulose and lignin from sugarcane bagasse and *Eucalyptus grandis*.

Chapter Two

2 FROM LIGNOCELLULOSE BIOMASS TO VALUE ADDED CHEMICALS AND MATERIALS, A LITERATURE STUDY

2.1 Lignocellulosic biomass structure

It is imperative to understand the nature of lignocellulose materials in terms of their physical and chemical structure and also the orientation in their lignocellulose complex. This understanding enables one to design a robust fractionation method that will optimize the end products qualitatively and quantitatively.

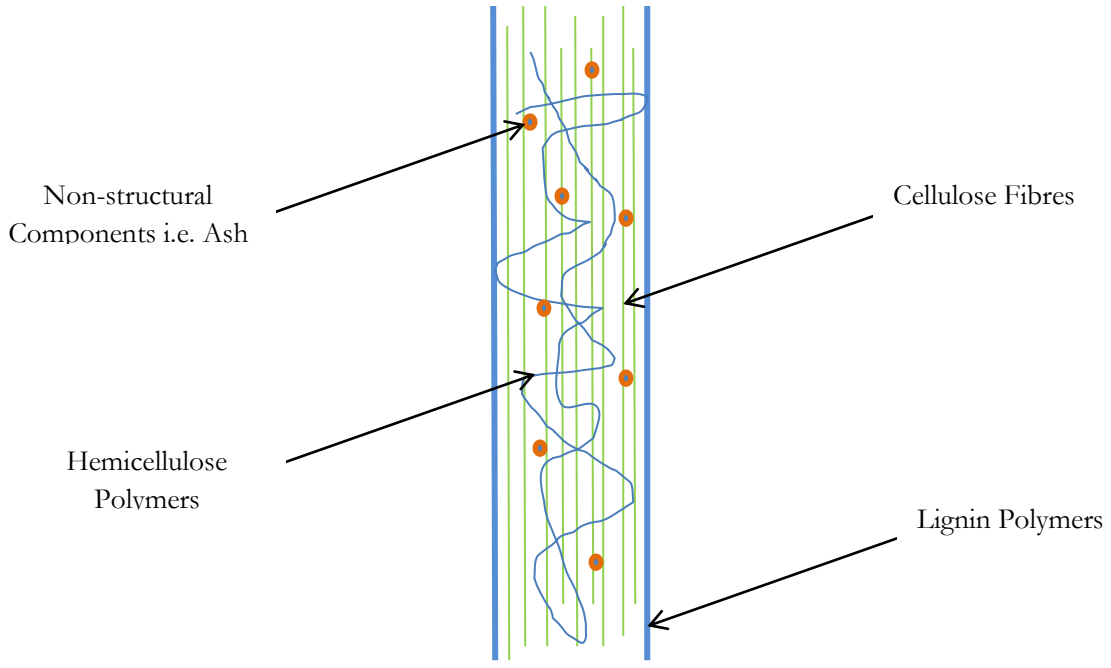


Figure 1: Spatial arrangement of components in the cell walls of lignocellulosic biomass[1] (reproduced from Harmsen, Huijgen, López, & Bakker, 2010)

[1]The colors used in this diagram do not necessary reflect the colors of individual components in real nature, it's for illustrations only

Essentially, the structure of lignocellulose dictates the combination of temperature, catalyst, or the type of disruption that has to be applied, in order to affect the desired product outcomes, because of the differing nature of the individual components. As illustrated in Figure 1, lignocellulosic biomass is typically comprised of both structural (carbohydrates, lignin) and non-structural (ash, waxes, water and alcohol extractives) components (Diedericks et al., 2012; M. Kim & Day, 2011; Pérez et al., 2002). Furthermore, extractives are an extensive mixture of other minor components such as sugars, terpenoid compounds, and monolignols (Davison, Parks, Davis, & Donohoe, 2013). The structural carbohydrates (cellulose and hemicelluloses) are polymeric units consisting of five to six carbon sugar building blocks while lignin is a phenolic polymer. Lignin phenolic structures form center points onto which other carbon chains branch from. Lignin is also considered as the most rigid of the three major components due to its highly branched structure (Leskinen, King, & Argyropoulos, 2013) which contributes to recalcitrance of lignocellulose and fractionation by solvents.

Table 1: Chemical composition (g/100g) of various lignocellulosic materials

Feedstock	Cellulose (g/100g)	Hemicellulose (g/100g)	Lignin (g/100g)	Feedstock Source[2]	Reference
Sugarcane bagasse	38.59	17.79	27.89	Arés, Brazil	(Guilherme, Dantas, Santos, Fernandes, & Macedo, 2015)
Sugarcane bagasse	45.28	22.10	22.39	Guangxi, China	(Yao, Nie, Yuan, Wang, & Qin, 2015)
Sugarcane bagasse	39.10	24.10	18.9	Malelane, South Africa	(Diedericks et al., 2012)
E. grandis	46.16	14.60	27.72	Gondwana, South Africa	(Postma, 2012)
E. grandis	44.65	15.23	25.77	Telemaco Borba, Brazil	(Emmel, Mathias, Wypych, & Ramos, 2003)
Eucalyptus globulus	44.99	16.00	27.65	Pontevedra, Spain	(Romani et al., 2013)
Eucalyptus globulus	45.60	17.50	26.20	Biobío, Chile	(Castro et al., 2013)

Chemical composition of lignocellulose biomass vary in terms of chemical and structural composition as presented in Tables 1-4, depending on a set of factors such as plant species, type, age and the region of growth (Davison et al., 2013). Raw lignocellulose materials' lignin, hemicellulose and cellulose values can

[2] The materials' chemical composition were also analysed at laboratories in these respective countries.

also vary due to the type of analysis method applied or the laboratory where the analysis is done as observed in Table 1. Therefore, sugarcane bagasse and *E. grandis* samples studied in this work required comprehensive analysis to determine their chemical composition before the fractionation processes.
Chemical composition of lignocellulose biomass vary in terms of chemical and structural composition as presented in Tables 1-4, depending on a set of factors such as plant species, type, age and the region of growth (Davison et al., 2013). Raw lignocellulose materials' lignin, hemicellulose and cellulose values can also vary due to the type of analysis method applied or the laboratory where the analysis is done as observed in Table 1. Therefore, sugarcane bagasse and *E. grandis* samples studied in this work required comprehensive analysis to determine their chemical composition before the fractionation processes.

Age of the tree at the time of harvesting or in the case of eucalyptus or stage of harvesting of the sugarcane bagasse is critical for fractionation processes because the composition of lignocellulose change with age as demonstrated in the studies of Miranda and Pereira, (2002) presented in Table 2.

Table 2: Chemical composition of *Eucalyptus globulus* wood chips from trees aged 2, 3 and 6 years (Miranda & Pereira, 2002)

	Age of *Eucalyptus globulus* at harvesting		
	2 years	3 years	6 years
Ash (g/100g)	0.8	1.7	0.6
Extractives (g/100g)			
Dichromate	0.4	0.8	0.5
Ethanol	1.8	3.1	1.8
Water	2.1	4	1.9
Total	4.2	7.9	4.2
Lignin (g/100g)			
Soluble	4.6	2.9	2.2
Klason	22.3	25.7	25.1
Total	26.9	28.6	27.3
Carbohydrates (g/100g)			
Glucose	50.0	40.2	43.8
Xylan	18.6	15.4	19.8
Total	68.6	55.6	63.6

Furthermore, composition of lignocellulose materials is also influenced by the plant specie and cell wall composition as demonstrated by Jansson, Näsman, & Francisco, (2013) in their study of eucalyptus species grown in Uruguay, results presented in Table 3, this variation is related to the genetic composition of each plant specie (Pauly et al., 2013). However, the variation does not appear to be significant as observed in Table 3.

Table 3: Chemical compositions of eucalyptus species grown in Uruguay (Jansson, Näsman, & Francisco, 2013)

Wood Specie	Ethanol Extractives (g/100g)	Lignin (g/100g)	Cellulose (g/100g)	Hemicelluloses (g/100g)	Ash (g/100g)
E.bicostata	2.1	32.6	37.0	16.7	0.6
E.dunii	1.3	28.1	41.8	17.3	0.8
E.globulus	1.1	28.9	40.7	18.2	0.4
E.grandis	1.7	30.4	42.3	15.3	0.4
E.maidenii	1.7	30.4	40.2	16.8	0.5

Table 4: Chemical and structural composition of lignin, hemicellulose, and lignin in lignocelluloses (Chen, 2014).

	Lignin	Hemicellulose	Cellulose
Subunits	Guaiacylpropane (G), syringylpropane (S), phydroxyphenylpropane (H)	D-Xylose, mannose, L-arabinose, galactose, glucuronic acid	D-Pyran glucose units
Bonds between the subunits	Various ether bonds and carbon-carbon bond, mainly β-O-4 ether bond	β-1,4-Glycosidic bonds in main chains; β-1.2-, β-1.3-, β-1.6-glycosidic bonds in side chains	β-1,4-Glycosidic bonds
Polymerization	4000	Less than 200	Several hundred to tens of thousands
Polymer	G lignin, GS lignin, GSH lignin	Polyxylose, galactoglucomannan, (Gal-Glu-Man), glucomannan (Glu-Man)	β-Glucan
Composition	Amorphous, inhomogeneous, nonlinear three-dimensional polymer	Three-dimensional inhomogeneous molecular with a small crystalline region	Three-dimensional linear molecular composed of the crystalline region and the amorphous region
Bonds between three components	Contain chemical bond with hemicellulose	Contains chemical bond with lignin	Without chemical bond

2.1.1 Cellulose

Cellulose is the most abundant wood polymer (Yoon et al., 2014). It is commonly known for its use in production of paper and pulp products (Bose, Francis, Govender, Bush, & Spark, 2009; Brandt et al., 2013). Cellulose appears in two structural forms in lignocellulose either crystalline or amorphous (Hendriks & Zeeman, 2009). Crystalline cellulose forms lateral structures held together by hydrogen bonds. Moreover, crystalline cellulose comprise 50–90% of celluloses in most lignocellulose materials (Jacobsen & Wyman, 2000). Crystalline cellulose significantly contributes to lignocellulose recalcitrance (Davison et al., 2013). The less structured amorphous cellulose makes up the other small proportion of cellulose and which is more susceptible to hydrolysis by solvents and enzymes (Kumar et al., 2009b).

Cellulose fibrils consist of glucose polysaccharides bound together by β-1, 4-glycosidic bonds. These independent and elongated fibrils are often connected by hydrogen and van der Waals bonding (Arantes & Saddler, 2010). Cellulose is tightly enclosed in a hemicellulose and lignin bracket, it is this arrangement which makes it difficult for cellulose accessibility by solvents or enzymes in lignocellulose materials. Cellulose intermolecular and intramolecular bonds are broken at varying degrees by several solvents such as acids, ionic liquids and alkaline solvents (Castro et al., 2013; Pérez et al., 2002). Isolated cellulose is dissolved by organic solvents such as ethanol and acetone (Carvalheiro, Duarte, & Gírio, 2008; Peng et al., 2012). In addition, cellulose thermal degradation is noted to begin at temperatures between 21-220 °C (Peng et al., 2012) and even more pronounced at higher temperatures. Isolation of cellulose from lignocellulose materials is possible through hydrolysis of its β-1, 4-Glycosidic bonds, hydrogen and van der Waals bonding to hemicelluloses and lignin. Further hydrolysis can lead to degradation of its subunits into degradation products such as furfurals and 5-hydroxymethylfurfural (Zhou, Xia, Lin, Tong, & Beltramini, 2011).

When isolated from the lignocellulose structure, cellulose polymers have various applications. The most common application being its conversion to fuel through saccharification (Arantes & Saddler, 2010; Jian, Meiqiang, & Gu, 2013; Winkler, 1981). This is largely so because of increasing interest in bio-based fuels which are considered more environmental friendly than fossil generated fuels. Other applications of cellulose include its use as a fat replacer, food stabilizer, wood composite and in the medical field it is used as a binder or filler for tablets (Terinte, Ibbett, & Schuster, 2011).

2.1.2 Hemicellulose

Hemicellulose is the second most abundant renewable carbohydrate polymers in the world (Peng et al., 2012), but until 1961 scientists were still unsure of what to call an interlinked non-cellulose polymer found in the seeds of *Tamarindzcs indica*. In his investigations Kooiman (1961) reported that the polymer

was water soluble and ethanol insoluble composed of xylose and cellobiose. In addition, Hemicelluloses did not have a particular polymeric structure, but rather comprised a group of polysaccharides characterised by being neither cellulose nor pectin having β-(1→4)-linked backbones of glucose, mannose or xylose, and dissolved easily in chaotropic agents such as alkalis (Pauly et al., 2013; Scheller & Ulvskov, 2010). Hemicellulose polymers just like cellulose consist of monomeric sugar units bonded by glycosidic bonds. However, hemicellulose polymers are much shorter, branched and substituted compared to cellulose and therefore usually non-crystalline (Kirk, 1983). In nature, the role of hemicelluloses is to chain cellulose microfibrils, strengthening the plant and its walls (Scheller & Ulvskov, 2010).

Hemicellulose polymers and oligomers have many industrial applications as presented in Figure 2 owing to their extended functional groups, reactivity and ease of chemical modifications through reactions such as alkylation, cross-linking and sulfonation in order to produce value added materials (Deutschmann & Dekker, 2012). Applications of hemicellulose polymers include food processing, pharmaceuticals and cosmetics. Film forming properties of xylan is used in the production of biopolymeric films used in both edible and biodegradable packaging materials (Carvalheiro et al., 2008). Additionally, hemicellulose polymers are known to have a water absorbing functionality required in food production and pharmaceutical recipes, as an example xylan used in bread making to decreasing syneresis and retrogradation of dough (Sedlmeyer, 2011). When combined with clays such as montmorillonite, hemicellulose polymer biocomposites are used in formulations of cosmetics as cleansing agents and as thickeners (Sedlmeyer, 2011). Other material applications of hemicellulose polymers includes their use in production of foams and plastics (Deutschmann & Dekker, 2012).

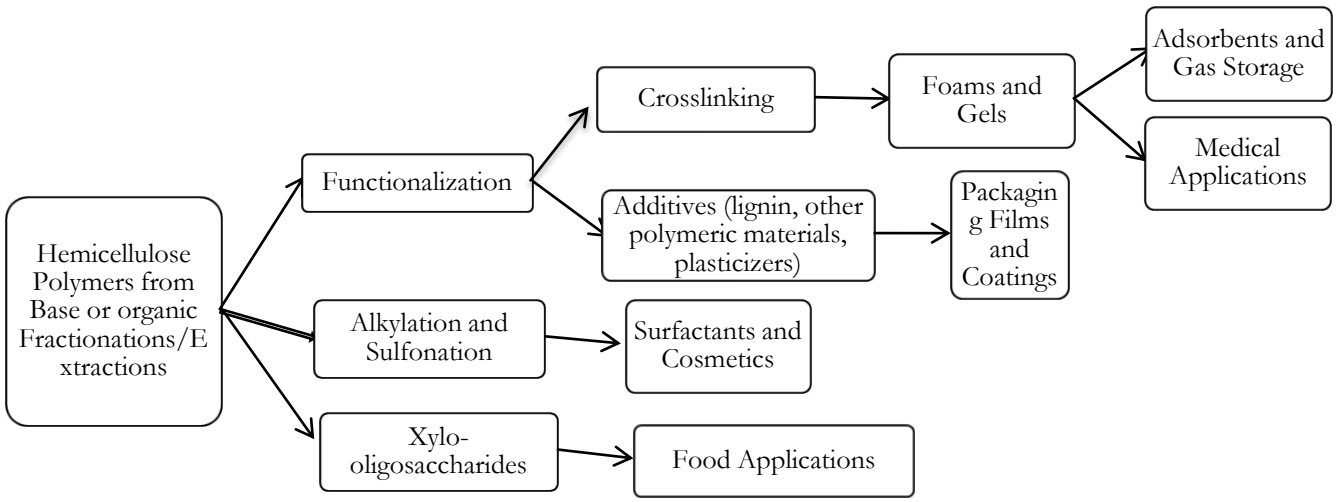


Figure 2: Pathways towards Hemicellulose Polymer based value added products and materials, adapted with modifications from Deutschmann & Dekker, 2012.

2.1.3 Lignin

Lignin is complex organic polymer which does not have a primary structure like the carbohydrates, it is heterogeneous and amorphous. Its structure is three dimensional consisting of three primary phenylpropane units (monolignol units), namely; coniferyl, sinapyl and p-coumaryl alcohol (Hendriks & Zeeman, 2009; Xuan Li, 2010; Pérez et al., 2002). When these three monolignols polymerise into a heterogeneous macromolecule (phenylpropanoids) in various proportions, three types of lignin polymer building units are distinguished;

1. Guaiacyl lignin (G), characterized by two lignin monomers, namely trace sinapyl alcohol units and mainly coniferyl alcohol, this lignin phenylpropanoid is common in mainly softwood and varying proportions in hardwoods (Aldaeus, Schweinebarth, Törngren, & Jacobs, 2011; Gírio et al., 2010).
2. Syringyl lignin (S), comprise two monolignols namely, coniferyl and sinapyl alcohol in different proportions, equally common in lignins of hardwoods with guaiacyl lignin (Rodrigues, Meier, Faix, & Pereira, 1999a; Yoo, 2012)
3. p-hydroxyphenyl (H) is mainly build of p-coumaryl alcohol common for lignin in grasses (Kirk, 1983).

Units of lignin partially enclose carbohydrates in the plant cell walls providing elastic and mechanical support to the plant and also facilitating transport of nutrients and water (Davison et al., 2013). Lignin is considered one of the contributors to lignocellulose recalcitrance (Hou, Li, & Zong, 2013; Palonen, 2004) and also an undesirable component in the paper making industry, thus removed by the pulping and bleaching process (Adler, 1977).

Lignin classification is dependent on its chemical structures and composition (Zhao, Zhang, 2012). Because it is not possible to isolate lignin in its natural form, technical (extracted) lignin is commonly identified by the type of process with which it was isolated from lignocellulose materials. Common lignins are kraft lignin and soda lignin (Moghaddam et al., 2014), lignosulphonates, organosolv lignin, hydrolysis lignin and ionic liquid lignin (Agrawal, Kaushik, & Biswas, 2014). The process of lignin isolation alters the chemical structures of native lignin; hence lignin from these processes differs in structure, functional group composition, molecular weight, type and composition of monomer units.

Applications of lignin vary depending on its chemical and physical properties after fractionation (Zhao, Zhang, 2012). Lignin has many functional groups which makes it reactive and responsive to a variety of chemical derivations. Reported functional groups of lignin include methoxyl groups, phenyl hydroxyl, benzyl hydroxyl, carbonyl and various others on the side chains such as aldehyde groups (Moghaddam et al., 2014). This functionality makes lignin a high potential precursor for value added materials and

chemicals in the biorefinery concept. Lignin derivatives are widely used in primary intermediate materials such the making of carbon fiber and adhesives (Peng et al., 2009) while end products, motor fuel, vanillin, sorbent and surfactants also have pathways from lignin (Agrawal et al., 2014).

2.2 Lignocellulose Fractionation: from raw materials to value added chemicals and materials

2.2.1 The conventional fractionation approach

Fractionation of lignocellulose is the isolation of its three main components; cellulose, hemicellulose and lignin. These components are separated into three streams each having an acceptable yield (compared to raw material inputs) and characteristic properties as per the intended downstream applications. These end products can be processed further into other materials, for example the cellulose stream is hydrolysed into monomeric sugars which are subsequently fermented into biofuels. Hemicelluloses stream can also be raw material for the synthesis of cationic polymers, thermoplastic xylan derivatives, hydrogels (Peng et al., 2009) and as natural barrier for packaging films (Brienzo et al., 2009)

Fractionation is achieved through application of an appropriate combination of reaction conditions, for instance; temperature, residence time, solvent, catalyst or no catalyst, and pressure. Fractionation procedures are carried out in at least two steps to obtain three product streams, more steps can be added depending on desired product outcomes. In the first step, a solvent would selectively dissolve one of the three components, for instance hemicellulose is dissolved with the remainder of the solid residue highly rich in cellulose and lignin. A second step would then be required to further isolate the lignin from cellulose. Alternatively, the first step would dissolve lignin and hemicellulose, and the solid pulp remainder would be rich in cellulose. The second step following this would be one that separates lignin and hemicellulose.

Whereas a single step fractionation step is also possible, product separation becomes a challenge, according to vom Stein et al., (2011), managing to fractionate beech wood in a one step at mild temperatures of 80–140 °C. The process was carried out in a biphasic system, in which two solvents, aqueous oxalic acid and 2-methyltetrahydrofuran, were injected into the reactor with the substrate at once. The end results were three streams; a cellulose rich solid, a hemicellulose aqueous fraction and a lignin dissolved organic fraction. Although their hemicelluloses were not recovered as polymers because of the use of oxalic acid, their approach benefitted from recovery and potential reuse of the oxalic acid and 2-methyltetrahydrofuran solvent.

One of the focus areas for this study is recovery of the hemicellulose component in polymeric/oligomeric form. As emphasized earlier, this requires application of appropriate and mild fractionation conditions since hemicellulose bonds are highly susceptible to hydrolysis and degradation during solvent treatment. Hemicellulose molecules are held together by weak bonds such as esters, which is comprised of an acetyl group bonded with a hydroxyl group, and has an irreversible hydrolysis reaction in the presence of acid is solvents or catalysts (Harmsen, Huijgen, Bermudez, & Bakker, 2010). A variety of dilute and concentrated acidic solvents are thus able to hydrolyse hemicellulose into monomers and oligomers. Therefore, for the purpose of this study and for achieving polymeric hemicelluloses as products from this fractionation design, acidic fractionation conditions are avoided. Hemicellulose polymers or oligosaccharides are raw material commodities in the food and pharmaceutical industries. Current volumes of value added products generated from hemicellulose oligosaccharides and polymers is surpassed by hemicellulose monosaccharide-derived products (Vilcocq et al., 2014). From literature reports, it appears there are a few studies carried out on the fractionation of lignocellulose with the aim to recover hemicelluloses as poly/oligosaccharides, together with separate product streams for lignin and cellulose. The primary goal of this study is a fractionation process that achieves close to a pure stream of hemicellulose polymers and oligomers, with simultaneous high yields and quality cellulose and lignin. Therefore, all fractionation methods that generate acidic reaction conditions or their design are of high severity which results in production of monomers are not suitable for the purpose of this study. These methods include, but may not be limited to fractionation methods which utilise acid catalysts, concentrated and dilute acidic solvents, liquid hot water (LHW), ammonia fibre explosion (AFEX), carbon dioxide explosion and oxidative methods such as wet oxidation and ozonolysis (Harmsen et al., 2010).

Other greener fractionation solvents considered for oligosaccharides production are ionic liquids but mostly for their selective lignin or cellulose dissolution abilities at temperatures as low as room temperature (Harmsen et al., 2010). Ionic liquids have been considered as solvents for sugarcane bagasse fractionation as reported in literature (Diedericks et al., 2012; Leskinen et al., 2013; Leskinen, King, Kilpelainen, & Argyropoulos, 2011). Most of them are neutral and achieve good fractionation yields at low temperatures and usually do not require catalysts. A most recent fractionation study was done by Hou, Li, & Zong, 2013 using a mixture of chlolinium amino acid ionic liquid and water at 90°C achieving more than 80% recovery of carbohydrates after 12 hours.

Production of hemicellulose polymers from ionic liquid fractionations is possible when coupled with hemicellulose alkaline extraction. By working with 5-100% w/w ionic liquid, 90-190 °C and 0.5-22 hours of fractionation time hemicellulose polymers are produced from *E. grandis* and sugarcane bagasse (Makhetha, 2016) in addition to highly digestible cellulose and good quality lignin. At severe conditions

(high temperatures and longer residence times) which favour lignin dissolution, hemicellulose polymers are hydrolysed to monomers (Makhetha, 2016) which is undesirable.

Besides the ability to yield hemicellulose polymers, ionic liquids are associated with side reactions such as esterification which affects the quality of the three components, particularly cellulose (Makhetha, 2016). Additionally, ionic liquids are quite expensive solvents (Wen, Sun, Yuan, & Sun, 2015) which is a challenge for up-scaling. While some ionic liquids are biodegradable (Socha, Plummer, Stavila, Simmons, & Singh, 2013) others are considered toxic, corrosive, hygroscopic and not biodegradable (Jian et al., 2013) although recyclable. Another concern for ionic liquids is their reported formation of cellulose gels, formed when cellulose dissolved by the ionic liquid is being recovered by an organic anti-solvent such as acetone (Jian et al., 2013), making the separation another challenge for fractionation.

2.2.2 Organosolv Fractionation: Polyols as potential solvents for fractionations

The organosolv approach to fractionation (Figure 3) and pretreatment is widely reviewed (Area et al., 2009; Brudecki, Cybulska, & Rosentrater, 2013; Castro et al., 2013; Harmsen et al., 2010). Organic solvents are employed to effect the fractionation of lignocellulose materials. Commonly used solvents include low boiling point alcohols such as acetone, ethanol and methanol (Gírio et al., 2010). Generally organosolv fractionations operates at up to 210°C depending on the solvent being used and other operating conditions (Gírio et al., 2010) due to high volatility of monoalcohols. Pressures of up to 30 Mpa may be required to contain solvent evaporation (Makhetha, 2016), especially for highly volatile solvents (low boiling alcohols). Fractionation with high boiling point alcohols may not necessarily require the use of pressure if the process does not exceed the boiling point of the alcohol in use. For instance, Sun et al., (2007) treated wheat straw with glycerol (boiling point 290 °C) at 240 °C recovering 95% of raw material cellulose while dissolving >70% (wt. %) lignin and >90% (wt. %) hemicelluloses (polymerization of hemicelluloses not specified). As shown in Figure 3, when lignocellulose materials are treated with an organic solvent the reaction produce two fractions, a solid residue rich in cellulose and a liquid mixture of mainly lignin and hemicelluloses (Oliveira et al., 2013).

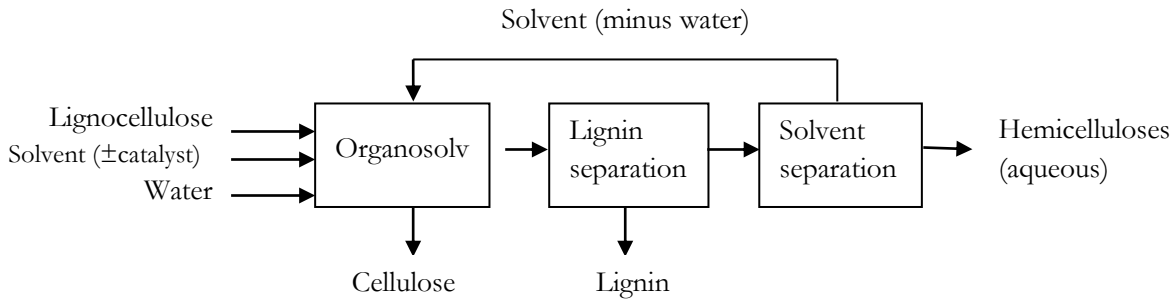


Figure 3: Simplified organosolv fractionation scheme (reproduced from Harmsen, et al., 2010).

The chemistry of organosolv fractionation is twofold; (1) selective dissolution of lignin and partly hemicelluloses (extend of dissolution depends on reaction conditions) and (2) preservation of cellulose in the solid residue (minimal dissolution of cellulose depending on conditions). Cellulose is insoluble in most organic solvents due to its crystalline structure. The interaction of mild organic solvents such as ethanol, methanol and ethylene glycol on cellulose is physical, limited to swelling of the macrostructure (Hendriks & Zeeman, 2009; Kumar et al., 2009b; Menon & Rao, 2012). However, dissolution of cellulose is activated by higher temperatures, use of selective catalysts and strong organic solvents such as pyridine, toluene and ionic liquids (Fort et al., 2007). Cellulose dissolution from the lignocellulose structure in the presence of strong organic solvents is preceded by swelling of the fibres, the swelling and stretching stress of the intramolecular and intermolecular bonds then results in the partial collapse of cellulose structure (dissolution).

Unlike the action of mild organic solvents on cellulose (swelling, minimal dissolution) hemicellulose and lignin are actively involved in chemical reactions with organic solvents, a process which results in dissolution of the two components at varying degrees (organosolv fractionation). Hemicellulose is dissolved from the lignocellulose structure by organic solvents through hydrolysis of its glycosidic bonds leaving enlarged pores in the cell walls of the lignocellulose structure (Perez & Curvelo, 2010), this is usually the first step of the organosolv process. Hydrolysis of hemicellulose in this organosolv step is also associated with partial removal and substitution of uronic acid and the acetyl group (Carvalheiro et al., 2008). Following partial hemicellulose removal from the lignocellulose structure is delignification (dissolution) of lignin polymers (further hemicellulose and cellulose dissolution can also happen subsequently) through the cleavage of *a*-O-4 and *β*-O-4 bonds with carbohydrates, resulting in immediate dissolution of lignin (Tejado, Peña, Labidi, Echeverria, & Mondragon, 2007). This was confirmed in the study of physio-chemical properties of organosolv lignin by Tejado et al., (2007) confirming the presence non-etherified phenolic hydroxyl groups (produced from cleaving of aryl-ether bonds) as visible in the infrared spectrums at 1365 cm^{-1}.

The use of catalysts to enhance organosolv fraction is reported in the literature as presented in Table 5. Depending on desired selectivity of the fractionation i.e. to produce oligomeric hemicelluloses, catalysts are used to enhance both lignin and hemicellulose removal from the structure of lignocellulose while cellulose is preserved in the solid residue. Mineral acids i.e., phosphoric, hydrochloric and sulphuric) and organic acids (i.e., oxalic, salicylic, formic and acetylsalicylic) can be used to enhance hemicellulose dissolution delignification (Zhao et al., 2009). As mentioned earlier, the use of acid catalyst creates an acidic reaction medium which promotes hydrolysis of hemicellulose to monosaccharides. Therefore, alkaline conditions are most preferable for production of hemicellulose oligomers and polymers whilst the quality of lignin and cellulose is simultaneously maintained (Gírio et al., 2010). Therefore, in order to recover polymeric hemicelluloses and good recoveries of cellulose and lignin, organosolv fractionation must be carried out with a combination of alkaline catalysts and low temperatures to avoid hemicellulose degradation and poor recovery reported with high temperatures.

Table 5: Organosolv pre-treatments of various feedstock (Harmsen et al., 2010)

Reference	Biomass	Remarks	Organic solvent	Catalyst	L/S (% w/w)	Temperature (°C)	Pressure (bar)	Cooking time (h)	Pulp yield (%)	Lignin removal (%)	Cellulose recovery (%)	Hemicellulose removal (%)
(Lee *et al.*, 1986)	Corn stover	Pre-treated with dilute H2SO4.	Methanol, butanol, aromatic alcohols	H_2SO_4	5	160		1		>90		
(Zhang *et al.*, 2007)	Corn stover	Knife-milled/ screened.	H3PO4/ acetone			50	Atmospheric Pressure	0.5 - 1		50	95	79
(O'Connor *et al.*, 2007)	Corn stover	Chopped, pre-soaked	Ethanol	H_2SO_4	6	170		0.5	40	85	92	91
(Carioca *et al.*, 1985)	Elephant grass		Ethanol		3 – 14	180		1 – 3		70	95	90
(Ibrahim *et al.*, 1999)	Oak (red)	After steam pre-treatment	Acetic acid		11	60		1		~60		
(Hasegawa *et al., 2004)*	Oil palm shell wastes/ Apricot tree shell wastes		Acetone			200				High		High
(Black *et al.*, 1994)	Poplar	Chips	Ethanol	H_2SO_4	9	140		1	64			
(Pan *et al.*, 2006)	Poplar	Chopped	Ethanol	H_2SO_4		180		1		74	88	
(Ghose *et al.*, 1983)	Rice straw	Chopped <1cm	Butanol	Organic catalysts		120		2	54	83		
(Kiran *et al.*, 1994)	Spruce (red)	Chipped <7mm, flow-through reactor	Acetic acid			180	250	3	51	93		
(Gonçalves *et al., 2003)*	Sugarcane bagasse		Ethanol		10			1-3	44-52	>75		
(Arora *et al.*, 1990)	Sugarcane bagasse/ Elephant grass		Ethanol	Various catalysts		180						
(Pasquini *et al.*, 2005)	Sugarcane bagasse/ Pine (P. taeda)		Ethanol, acetic acid, methanol, dioxane	sc CO_2		142 - 198	147 – 232	0.5 - 2.5	33 - 44	88 - 93		
(Papatheofanous et al., 1995)	Wheat straw	Pre-treated with acid hydrolysis	Ethanol	H_2SO_4		81		1.5	63	>70	>98	50
(Sun *et al.*, 2007)	Wheat straw		Glycerol		15	240	Atmospheric pressure	4		>70	95	>90
(Arato *et al.*, 2005)	Woody biomass		Ethanol		9 - 20	180-195	30	0.5 - 1.5				

Organosolv fractionation of lignocellulose is also reported with the use of highly branched alcohols such as polyols. There appear to be only a few reported case studies on lignocellulose fractionation using polyols as solvents. Most polyol studies mainly focused on lignocellulose pretreatment for biofuel production and pulping, for instance ethylene glycol (Moghaddam et al., 2014), glycerol carbonate (Zhang, Rackemann, Doherty, & O'Hara, 2013) and propylene carbonate (Zhang, O'Hara, Rackemann, & Doherty, 2013). The advantage of using polyols is their low volatility, which makes them suitable to work with at atmospheric pressure (Brandt et al., 2013). Their high boiling points also make them suitable to design fractionations reactions at low temperatures without surpassing the solvents' boiling points i.e. 197.3 °C for ethylene glycol and 216 °C xylitol. This means polyol fractionation can be carried out safely up to 190 °C, at atmospheric conditions. Zhang et al, 2013 studied fractionation of sugarcane bagasse with glycerol at 130°C under atmospheric conditions with recovery of ≥90% for all the three components (nature of recovered hemicellulose component not specified). Conventional organosolv solvents such as ethanol and methanol are highly volatile, which creates a need for them to be well contained during the process.

In the present study, two polyols are investigated for their lignocellulose fractionation abilities, namely; xylitol with chemical formula $C_5H_{12}O_5$ and ethylene glycol with chemical formula $C_2H_6O_2$. Xylitol is a polyol solid at room temperature with a melting point between 94-97 °C. Xylitol dissolves relatively well in water at 50 mg/ml (Martínez et al., 2015a) and a variety of other solvents including ethanol (Martínez et al., 2015). Xylitol is produced from hemicellulose (Deutschmann & Dekker, 2012; Fatehi, Catalan, & Cave, 2014). In the food industry, xylitol is widely used as a food additive alongside other polyols such as sorbitol and mannitol (Sedlmeyer, 2011). In food, xylitol is considered as a calorie-free sweetener; it is preferred over other glycols such as sorbitol for its ability to act as artificial non-sugar sweetener. But being a polyol, it could be a potential delignifying agent when in solution as other polyols have demonstrated such as glycerol (Romani et al., 2013), ethylene glycol, ethylene carbonate and glycerol carbonate (Zhang, Rackemann, et al., 2013). However, from literature review, no experiment data on fractionation of lignocellulose material using xylitol solutions is reported. Therefore, considering a green process, xylitol as a polyol can be used as a solvent dissolved in water and recovered from the aqueous solution on a large scale. Recrystallization studies of xylitol from solution have been done before with success (Martínez et al., 2015b).

In conclusion, organosolv lignocellulose fraction and treatment process is optimised with most solvents both at laboratory and pilot scale. Moreover if not catalysed by acids, organosolv fractionation has an advantage of high selectivity towards high purity and low molecular weight lignin, oligomeric hemicelluloses and relatively pure cellulose (Harmsen et al., 2010). Other economic considerations for

organosolv fractionations are the non-complicated recovery of solvents. Additionally, based on information gathered from literature, concrete understanding of lignocellulose fractionations specifically sugarcane and *E. grandis*, fractionations identified two additional design gaps and shortcomings in these fractionation processes (1) there are no literature reports which studied fractionation of sugarcane bagasse or *E. grandis* using xylitol as a solvent, (2) there are also no reports on fractionation of sugarcane bagasse or *E. gr*andis using polyols combined with a hemicellulose pre-extraction step using NaOH as pre-extracting solution.

2.2.3 Hemicellulose pre-extraction

Pre-extraction of hemicelluloses is a necessary step prior to fractionation, especially if the fractionation process is known to degrade hemicelluloses (Diedericks et al., 2012). Literature extractions of hemicelluloses are summarised in Table 6. While low pH or acids enhance monomerization or hydrolysis of hemicelluloses (Xuan Li, 2010) alkaline solvents are known to enhance the deacetylation of hemicellulose, cleaving them from lignin only while maintaining the bond between hemicellulose monomers (Halog & Mao, 2011; Palonen, 2004). For this reason, alkaline solvents have been widely used to pre-extract hemicellulose, while lignin is partially solubilised in the process (Peng et al., 2012; Postma, 2012; Vena, 2013; Wyman et al, 2005). Pre-extraction of hemicellulose from the lignocellulose material results in a liquid fraction with high hemicellulose content, some lignin and cellulose and also a solid residue with mainly lignin, cellulose and minor hemicelluloses remaining (Diedericks et al., 2012). The solid residue can then be fractionated further into cellulose and lignin with a solvent of choice (Diedericks et al., 2012).

Previous studies on hemicellulose pre-extraction from sugarcane bagasse used alkaline hydrogen peroxide catalysed by magnesium sulfate to recover 94.5% of hemicelluloses alongside with 88% of lignin, between 4 to 16 hours and temperatures from 20 to 60 °C (Brienzo et al., 2009). Almost pure xylose and xylan rich hemicelluloses were recovered using sodium hydroxide process alone with up to 802.2 g/kg of the original hemicelluloses recovered from pear pomace (Rabetafika et al., 2014). Vena., (2013, carried out hemicellulose extraction studies for *E. grandis* and sugarcane bagasse, comparing extraction by mild alkaline sodium hydroxide and dilute sulphuric acid. The study found that the alkaline process was better as more hemicelluloses were recovered and also as polymeric xylans with up to 69% was recovered from sugarcane bagasse. Vena, (2013) as shown in Table 6 also investigated pre-extraction of bagasse with hot water which recovered 5.7% (wt. %) of the xylo-oligomers.

Table 6: Alkaline fractionation/treatment/hemicellulose pre-extraction of lignocellulose materials

Substrate	Reaction Conditions	Solid yield (%)	Cellulose yield (glucan) (%)	Hemicellulose yield (xylan) (%)	Lignin yield (%)	Cellulose Enzymatic digestibility (%)	Reference
E. grandis	1.5 M NaOH, 1:10 solid-liquid loading, 90 °C, 4 h	78.06	95.63	55.39	26.16	66.09	Makhetha, 2016
E. grandis	1 M NaOH, 1:10 solid-liquid loading, 90 °C, 4 h	0.70	76.25	8.50	8.98	-	Vena, 2013
	2 M NaOH, 1:10 solid-liquid loading, 40 °C, 4 h	0.69	75.00	12.40	8.38	-	
	2 M NaOH, 1:10 solid-liquid loading, 90 °C, 2 h	0.76	81.04	10.30	2.99	-	
	2 M NaOH, 1:10 solid-liquid loading, 90 °C, 4 h	0.63	73.54	16.00	13.17	-	
E. grandis	1 M NaOH, 1:10 solid-liquid loading, 120 °C, 1 h, 105 kPa	73.10	103.69	-	4.09	75.00	Lima et al., 2013
Hybrid *E. grandis* x urophylla	1 M NaOH, 1:10 solid-liquid loading, 120 °C, 1 h, 105 kPa	63.40	100.85	12.35	40.72	100.00	Lima et al., 2013
Eucalyptus residues	1 M NaOH, 1:10 solid-liquid loading, 60 °C, 24 h	83.20	94.02	42.78	17.61	8.00	Park and Kim, 2012
	1 M KOH, 1:10 solid-liquid loading, 60 °C, 24 h	83.50	94.98	36.36	18.60	7.00	
	1 M Na_2CO_3, 1:10 solid-liquid loading, 60 °C, 24 h	93.30	97.61	16.04	8.64	5.20	
	15 % aq. NH_3, 1:10 solid-liquid loading, 60 °C, 24 h	90.10	96.41	17.65	14.29	6.00	
	1 M Na_2CO_3 percolation	79.10	98.33	28.88	21.93	19.00	
E. globulus	2.5 M NaOH, 1:10 solid-liquid loading, 100 °C, 1 h	82.80	97.61	13.75	5.41	-	Júnior et al., 2013
	12.5 M NaOH, 1:10 solid-liquid loading, 100 °C, 1 h	82.80	92.19	33.13	21.24	-	
Sugarcane bagasse	1.5M NaOH, 1:10 solid-liquid loading, 65 °C, 1.53 h	59.31	95.50	71.17	65.72	80.14	Makhetha, 2016
Sugarcane bagasse	1.5M NaOH, 1:10 solid-liquid loading, 65 °C, 1.53 h	69.10	94.60	69.10	18.70	-	Vena, 2013
Sugarcane bagasse	1.25 M NaOH, 1:10 solid-liquid loading, 121 °C, 1 h, 105 kPa	73.10	81.60	96.20	89.10	-	Khuong et al., 2014
	0.25 M NaOH, 1:10 solid-liquid loading, 121 °C, 1 h, 105 kPa	63.00	81.90	65.00	79.50	-	
	0.2 M NaOH, 1:10 solid-liquid loading, 121 °C, 1 h, 105 kPa	83.00	98.30	49.40	63.80	-	

2.3 Research aim and scope

The general aim of the study was to fractionate both sugarcane bagasse and *E. grandis* individually into three streams, namely; cellulose, hemicellulose and lignin respectively according to schematic flow process shown in Figure 4. The fractionation was carried out using two polyol solvents, xylitol and ethylene glycol at moderate conditions of temperature, time and sodium hydroxide as catalyst in a design that achieves both high yield and good quality of products as specified (section 2.3.1). The research experimental runs were designed statistically in order to generate meaningful results for interpretation and comparison to other fractionation solvents and processes.

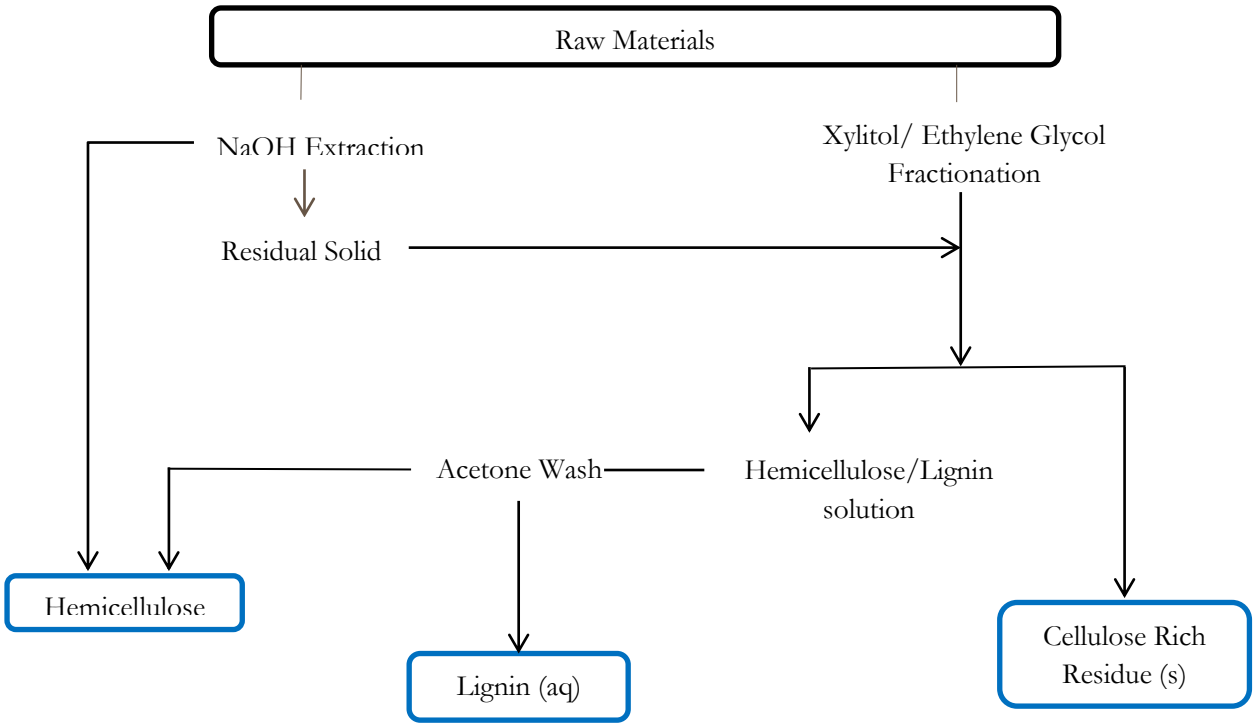


Figure 4: Polyol Fractionation Schematic Flow

For comparison purposes, ethylene glycol and xylitol fractionations are carried out under similar reaction conditions (except solvent concentrations) to establish the efficiency of the xylitol-water fractionation process when compared to ethylene glycol. It is expected that the polyols will delignify the substrates and also dissolve a significant portion of hemicelluloses resulting in a cellulose rich pulp and a liquid mixture of mainly lignin and hemicellulose biopolymers. The liquid fraction is further fragmented using an antisolvent (acetone) to give two distinctive streams of aqueous lignin and crystals of hemicellulose biopolymers at optimum conditions. The antisolvent precipitates hemicelluloses out of the liquid matrix.

Additionally, prior to fractionation, a selected set of experiments are subjected to a hemicellulose pre-extraction step introduced in order to preserve hemicellulose polymers in the case where solvents are too destructive of the hemicellulose component.

2.3.1 Specific objectives identified for the study

The approach to this research was based on four main deliverables for both substrates;

- To retain 80% or more cellulose in the solid fraction. The cellulose should also be enzymatically digestible by more than 80% efficiency.
- Dissolve >80% and recover more than 70% of hemicelluloses in the liquid fraction with subsequent extraction with an anti-solvent at optimum fractionation conditions. Recovered hemicelluloses should also be of polymeric form with minimum molecular weight average of 10 000 $gmol^{-1}$.
- Remove more than 70% lignin from the solid fraction, while maintaining high quality of lignin (carbon content of >30%, syringyl-guaicyl ratio of >1.52 and 3.06 for sugarcane bagasse and *E. grandis* respectively).
- To determine and understand the effect of set fractionation parameters such as temperature, catalyst and solvent concentration and time of retention on the fragmentation of *E. grandis* and sugarcane bagasse into the respective components (lignin, hemicellulose and cellulose) and use these to optimize best fractionation conditions that maximize components' yields and purity.

2.3.2 Statement of novelty

Several studies reported the use of polyols as potential pre-treatment solvents with focus on achieving maximum digestibility for the cellulose component. This study is unique in its approach of fractionating the three components of lignocellulosic sugarcane bagasse and *E. grandis* into individual streams, giving weight to all three components. There has been no report or studies of either pre-treatment or fractionation done using xylitol as a solvent alone or as a solvent coupled with a catalyst. There have also been no reports documented on pre-extracting hemicellulose from these two substrates prior to xylitol or ethylene glycol fractionation. Findings from this study bring a completely novel dimension to fractionation studies and addition to existing knowledge on polyol solvents and the fractionation concept as a whole.

2.3.3 Thesis Outline

This dissertation is arranged accordingly and in the following sequence for smooth understanding of the contents and the idea behind the project.

Chapter 1 Introduction

This chapter gives a brief introduction to lignocelluloses fractionation and its contribution to efficient biomass refineries, the prospects of this industry and current shortcomings in lignocellulose fractionation studies. The chapter also discusses industrial applications of cellulose, hemicelluloses and lignin and conditions which are favourable for producing these three components in yields above 70% and with minimal degradation.

Chapter 2 From lignocellulose biomass to value added chemicals and materials, a literature study

The nature and structure of lignocellulose materials are reviewed with particular focus on cellulose, hemicellulose and lignin. The reaction of the three components under a variety of conditions including use of polyols as fractionating solvents is also discussed. The chapter further identified gaps and shortcomings of established organosolv lignocellulose fractionation processes. The chapter incorporated the statement of the research study, aims and specific objectives targeted. And finally the chapter ends with a discussion of possible and appropriate approach to this research work.

Chapter 3 Alkaline Polyol Fractionation of *E. grandis* and Sugarcane Bagasse

This chapter outlines the design of the fractionation experiment, the design of the reactor used, treatment of feedstock before fractionation and specific working conditions for temperature, time, catalyst concentration and solvent concentration. The chapter also describes in detail how the liquid and solid residue fractions were treated after a fractionation run including methods used to determine the carbohydrate and lignin composition in each fraction.

Chapter 4 Quantitative assessments of polyol fractionation yields and component recoveries

This chapter presents yields and recoveries of the three components and their respective fractions. The chapter analyses the influence of independent variables (temperature, time, catalyst and solvent concentrations) both individually and through their interactions on cellulose preservation in the solid fraction, hemicellulose and lignin dissolution. The chapter also partly discusses the extent to which the research specific objectives addressed by this analysis are met and providing the chemistry which influenced these outcomes.

Chapter 5 Quantitative assessment of the quality of products from *E. grandis* and sugarcane bagasse fractionations

This chapter is an assessment of the quality of cellulose, lignin and hemicellulose products from the fractionation process. Methods used for product analysis are detailed in this chapter in addition to scientific explanations of the chemical and structural characteristics of the products as influenced by the solvents and other reaction conditions. Cellulose was assessed for enzymatic hydrolysis, crystallinity and functional group composition, while isolated hemicelluloses were assessed for molecular weight, functional groups and lignin composition and the lignin component was assessed for functional groups and its proximate analysis. A section specific to possible industrial applications of products derived from these processes is also proposed.

Chapter 6 Conclusions and Recommendations

Conclusions from results presented in chapter four and five are summarized here with clear conclusions on the choice of the best fractionation process based on the yields of the individual products and their quality properties. The chapter also discusses possible improvements to the current work for further research and development.

Bibliography	A list of all referenced work in the dissertation
Appendices	A collection of supporting raw data calculation sheets, mass balance sheets and other supporting work that could not be fitted in the main body of the thesis.

Chapter Three

3 EXPERIMENTAL METHODS AND DESIGN FOR ALKALINE POLYOL FRACTIONATION OF *E. GRANDIS* AND SUGARCANE BAGASSE

3.1 Research Design and Methodology

3.1.1 Chemicals and Substrates

The selected lignocellulose feedstock, sugarcane bagasse supplied by TSB Sugar Mill, Mpumalanga Province, South Africa and E. grandis was sourced from Tzaneen, Limpopo Province, South Africa. The substrates were stored in a conditioning room at ±20 °C to maintain moisture content of less than 10% (w/w) for the duration of the project. Keeping the moisture content low prevents the materials from spoilage and degradation (Diedericks et al., 2012).

The substrates were then mixed thoroughly on a wide bench and sampled using the coning and quartering method as described by the British Standards DD CEN/TS 14780:2005 "Solid biofuels - Methods for sample preparation" (British Standards, 2005). For sugarcane bagasse, a portion of the sample representative of the bulk material received was milled with a centrifugal mill (Retsch ZM 200, Haan, Germany) to less than 2 mm and further sieved using a series of mesh to achieve particle size range of 0.425 to 0.850 mm. The portion which was more than 0.850mm was further ground milled to fall within range using a centrifugal mill (Retsch ZM 200, Haan, Germany) operating at 6500 rpm. The cone-and-quartering method was applied further, subsequent to size reduction to obtain a more representative sample.

Because *E. grandis* chips were much bigger in size, a Condux-Werk Wolfgang bei Hanau mill was used to mill the chips to approximately 8 mm. The sample was further milled with using a Retsch ZM200 mill with a 1 mm circular blade generating samples of approximately less than 2 mm. The samples were then screened through a Retsch AS200 shaker to recover sample size fraction between 0.425 and 0.850 mm. The homogenous samples collected for both substrates were used for compositional analysis and for the fractionation experiments.

However, prior to use, the substrates were oven-dried at 45 °C until a moisture content of less than 1%(w/w) was reached. Preparation and chemical composition analysis substrates were determined according to National Renewable Energy Laboratory (NREL) procedures;

a) Sample preparation for compositional analysis (Hames et al., 2008),

b) Extractive content (A. Sluiter, Ruiz, Scarlata, Sluiter, & Templeton, 2008),

c) Structural carbohydrates and lignin composition (A. Sluiter et al., 2012),

d) Moisture content (A. Sluiter, Hames, Hyman, et al., 2008)

e) Ash content (A. Sluiter, Hames, Ruiz, et al., 2008)

Sodium hydroxide pellets, purity ≥98%, xylitol crystals with a purity of ≥99% were both sourced from Sigma Aldrich (Sweden and United States of America respectively). Ethylene glycol with purity of ≥99% was obtained from Merck (South Africa).

Spezyme CP cellulase cocktail, with a calculated average cellulose activity of 64 filter paper units (FPU)/ml, according to the NREL procedure, was obtained from Genencor (Palo Alto, CA, USA). The cellulase was supplemented with β-glucosidase (Novozyme 188), obtained from Novozyme (Bagsværd, Denmark).

Avicel, purity of ≥ 99%, was sourced from Sigma Aldrich, Unite States of America. Soda Lignin used for comparison purposes in the proximate analysis of lignin rich solids produced from this study was isolated from black liquor provided by Felixton Mill, Emphangeni, South Africa; lignin was isolated using sulphuric acid followed by a series of water washings in order to reduce ash content and then finally dried in the oven at 40 °C until a moisture content below 10% was achieved.

3.1.2 Experimental setup and operations

The approach to this experiment Was designed in a 2^4 ($Levels^{Independent\ Factors}$) Central Composite Design (CCD) using a statistical software by StatSoft Inc, Statistica 12.6, 2015, so that comprehensive and useful information can be collected from the research. A central composite design also allows you to statistically, with ease, analyse the effect of independent variables on the dependent variables and the interaction between them. The CCD designs are listed in Appendix A. Variables used to design the CCD are listed in Table 7. The ranges used for the variables were determined based on thorough literature studies, except for xylitol concentration which was determined through a set of trial fractionation experiments (data not

presented in the thesis) in which all other variables were constant while xylitol experimented at various concentration. This was based on assumption that dependent variables will respond more to change in xylitol concentration change than the constant independent variables.

For fractionation experiments that used raw substrates (non-hemicellulose extracted), a CCD with a three-level design, four variables; temperature, time, sodium hydroxide concentration and solvent concentration (xylitol-water solutions or ethylene glycol-water solutions) was designed and applied to each substrate (*E. grandis* or sugarcane bagasse). Four assays at the midpoint of the design were also included in order to give an estimate of the random error needed for the analysis of variance (ANOVA).

Table 7: Experiment independent variable range

Variable	Variable Range		
	Lowest	*Midpoint*	*Highest*
Temperature (°C)	140	160	180
Time (h)	2	3	4
Catalyst Concentration (NaOH) (wt. %)	1	1.5	2
Xylitol Concentration (wt. %)	20	25	30
Ethylene Glycol Concentration (%, v/v)	50	60	70

Dissolved xylose (hemicellulose) and lignin in the liquid fraction and glucose (cellulose) remaining in the solid fraction were used as dependent variables. Cellulose dissolved, lignin and xylose remaining in the solid residue were also determined for mass balance closure. These dependent variables were also used to evaluate the best fit to which optimum conditions were determined with Statistica 12.6, 2015. The best fitness was expressed by the coefficient of determination R^2, and statistical significance was checked by F-test at a probability (p) of 0.005 and alpha value 0.10. The designs also included four star points with star high and star low values for each independent variable as determined by the software included in the CCD's at runs 17-24.

Table 8: Conditions for ethylene glycol fractionation of hemicellulose pre-extracted materials

	Variable Range		
Parameter	**Lowest**	**Midpoint**	**Highest**
Temperature (°C)	140	160	180
Time (h)	2	3	4
Catalyst Concentration (NaOH) (wt. %)	1	1.5	2

Additionally, fractionation experiments that used hemicellulose extracted substrates were also run in a CCD design. The only difference was that three variables were used as indicated in Table 8; temperature, time, and sodium hydroxide concentration, ethylene glycol concentration was kept constant at 60%.

3.1.3 Sodium hydroxide hemicellulose pre-extraction

As demonstrated in the flow diagram shown in Figure 4 in Chapter Two, some of raw materials fed into the polyol fractionation procedure were hemicellulose pre-extracted prior to ethylene glycol fractionation. Conditions for the pre-extraction were borrowed from previously optimized studies (Vena, 2013; Makhetha, 2016), as they are considered most optimal for hemicellulose extraction in both feedstock. For all treatments a 10% solid loading was used. In a 500ml closed squash bottle, a 20g solid substrate was weighted and soaked with 200ml sodium hydroxide. The slurry was allowed to equilibrate for an hour and then heated in a preheated water bath at 50rpms and the desired temperature. Sugarcane bagasse was pre-extracted at 65°C, 1.5M NaOH for 92 minutes (Vena, 2013), while *E. grandis* was pre-extracted at 90°C, 1.5M NaOH, 240 minutes (following a factorial design at 0.5M, 1.0M and 1.5M NaOH; and 4, 5 and 6 hours by Makhetha, (2016).

At the end of reaction time, the slurries were cooled in a water bath and then washed with lukewarm water until neutral, approximately ten times the reaction volume. The washouts were vacuum filtered through a 90mm Munktell Ahstrom filter paper. The solid was divided into two portions using quarter sampling after the solid was thoroughly mixed with a spatula during the washing process in order to ensure homogeneity, one portion stored as is, wet, in an airtight container and analysed for enzyme digestibility within two weeks. The other solid portion was dried at 45°C for 48 hours and analysed for sugars and lignin. The liquid fraction from the washing was also analysed for dissolved sugars and lignin.

3.1.4 Xylitol and Ethylene glycol treatment

The fractionation of *E. grandis* and sugarcane bagasse substrates corresponding to a dry weight of 1.0g was carried out in tubular reactors. Seamless Hastelloy C276 stainless steel was used for construction of the reactors. The tubular reactors were designed with inner diameter of 11.8mm, an outer diameter of 12.7mm and a total length of 152.0 mm according to Jacobsen & Wyman, (2002). To avoid evaporation of the solvent and also to contain entire content of the reactor, stainless steel Swagelok fittings (Solon, Ohio, USA) were used to provide a leak-tight closure on the two open ends of the reactors. The leak-tight closure was also supported by in-house modified DuPont Teflon (Wilmington, Delaware) stoppers which were fitted inside the steel fittings. The substrate inside the reactor was compressed by application of a stainless steel rod fitted through the open end of each reactor. Manual Compression of the substrate was done to account for mass and heat transfer limitations (Diedericks et al., 2012) and it also create space to accommodate the solvent poured on top. The substrate was then soaked with 10ml of appropriate solvent dissolved with the right amount of catalyst, sodium hydroxide. The content of the reactor was equilibrated at room temperature overnight after which they were ready for reaction. The reactions of the reactors' content at the set conditions took place in a sand medium using two similarly designed sand baths, fluidized, namely; Techne SBL-2D (Minneapolis, MN, USA). Their use was twofold; one sand bath was temperature controlled by a Techne TC-8D (Minneapolis, MN, USA) whereas the other sand bath, that was approximately 50 °C more than the desired temperature. Reactors were first inserted in the temperature uncontrolled sand bath for with a thermostat until they reach the set temperature for that particular test point and then quickly transferred to the sand bath which is temperature controlled. For consistency of the reactions, the temperature controller ensured temperature within a range of ±0.3°C. The temperature of the second sand bath and the reactors was maintained in this range until the lapse of set residence time for reaction.

At the end of the reaction time, the tubular reactors were removed from the sand bath and rapidly cooled in a water bath. After uncapping the reactors, the reaction slurry was washed with lukewarm water in small portions until neutral. The slurry was further separated into two fractions; the liquid fraction and the wet solid fraction by vacuum filtration through a 90mm Munktell Ahstrom filter paper. The liquid fraction was further analysed for sugars; glucose, xylose and lignin using NREL method ("Analytical Procedures: Determination of Sugars, Byproducts and Degradation products in liquid process samples") (Sluiter, Hames, et al., 2008) without any alterations. The liquid fraction was also analysed for dissolved lignin using a UV-vis spectrophotometer at a wavelength of 240 nm.

The wet solid fraction was weighted and separated into two equal portions, one stored in an airtight container and further hydrolysed enzymatically within two weeks of production, also according to an

NREL procedure (Resch, Baker, & Nrel, 2015); explained further in Chapter Five. The second solid portion was dried at 45 °C for 48 hours and analysed for sugars and lignin using NREL method ("Analytical Procedures: Determination of Structural carbohydrates and lignin in biomass") (A. Sluiter et al., 2012). In the case of pre-extracted raw materials, the reminder of the dried solid was used as feed for ethylene glycol fractionation.

3.1.5 Analytical Procedures: characterization of liquid and solid fractions

Composition of the solid residues from fractionation process were determined according to NREL procedure (A. Sluiter et al., 2012). This method was a two-step hydrolysis of the solid using sulphuric acid which in the end enabled determination of the solid content in terms of acid-soluble lignin, acid-insoluble lignin, xylose and glucose. The first step involved hydrolysis using 3ml of 72 % (w/w) H_2SO_4 per 0.3g on dry weight basis of solid material, at 30 °C for 1 hour in a water bath with five minute stirring intervals. After reaction was completed, the solid substrate-acid reaction mixture was further diluted with distilled water up to 4% (w/w) and hydrolysed further for another hour at 121 °C in an autoclave. Sugar contents in the hydrolysates was determined by a high performance liquid chromatography (HPLC) analyser (Waters Breeze System, Milford, MA, USA) using an H ion exchanger column Bio-Rad Aminex HPX-87 (Hercules, CA, USA) at a temperature of 65 °C. Furthermore, the ion exchanger was fitted with a guard column, Bio-Rad H cartridge. The mobile phase used to carry the ions was a low concentrated with 5 mM H_2SO_4 at a flow rate of 0.6 ml/min. The detection of peaks was completed by a refractive index detector, Waters 2141 (Milford, MA, USA). Quantification of sugar content was done using standard curve(s) of the various sugar components combined.

Vacuum filtration using a porous number 3 glass filter was employed to separate acid-insoluble lignin liquid fraction. Separated acid soluble lignin was then dried for at least 4 hours at 105 °C after which it was weighed. The amount of ash in the dried acid-insoluble lignin was determined gravimetrically. First, the samples were combusted in a Gallenkamp furnace (Loughborough, UK) for 4h at 575 °C. The ash content was then determined as the mass difference between the mass after combustion and before combustion. For the acid-soluble lignin in the hydrolysate, a UV-visible spectrometer (Pharmacia, Cambridge, UK) was used to detect lignin molecules at a wavelength of 240 nm as recommended by Sluiter et al., 2012.

3.1.6 Analytical procedures: quantitative analysis of the quality of fractionation products

Analysis of the quality of products was only done on streams recovered at optimum conditions; residual cellulose rich solids, hemicelluloses isolated with acetone and the remaining lignin rich solutions which were later freed of water by evaporation at 40 °C to collect solid rich lignin. Analytical methods employed for quality assessments of the fractions are summarized in Table 9. Instrument specifications and analytical method details are discussed in sections 3.16a to 3.16f that follows.

Table 9: Analytical test methods for fractionation product quality

	Analytical Method Used					
Material	XRD (Crystallinity)	FTIR (Functional groups)	GC-MS (S/G ratios)	TGA (proximate analysis)	Enzymatic hydrolysis	GPC (Molecular weights)
Residual Cellulose Solids	√	√			√	
Hemicelluloses		√				√
Lignin		√	√	√		
Raw Biomass	√	√	√	√	√	

a) Crystallinity Index

Measuring the Crystallinity Index (CI) of cellulose CI by the x-ray diffraction (XRD), FTIR and NMR methods provides a qualitative or semi-quantitative evaluation of the amounts of amorphous and crystalline cellulosic components there are in a sample (Park, Baker, Himmel, Parilla, & Johnson, 2010; Park, Johnson, Ishizawa, Parilla, & Davis, 2009). To some extent, although not sufficiently established in literature, Park et al., 2010 argues that the CI has an influence of the accessibility of cellulose during enzymatic hydrolysis.

For this study, the crystalline and amorphous portions of cellulose in the raw materials and treated residual solid samples were determined with an XRD. About 2g of ground sample (250 µm) was pressed into a metal holder with applied pressure and scanned with X'Pert High Score PW3209 diffractometer, using Ni-filtered CuKa radiation (0.15406 nm) generated at 30 kV and 40 mA. The X-ray diffractogram were recorded through a 2θ (Bragg angle) equivalent from 4° to 40°, with a step size of 0.05°. A background subtraction was done on all samples. A random sample was also analysed twice to ensure

repeatability of the method. Additionally, a commercial sample containing 99% of microcrystalline cellulose (Avicel PH-101, Fluka) was also measured for comparison purposes.

The crystallinity index of cellulose from XRD patterns is calculated according to the peak height method described in the literature (Chikouche, Merrouche, Azizi, Rokbi, & Walter, 2015; M. a Lima et al., 2013); After baseline subtraction, CI is expressed as a percentage based on equation 1;

Equation 1: Crystallinity Index

$$CI\ (\%) = 100\ [(I_{002}-I_{am})/I_{002}]$$

where I_{002} is the height of the principal cellulose I peak at 2θ angle between 22° and 23°, I_{am} is the height of the minimum (attributed to amorphous cellulose) between the 002 and 101 peaks, given at 2θ angle between 18° and 19° as depicted in Figure 5.

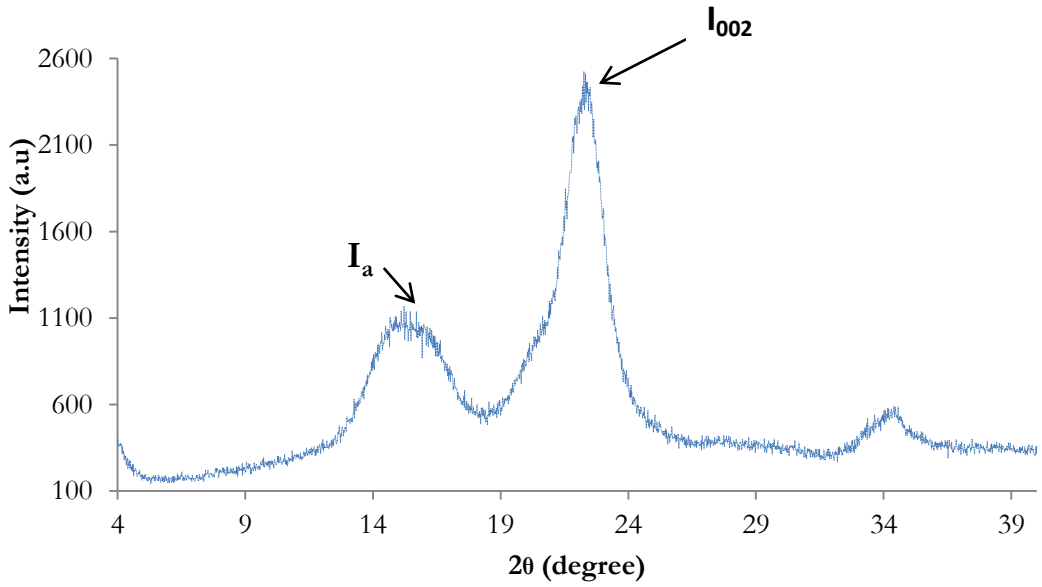


Figure 5: X-Ray diffractogram of commercial cellulose (Avicel)

b) Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose was analysed in the raw materials, hemicellulose pre-extracted solids, and on cellulose fraction after the fractionation process. The first step in the process was to determine the activity of enzymes. Enzyme activities for cellulase in Optiflow and endoglucanase in Novozyme 188

were determined according to a previously described standard (Ghose, 1987), using Whatman No. 1 filter paper (1 x 6 cm, 50 mg) and 1.0 wt. % Glucose as substrates. The enzyme activities were found to be 150 FPU/mL for Optiflow and 584IU/mL for Novozyme 188 in agreement with specified range of the manufacturers.

The enzymatic digestibility of the substrates and fractionation products were then determined according to the NREL Laboratory Analytical Procedure (Resch et al., 2015). A solid containing an equivalent of 0.55g glucose based on 105°C dry weight basis was transferred to a 100-ml Erlenmeyer flask. About 150µl of a 2 % (w/v) sodium azide solution was added to prevent microbial contamination. A buffer supplement with enzyme was prepared and 25mL added to the flask. The amount of distilled water required to bring total volume to 50ml was calculated and added assuming all total biomass and solutions have specific gravity of 1.0g/mL (Resch et al., 2015). This mixture had a final pH of 4.8, 0.05 M sodium citrate and an enzyme loading of 30FPU cellulase/g glucose and 15IU of β-glucosidase/g glucose. The saccharification was carried out for 72 hours at 50 °C and 150 rpm rotation. Samples were taken at time 0 and at 72 hours respectively. Total reducing sugars released during the saccharification process were quantified by HPLC using glucose standards.

c) Functional group determination by FT-IR

Residual solid samples, also enriched with cellulose as discussed in Chapter Two, were pulverised to fine powder and analysed with an IR spectrometer. About 20mg of the sample, previously dried to 60°C, was read for IR spectra in reflectance mode using a Smart Performer detector from Thermo equipped with ZnSe lenses. A portion of the sample was placed on the ZnSe horizontal ATR and 32 scans with a resolution of 4cm-1 collected. FTIR spectra were obtained directly from raw substrates, and substrates from the optimum conditions, utilising diffuse reflectance infrared with Fourier transform technique (Perkin Elmer - Spectrum GX). The spectra were normalised by the absorption at 600– 3000cm-1 after baseline correction and analysed using OMNIC software after which the data points were extracted into Microsoft Excel to draw peaks with better resolution.

d) Average molecular weight determination

Isolated hemicelluloses were analysed for their molecular weights as weight average molecular weight. A Gel Permeation Chromatograph (GPC), Ultimate 3000 HPLC by Dionex system was used. The system was made of SUPREMA columns (PPS, Germany), two 3000A 300×8mm columns and one 30A 300×8mm. Isocratic separation using water at 70 °C with ELSD detection was used to detect weight

distribution. Generated results were analysed on Chromeleon® Version 6.80 software package. About 1g of hemicelluloses was dissolved in water to make a concentration of 1 g/L. The solution was then filtered through a 0.2 micrometer filter and analysed on the chromatogram.

e) Functional group determination by Gas Chromatography Mass Spectrometry (GC-MS)

Compositional information of lignin such as the ratio of their syringyl/guaiacyl, their presence or lack therefore can give an indication of the severity of the fractionation process, thereby giving information on the quality of the lignin generated.

Air dried lignin samples from the hemicellulose pre-extraction and fractionation process were prepared for functional group analysis using a method by Foster et al., 2010. This analysis also included untreated material. The method used is based on the ability of the Thioacidolysis reaction in which the lignin complex structure is defragmented into its monomeric building blocks syringyl, guaiacyl and p-hydroxyphenyl. The reaction selectively cleaves off the alkyl aryl ether bonds to achieve this (Lapierre, 2008). Thioacidolysis products, the three lignin monomers are then diagnosed and quantified chromatographically using instruments such as gas chromatography−flame ionization detector (GC/FID) or gas chromatography−mass spectrometry (GC/MS) (Yue, Lu, Sun, & Ralph, 2012).

In this study lignin samples were analysed with a GC/MS equipped with a quadrupole mass-spectrometer, Agilent HP-5MS column (30 mm× 0.25 mm× 0.25µm film thickness). The following temperature gradient is used with a 30 min solvent delay and a 1.1 ml/ min flow rate: Initial hold at 130 °C for 3 min; a 3 °C/ min ramp to a 250 °C and hold for 1 min; allow equilibration to the initial temperature of 130 °C.

Peaks were identified by relative retention times using tetracosane internal standard (optional) or by characteristic mass spectrum ions of 299 m/z, 269 m/z, and 239 m/z for S, G, and H monomers, respectively (see Fig. 2). The composition of the lignin components is quantified by setting the total peak area to 100%. The syringyl/guaiacyl (S/G) ratio was calculated by dividing the sum of peak areas from syringyl units by the sum from the peak areas of guaiacyl derivatives of the selected markers, obtained by integration of the peak areas and considering the total peak area as 100%.

f) Proximate analysis

Thermogravimetric analysis (TGA) was used to characterise the combustion profile of the lignins isolated from the fractionation process. Prior to analysis, the lignin samples from the process were oven dried at 50° until moisture content below 10% was achieved. For the analysis, ASTM standard method D3172-13 as described by (Rubio, Mayoral, Izquierdo, & Andre, 2001) was carried out using a TGA Instrument, TA Instruments Q500 in an oxygen flowing atmosphere of 15cm^3 /min. Approximately 20 mg lignin sample weighted and heated in the TGA at a rate of 10 K/min from room temperature to 900°C. Two random samples were replicated to ensure the repeatability of the method. The data were derivatized according to models used in literature (Rubio et al., 2001).

Chapter Four

4 QUANTITATIVE ASSESSMENTS OF POLYOL FRACTIONATION YIELDS AND COMPONENT RECOVERIES

4.1 Introduction

This Chapter presents a quantitative evaluation on the effectiveness of fractionation conditions and designs presented in Chapter Three and in answering some of specific objectives of the study highlighted in Chapter Two, namely;

- To retain 80% or more cellulose in the solid fraction.
- Dissolve > 80% and recover more than 70% of hemicelluloses in the liquid fraction.
- Remove more than 70% lignin from the solid fraction.
- To determine and understand the effect of set fractionation parameters such as temperature, catalyst and solvent concentration and time of retention on the fragmentation of *E. grandis* and sugarcane bagasse into the respective components (lignin, hemicellulose and cellulose) and use these to optimize best fractionation conditions that maximize components' yields.

In response to these targets, the chapter firstly presented compositional data for the raw feedstock and compared between the feedstock and also with information found in the literature with respect to grams/100 grams of cellulose (glucose), hemicellulose (xylose), lignin (sum of acid soluble and acid insoluble). This data is important in calculations of total mass balances of individual components after the fractionation processes. The raw material compositional information was followed by a discussion of results from hemicellulose pre-extraction process establishing the amount of hemicelluloses dissolved and recovered in the liquid fraction and those retained in the solid residue in addition to information on other components, i.e. cellulose and lignin dissolved, recovered and retained in the solid residue after the pre-extraction process. These data were also compared to pre-extraction studies reported by Makhetha, 2016 and Vena, 2013 which established the conditions used. Hemicellulose pre-extraction step is presented right after raw material composition results and discussions of component fractionations since hemicellulose extracted solids were also subjected to further fractionation with ethylene glycol.

Later in the chapter, mass balances of components (cellulose, hemicelluloses and lignin) and their recoveries are presented. This is discussed with support of graphical interpretations and detailed analysis of the relationship between independent variables, interactions and their influence on dependent variables. Analysis of variance (ANOVA) and other statistical tools were also employed to establish the significance of the models presented at 95% confidence (p-value <0.05)

From the models fitted with minimum r-squared value of 0.5 the most optimum fractionation conditions were established in section 4.4.5. The process of optimum condition determinations is explained in detail in this section. Statistically predicted results of optimum conditions and those of actual runs from established conditions were discussed in conjunction with aims of the project and other results obtained from literature. The chapter is concluded with a summary of significant findings and a discussion on the preferred fractionation route in respect of targets specified earlier.

4.2 Chemical composition of raw materials

The approximate (variable) composition of both materials are summarised in Table 10. Reported values are average of three replicates per component. The composition of raw materials did not vary significantly during the execution of experimental work (data not shown), despite reports of natural lignocellulose degradation during storage elsewhere (B. Yang, Dai, Ding, & Wyman, 2011).

Table 10: Substrate composition

	Composition (g/100g)					*Total*	*Reference*
Biomass	Glucose	Xylose	Lignin	Ash	Extractives	*(g)*	
SCB	40.24±1.45	23.35±1.26	22.96±1.44	3.20±0.08	5.91±0.22	84.55±4.13	This Study
SCB	42.40	25.20	19.60	1.6	n/a	88.8	(Brienzo et al., 2009)
E. grandis	47.45±1.82	20.90±0.28	25.52±0.57	0.16±	2.73±0.04	93.88±4.83	This Study
E. grandis	44.65	15.23	25.77	n/a	3.25	88.9	(Emmel, Mathias, Wypych, & Ramos, 2003)

Summative analysis of sugarcane bagasse and *E. grandis* compositional analysis totals 84.55g/100g and 93.88g/100g of raw material respectively. All reported values have an error margin <5% corresponding to a confidence interval of 95%. Extractives content for *E. grandis* (2.73wt. %) is in accordance with values of other eucalyptus varieties reported in literature indicated in Table 2-3, with a range from 1.1-7.9wt. %. Sugarcane bagasse reported high extractive content 5.91 wt. % higher than 2.72wt. % reported by Guilherme et al., (2015) but lower than the 6.0wt. % extractives content reported in the work of Diedericks et al., (2012). High extractive content in lignocellulose is attributed to presence of waxes and low molecular weight aromatics (Masarin et al., 2011), free sugars or pectin and dust (Yao et al., 2015). Ash content for both materials is commonly reported below 2% in literature (Brienzo et al., 2009; Castro et al., 2013; van der Hage, Mulder, & Boon, 1993; Zhang, Rackemann, et al., 2013). However, sugarcane bagasse' ash content analysed in this study is higher at 3.2 wt. %, slightly comparable to 4.0 wt. % reported by Diedericks et al., 2012. High ash content in the feedstock is attributed to the presence of high concentration of inorganic matter such as silica from sand particles (Vena, 2013).

Cellulose content (measured as glucose) for sugarcane bagasse is lower than that of *E. grandis*, 40.24 wt. % and 47.45 wt. % respectively. Eucalyptus literature reports indicated cellulose content between 37.0 to 53.10 wt. % as shown in Tables 2-3 (Emmel et al., 2003; Magaton et al., 2009), a range covering the value obtained for *E. grandis* (47.45 wt. %) in this study. Cellulose content reported in literature for sugarcane bagasse range from 39.1 to 45.28 wt. % (Diedericks et al., 2012; Moghaddam et al., 2014; Yao et al., 2015; Zhang, O'Hara, et al., 2013). Sugarcane bagasse cellulose composition analysed in this study (40.24 wt. %) falls within this range but most comparable with less than 5% difference to values reported in literature, 42.40 wt. % by Brienzo and co-workers (2009) and 39.1 wt. % by Diedericks et al., 2012. Whereas the range of hemicelluloses (measured as xylose) reported in literature reports in Table 2-3 is 15.4 to 18.6 wt. % for eucalyptus and 20.2 to 25.2 wt. % (Brienzo et al., 2009; Diedericks et al., 2012; Zhang, O'Hara, et al., 2013), values obtained in this study are closely comparable; 20.9 wt. % for *E. grandis* and 23.35 wt. % for sugarcane bagasse.

Total lignin content of sugarcane bagasse (22.96 wt. %) was less than that of *E. grandis* (25.52 wt. %), which is typical of grasses (Fengel and Wegener, 2003). Lignin content for *E. grandis* is most comparable to 25.77wt. % recorded by Emmel, Mathias, Wypych, & Ramos (2003) but lower than values reported for other eucalyptus varieties 26.9-32.6wt. % (Jansson, Näsman, & Francisco, 2013) and also lower than two *E. grandis* samples (28.6 and 29.6 wt. %) reported elsewhere (Dutt & Tyagi, 2011). Additionally, it was demonstrated by Miranda & Pereira (2002) that lignin composition is slightly dependent on the age of the tree, sometimes representing an increase of up to 2.0 wt. % within 2 years which may add to the variation. Furthermore, sugarcane bagasse lignin content obtained in this study (22.96wt. %) is lower than the 26.0

wt. % lignin analysed in sugarcane bagasse by Moghaddam et al., 2014, but higher than 18.93wt. % reported by Mesa et al., (2011). The lignin content value recorded for sugarcane bagasse in this study also compares to other South African sugarcane varieties (20.3 to 22.4wt. %) in a cultivar selection study reported by Benjamin, García-Aparicio, & Görgens, 2014.

Determined compositions of both materials are in agreement with reported literature values. The small variations in component contents between our substrates and literature reports can be explained by lignocellulose variety, harvesting, growing and storage conditions (Templeton, Scarlata, Sluiter, & Wolfrum, 2010).

4.3 Hemicellulose alkaline pre-extraction

Results of hemicellulose extracted from the two substrates (eucalyptus and bagasse) through pre-extraction process are presented in Table 11 while individual component mass balances are presented in Tables 11-14. Sugarcane bagasse was pre-extracted at 65°C, 1.5M NaOH for 92 minutes according to an optimazation done by Vena, (2013) while *E. grandis* was pre-extracted at 90°C, 1.5M NaOH for 240 minutes based on optimized study of Makhetha, (2016). Summative mass total (Table 12-14) of sugarcane bagasse (cellulose based on glucose, hemicellulose based on xylose and lignin) were found to be 92.94%, 87.02% and 78.22% respectively, while *E. grandis* was reported to be 91.61, 82.8 and 91.84%. Sugarcane bagasse mass balances for hemicellulose (see Table 12) analysed in this study are comparable (less than 10% difference) to 95.6% and 94.6% obtained by Makhetha, (2016) and Vena, (2013) respectively. Similarly, this study's hemicellulose mass balances for *E. grandis* corresponded (less than 5% difference) with 90.1% and 94.4% reported by Makhetha, (2016) and Vena, (2013) respectively. Cellulose mass balances (see Table 12) reported in this study are slightly lower than 103.5% and 104.0% reported by Makhetha (2016) for *E. grandis* and sugarcane bagasse respectively, but much more comparable to 95.3% and 97.3% obtained Vena (2013) for *E. grandis* and sugarcane bagasse respectively. Additionally, lignin mass balance for sugarcane bagasse falls within the range determined between Makhetha, (2016) and Vena, (2013) of 90.9% to 100.0%, whilst a much lower mass balance was recorded for *E. grandis'* lignin (78.22%) below 94.1% and 104% reported by the same researchers respectively. This can be attributed to the nature of the substrates with varying lignin compositions.

As shown in Table 11, 81.88% of raw sugarcane bagasse hemicelluloses were solubilized while only 53.25% was solubilized from *E. grandis*. Sugarcane hemicellulose dissolution is slightly higher than 71.17% and 69.1% obtained under similar extraction conditions in the works of Makhetha, (2016) and Vena, (2013) respectively. However, 81.88% dissolution of hemicelluloses from sugarcane bagasse falls within

the 16-96% bracket reported by other researchers as presented in section 2.2.3. The 53.25% dissolution of hemicellulose from E. grandis' corresponds with 55.39% obtained by Makhetha (2016). The results also indicate significant dissolution of lignin in the liquor from sugarcane bagasse (dissolution of 65.98% of raw material lignin content) while 33.26% of the raw *E. grandis* lignin was also dissolved into solution alongside hemicelluloses, results presented in Table 14. Both of these results corresponds to 24.00-89% and 3.00-26.16% dissolution of raw material lignin from sugarcane bagasse and eucalyptus respectively (Makhetha, 2016; Section 2.2.3). However, the percentage of lignin dissolution reported for *E. grandis* is slightly higher previously reported values; this can be attributed to the variance in the nature of the feedstock composition as reported by Vena, (2013). Dissolution of a greater proportion of lignin during the hemicellulose pre-extraction step for sugarcane bagasse means that less severe conditions would be required to further fractionate the remainder of components in the lignocellulose structure matrix, while moderately severe conditions would be required to remove the remaining lignin from *E. grandis*. However, higher lignin content in the hemicellulose hydrolysate at this step also suggests further potential complicated separations or multi-steps to get a pure fraction of hemicelluloses such as its precipitation at neutral pH or mild acid or by use of excess ethanol or acetone (Peng et al., 2012) after alkaline extraction.

Alongside dissolved hemicelluloses, cellulose was also dissolved as presented in Table 13. Less than 5.1g/100g of raw material, cellulose was dissolved from eucalyptus (5.01g/100g of initial cellulose) and sugarcane bagasse (4.85g/100g of initial cellulose), these represents dissolution of less than 12.5% of the raw cellulose in both materials i.e. 12.05% and 10.56% for SCB and EC respectively. Cellulose was therefore hardly disrupted during the hemicellulose pre-extraction process with 87.9% (sugarcane bagasse) and 89.4% (*E. grandis*) of the initial cellulose in the raw material remaining in the residual solid. Sodium hydroxide (alkaline solvents) is known to selectively hydrolyse hemicelluloses and depolymerise lignin from lignocellulose materials whilst only causing swelling and minimal dissolution of the cellulose microcrystalline structures (Arantes & Saddler, 2010; Brandt et al., 2013; Kumar et al., 2009b; Ramos, Morgado, Gessner, Frollini, & El Seoudb, 2011)

Table 11: Results of hemicellulose alkaline pre-extraction

		Liquid Fraction						Residual solid (g/100g)			
		Dissolved Components (g/100g)			Recovered Components (g/100g)						
Substrate	Solid Yield (%)	Glucose	Xylose	Lignin	Glucose	Xylose	Lignin	Glucose	Xylose	Lignin	Total (g/100g)
Raw SBC								40.24	23.35	22.96	86.55
Sugarcane bagasse	59.13%	4.85	19.12	15.55	2.01	16.09	10.55	35.39	4.23	7.41	75.68
Raw E. grandis								47.45	20.9	25.52	93.87
E. grandis	74.48%	5.01	11.13	8.40	1.03	7.55	6.32	42.44	9.77	17.12	84.23

Based on the specific objectives of this study, specific to hemicellulose fractionation, the objective was to dissolve > 80% and recover more than 70% of initial raw material hemicelluloses in the liquid fraction while simultaneously removing more than 70% and preserving 80% of raw material lignin and cellulose respectively. The target objective of dissolving 80% of initial raw material hemicelluloses was only achieved slightly above target with sugarcane bagasse (81.88%) while only 53.25% of raw material hemicellulose was dissolved from *E. grandis*. Target recovery of 70% of dissolved raw material hemicelluloses from the liquor solution was met with sugarcane bagasse extraction and failed with *E. grandis*. Recovery of hemicelluloses dissolved from sugarcane bagasse was 84.12% of the dissolved hemicelluloses which represent 68.9% of raw material hemicelluloses. Only 67.83% (see Table 11-14) of the dissolved hemicelluloses were recovered from *E. grandis* hemicelluloses extraction liquor, which corresponds to 36.12% of hemicelluloses in the raw material. Low dissolution of hemicelluloses from *E. grandis* can be explained by the high content of lignin in its structure which acts as a physical barrier to hemicelluloses (section 2.2.2). Therefore, it can be concluded that under current hemicellulose extraction conditions of both materials, the specific objective on hemicellulose dissolution cannot be achieved. Khuong et al., (2014) suggested application of other reaction activators such as pressure can improve dissolution by introduction of a regulated mechanical disruption to the lignocellulose structure under alkaline conditions without compromising the quality of other components. In their pretreatment study, Khuong and co-workers treated sugarcane bagasse with 1.25 M NaOH at a 1:10 solid-liquid loading, 121 °C, 105 kPa for 1 hour to dissolve 96.2% and 89.10% of raw material hemicelluloses and lignin respectively while preserving 81.60% of initial raw material cellulose in the residual solid.

Table 12: Mass balance of hemicelluloses (measured as xylose) after hemicellulose pre-extraction process with NaOH

	NaOH (M)	Time (Hours)	Temperature (°C)	Solid Yield (%)	Liquid Fraction		Residual solid xylose (g/100g)	Total (g/100g)	Degraded xylose[3] (g/100g)	Hemicellulose Recovery (%)
					Dissolved xylose (g/100g)	Recovered xylose (g/100g)				
Raw SBC							23.35	23.35	0	100.00
Sugarcane bagasse	1.5	1.53	65	59.13	19.12	16.09	4.23	20.32	3.03	87.02
Raw *E. grandis*							20.90	20.90	0.00	100.00
E. grandis	1.5	4	90	74.48	11.13	7.55	9.77	17.32	3.58	82.87

Table 13: Mass balance of cellulose (measured as glucose) after hemicellulose pre-extraction process with NaOH

	NaOH (M)	Time (Hours)	Temperature (°C)	Solid Yield (%)	Liquid Fraction		Residual solid glucose (g/100g)	Total (g/100g)	Degraded Glucose (g/100g)	Cellulose Recovery (%)
					Dissolved glucose (g/100g)	Recovered glucose (g/100g)				
Raw SBC							40.24	40.24	0	100.00
Sugarcane bagasse	1.5	1.53	65	59.13	4.85	2.01	35.39	37.40	2.84	92.94
Raw *E. grandis*							47.45	47.45	0	100
E. grandis	1.5	4	90	74.48	5.01	1.03	42.44	43.47	3.98	91.61

[3] Grams/100g difference between dissolved and recovered component assumed to be degraded/lost in the liquid fraction and/or unaccounted for with HPLC measurement (degradation not confirmed with analytical tests)

Table 14: Mass balance of total lignin after hemicellulose pre-extraction process with NaOH

	NaOH (M)	Time (Hours)	Temperature (°C)	Solid Yield (%)	Liquid Fraction		Residual solid lignin (g/100g)	Total (g/100g)	Degraded lignin (g/100g)	Lignin Recovery (%)
					Dissolved lignin (g/100g)	Recovered lignin (g/100g)				
Raw SBC							22.96	22.96	0	100
Sugarcane bagasse	1.5	1.53	65	59.13	15.15	10.55	7.41	17.96	5.0	78.22
Raw *E. grandis*							25.52	25.25	0	100
E. grandis	1.5	4	90	74.48	8.4	6.32	17.12	23.44	2.8	91.84

4.4 Fractionation runs: component recoveries and mass balances

The central composite design experimental runs for the fractionation of bagasse and *E. grandis* using the two solvents xylitol and ethylene glycol under the conditions specified in Chapter 3, resulted in two major fractions; a cellulose rich pulp and an aqueous mixture of hemicellulose, lignin and some dissolved cellulose. The two fractions were analysed for recovered glucose, lignin and hemicellulose content using analytical methods also specified in Chapter 3. Discussions of results from these experimental runs based on the yield of remaining solid after fractionation, dissolved and recovered cellulose, hemicellulose and lignin and mass balances of these respective components are presented in the following sections 4.4.1 to 4.4.4.

4.4.1 Fractionation's solid yield

Yields of residual solids were calculated based on recovered after the respective fractionations using Equation 2, expressed as a percentage of solid remaining from fractionation/pre-extraction process, compared to initial solid fed into the process, based on dry weight using a method prescribed by Harmsen et al., 2010. Calculated solid yields of CCD's are presented in Tables 15 and 16.

Equation 2: Solid yield Calculation

$$Solid\ Yield\ (\%) = \frac{Weight\ of\ Dry\ Residual\ Solid\ (g)}{Initial\ Weight\ of\ Solid\ fed\ into\ the\ Fractionation\ Process\ (g)} \times 100$$

The solid yield for all CCD's varied depending on the reaction conditions as reported in Tables 15-16. The yields range from 37 to 88.8% covering the records 51 to 81% obtained by Romani et al., 2013 for eucalyptus using polyol fractionations (180−200 °C, 40−90 min, 40−80% (w/w) glycerol−water solutions). The lowest solid yield was recorded at very harsh temperature conditions for eucalyptus at run 18 of raw EG fractionations (200 °C, 180 min, 15.g/100g NaOH, 60% (w/w) EG−water solutions) which when compared to Romani et al., 2013's findings they used much shorter reaction times which is why their minimum solid yield was high than what is recorded from this study. It is also observed from Tables 15-16 that solid yield is high around low conditions in runs 1-10 and 17 (120−180 °C, 2−4 hours, 0.5-1.0% NaOH, 50−70% (w/w) ethylene glycol or 20-25% xylitol-water solutions) and declines towards medium-harsher fractionation runs 11-16, 8 and 19-28 (160−200 °C, 1−5 hours, 1. 0−2.5% NaOH, 40−80% (w/w) ethylene glycol, or 15-35% xylitol solutions).

Table 15: Fractionation Solid Yields

Run	Solvent Conc. (%) (Xylitol/EC)[4]	Catalyst Conc. (%, w/v)	Temp (°C)	Solid Yield (%)				
				Time (Hours)	EC-xylitol	EC-EG	SCB-xylitol	SCB-EG
1	20.0/50.0	1.0	140.0	2.0	79.6	77.17	77.36	79.05
2	30.0/70.0	1.0	140.0	2.0	81.6	68.74	82.37	81.25
3	20.0/50.0	2.0	140.0	2.0	80.0	61.74	76.34	65.43
4	30.0/70.0	2.0	140.0	2.0	78.3	69.43	73.86	76.09
5	20.0/50.0	1.0	140.0	4.0	83.0	55.42	73.41	79.88
6	30.0/70.0	1.0	140.0	4.0	81.7	62.89	71.17	79.68
7	20.0/50.0	2.0	140.0	4.0	79.7	65.98	58.52	69.12
8	30.0/70.0	2.0	140.0	4.0	76.0	63.43	73.87	72.06
9	20.0/50.0	1.0	180.0	2.0	66.8	53.48	64.39	83.13
10	30.0/70.0	1.0	180.0	2.0	66.7	64.59	63.59	66.95
11	20.0/50.0	2.0	180.0	2.0	57.7	52.47	54.80	54.66
12	30.0/70.0	2.0	180.0	2.0	59.0	57.66	59.79	62.75
13	20.0/50.0	1.0	180.0	4.0	64.8	55.27	62.02	49.72
14	30.0/70.0	1.0	180.0	4.0	47.0	58.03	63.81	82.89
15	20.0/50.0	2.0	180.0	4.0	52.4	46.04	56.11	68.36
16	30.0/70.0	2.0	180.0	4.0	54.3	43.17	59.90	55.79
17	25.0/60.0	1.5	120.0	3.0	82.1	75.41	84.28	77.29
18	25.0/60.0	1.5	200.0	3.0	63.9	36.96	54.98	57.44
19	25.0/60.0	1.5	160.0	1.0	81.8	69.86	83.53	79.87
20	25.0/60.0	1.5	160.0	5.0	63.1	56.09	63.50	69.71
21	25.0/60.0	0.5	160.0	3.0	57.6	67.92	79.55	75.56
22	25.0/60.0	2.5	160.0	3.0	62.0	68.58	73.37	69.06
23	15.0/40.0	1.5	160.0	3.0	70.6	68.19	69.88	72.83
24	35.0/80.0	1.5	160.0	3.0	67.4	69.47	67.20	76.87
25	25.0/60.0	1.5	160.0	3.0	68.8	64.86	64.04	68.16
26	25.0/60.0	1.5	160.0	3.0	76.2	61.00	62.83	71.22
27	25.0/60.0	1.5	160.0	3.0	70.1	57.70	67.69	73.20
28	25.0/60.0	1.5	160.0	3.0	72.2	61.58	64.17	86.98

[4]Xylitol concentrations on w/v basis while EG concentrations on a v/v basis.

Table 16: Solid yield recoveries of ethylene glycol fractionations of hemicellulose pre-extracted solid residues

Run	Solvent Conc. (%, v/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Solid Yield (%)	
					EC	SCB
1	60	1	140	2	88.88	60.94
2	60	2	140	2	83.89	56.61
3	60	1	140	4	84.93	55.99
4	60	2	140	4	75.33	56.40
5	60	1	180	2	70.40	59.99
6	60	2	180	2	44.69	69.72
7	60	1	180	4	63.42	74.48
8	60	2	180	4	55.55	68.59
9	60	1.5	126.4	3	87.09	77.87
10	60	1.5	193.6	3	54.49	68.72
11	60	1.5	160	1.3	74.52	55.26
12	60	1.5	160	4.7	65.87	73.98
13	60	0.7	160	3	78.33	76.78
14	60	2.3	160	3	64.76	70.38
15	60	1.5	160	3	69.65	74.93
16	60	1.5	160	3	68.03	75.40
17	60	1.5	160	3	70.80	74.71
18	60	1.5	160	3	69.84	74.71

4.4.2 Cellulose recovery in the solid fraction

One of the four objectives of this study was to preserve 80% or more cellulose (glucose) in the solid fraction as highlighted in section 2.3.1, meaning a solubilisation of <20% of cellulose in the raw material. Mass balances of cellulose retained in the solid fraction and dissolved in the liquor are presented in Tables 17to 22. High dissolution of cellulose with simultaneous low recovery in the liquid fraction was observed in all fractionation runs. Cellulose dissolution accompanying fractionations with xylitol solutions range from 4.55-14.65g/100g and 2.14-12.44g/100g of raw glucose in *E. grandis* and sugarcane bagasse respectively. Ethylene glycol fractionations reported 8.66-18.55g/100g and 0.14-16.24g/100g of raw glucose in *E. grandis* and sugarcane bagasse respectively. These represents dissolution of up to 30.87% and 30.91% of raw glucose in *E. grandis* and sugarcane bagasse under xylitol fractionations, while up to 40.36% and 39.73% of raw glucose was dissolved from *E. grandis* and sugarcane bagasse in ethylene glycol fractionations. These trends bring forth three general observations namely; firstly there are high dissolutions of cellulose (grams per 100grams of initial cellulose) from eucalyptus than sugarcane bagasse (irrespective of solvent) which can attribute to the proportionality of cellulose between the two residues, 47.45g (*E. grandis*) versus 40.24g/100g (sugarcane bagasse) of material. The second observation is that

solubilisation of cellulose from the solid matrix is generally more under ethylene glycol fractionations with up to 10% difference under similar conditions (temperature, time, NaOH concentration) when compared to xylitol fractionations (see Table 18-21). This suggests that ethylene glycol disrupts the lignin and hemicellulose physical barrier to cellulose far better than xylitol which enables hydrolysis of the cellulose matrix. This can be explained by the small molecule structure of ethylene glycol (62.07g/mol and 2 carbons atoms) as compared to xylitol (152.15g/mol and five carbon atoms), smaller molecules are able to penetrate the pores of the lignocellulose structure to reach enclosed structures such as cellulose easily and also relative to their low viscosities, dissolution of cellulose is eminent (Jian et al., 2013; Singh & Ekhe, 2014).

The third observation is the high solubilisation of cellulose in this study when compared to other reports in literature. It is generally reported that organosolv dissolves between 1-20% of raw material glucose as discussed earlier in the presentation of Table 5. The amount of cellulose dissolved from some fractionation runs in this study falls within this range while other runs dissolve up to 40.36% of raw material glucose which is outside commonly reported cellulose dissolution bracket. This observation can be attributed to several factors;

- The NaOH catalyst factor partly contributes to cellulose dissolution as demonstrated in Figures 6-7. NaOH has an alkaline effect which causes swelling and eventual disruption of cellulose bonds which results in dissolution. NaOH was used as a catalyst in all fractionation runs between 1-2g (wt. %) which is 0.25-0.5M concentration. The action of the catalyst alone dissolves up to 20% of cellulose from the substrates, as reported for *E. grandis* under close conditions of Vena, (2013) with dissolution of 23.75% initial cellulose with l M NaOH at 90 °C for 4 hours. Similarly, using 0.25M NaOH at 121 °C for 1 hour Khuong et al., (2014) also dissolved 18.10% of initial cellulose from sugarcane bagasse. These two cited studies used NaOH reaction conditions compared to those used in this study (see Table 17-22) which therefore partly explains the high dissolution of cellulose in addition to other fractionation conditions (temperature, fractionation time and solvent concentration) which in themselves contributes to dissolution of cellulose (Figure 6-9).

- The severity of other fractionation conditions (temperature, fractionation time and solvent concentration) also determines the amount of cellulose dissolved as observed in Tables 17-22 and Figure 6-9. Their action changes the structure of the solid matrix by reducing its crystallinity, defibrillation and defibration (Garrote, Dominguez, & Parajo, 1999). Dissolution of cellulose enclosed in a lignin-hemicellulose matrix with an organic solvent is minimal (up to 20%) under 200°C due to its stiffness and single-chain conformation (Cao, Pu, Studer, Wyman, & Ragauskas, 2012; Hu, Lin, Wu, Zhou, & Liu, 2015; Kobayashi, Wen, & Shoda, 1996; Leskinen et al., 2015;

Terinte et al., 2011) which makes it difficult to break the covalent bonding within the cellulose polymer structure. However, longer fractionation times and increased ethylene glycol or xylitol concentration increase dissolution of cellulose as shown by the paretto charts in Figure 6-9.

- Structural modifications induced by the reaction conditions i.e. catalyst (NaOH), temperature and solvents over the time of reaction improves the surface area and pore volumes of the lignocellulose structure through delignification (polyols are highly delignifying solvents) and hemicellulose removal/degradations which increases reactivity of the remainder solid residue (mainly cellulose) (Garrote, Dominguez, et al., 1999). Delignification is reported high in these fractionations as reported in section 4.4.3 which partly explains the dissolution of cellulose.

Despite high dissolution of cellulose, under all fractionation conditions the amount of glucose recovered from the liquid fractions is below 2.5g/100g of glucose initially in the material. This is a recovery of 5.3% and 6.2% of raw material glucose in the liquor for *E. grandis* and sugarcane bagasse respectively. This finding is similar to SCB fractionation studies of Moghaddam et al., (2014) who reported insignificant (<10% of raw material glucose) cellulose recovery with ethylene glycol solutions for 30minutes at 130°C. Similar results of <1.1g/100g of glucose from raw *Eucalyptus globulus* hydrothermolysis treatment at temperatures up to 200 °C for different time periods was reported by Castro et al., (2013). This occurrence is attributed on two main factors;

- Partial degradation of cellulose dissolved in the liquor. Based on estimated degraded cellulose in Tables 17-22, the fractionation processes indicates major degradation of estimated solubilized cellulose (solubilisation of up to 30.87% and 30.91% of raw glucose in *E. grandis* and sugarcane bagasse under xylitol fractionations, while up to 40.36% and 39.73% of raw glucose was dissolved from *E. grandis* and sugarcane bagasse in ethylene glycol fractionations) as confirmed by poor recoveries (<6.2% of initial glucose in both raw material). Drastic reaction conditions are said to contribute to the loss by means of thermal destruction of carbohydrates at temperatures above 200°C for cellulose during fractionation (Emmel, Mathias, Wypych, & Ramos, 2003) and with the aid of longer residence times (Emmel et al., 2003) such as up to 5 hours utilized in this study, low recoveries of cellulose in solution are evident (Table 17-22). Additionally, Emmel et al., 2003, further argues that at extreme conditions, some components may be lost as volatiles, which may have been tricky to contain in this experimental setup. Others (Jacobsen & Wyman, 2000; Lima et al., 2013) also suggest the uncounted cellulose may have been dissolved into its glucose units in solvent solution first and then degraded/broken to smaller HPLC detectable components such as 5-hydroxymethylfurfural (5-HMF). Analysis of these components was not done in this study.

- Carbohydrate molecules such as glucose are known to form a class of stable molecules called alkyl glucosides (Deng et al., 2014) also referred to as glycol-glucoside for glucose derivatives (Zhang, Rackemann, et al., 2013) which are not necessarily degraded glucose molecules but its polymorph or conformation. This reaction route for glucose into glucosides is reported for ethylene glycol fractionations of sugarcane bagasse (Zhang et al., 2013) and glycerol (Zhang, Rackemann, et al., 2013; Zhang, Wong, et al., 2013) both occurring in acidic medium. Although acid was not used as part of the main fractionation processes, acid was used in the liquid fraction compositional analysis step described in section 3.1.5 (the liquid fractionation contained ethylene glycol and xylitol in various compositions), which presented a medium for the formation of glucosides (Jacobsen & Wyman, 2000; Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013; Zhang, Wong, et al., 2013) and which in-turn contributed to non-detection of these glucose bound molecules. Due to limited capacity, the HPLC utilized in this study could not quantify glucosides to ascertain this finding, however brown precipitates (known for glucosides precipitates) were observed in the Erlenmeyer flasks after autoclaving the hydrolysis solution.

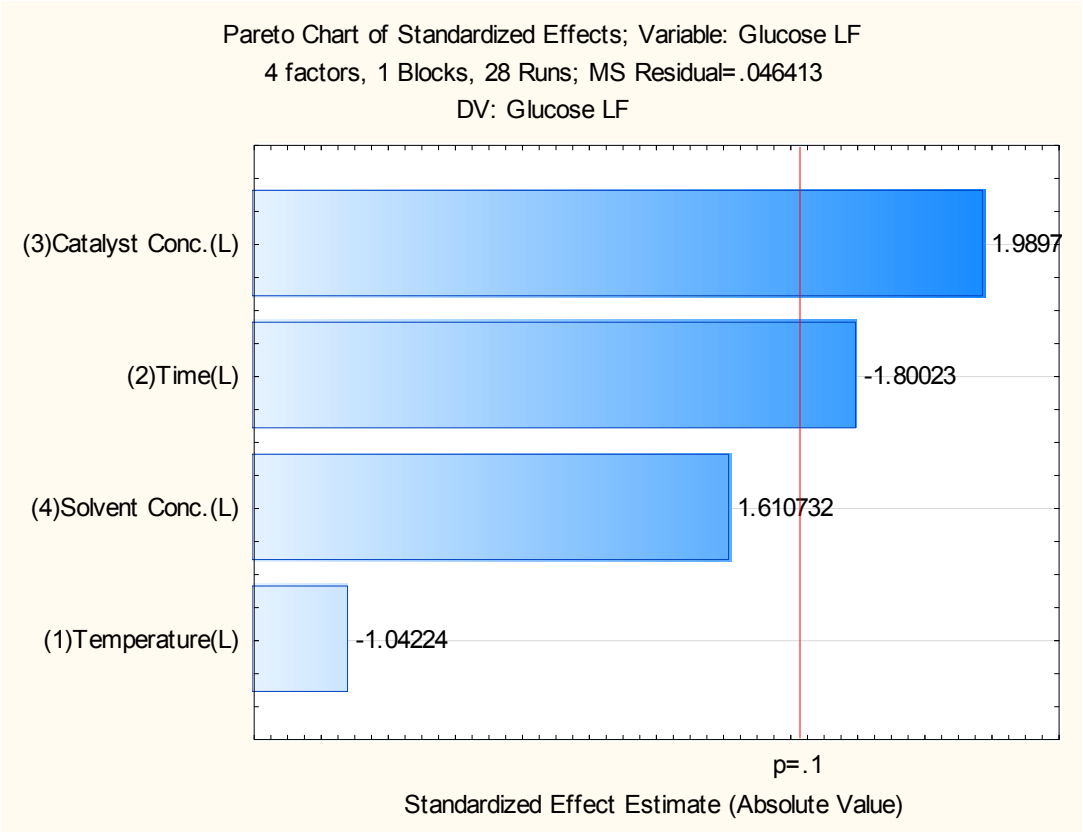


Figure 6: Pareto chart of effects EC-xylitol fractionation on glucose dissolution

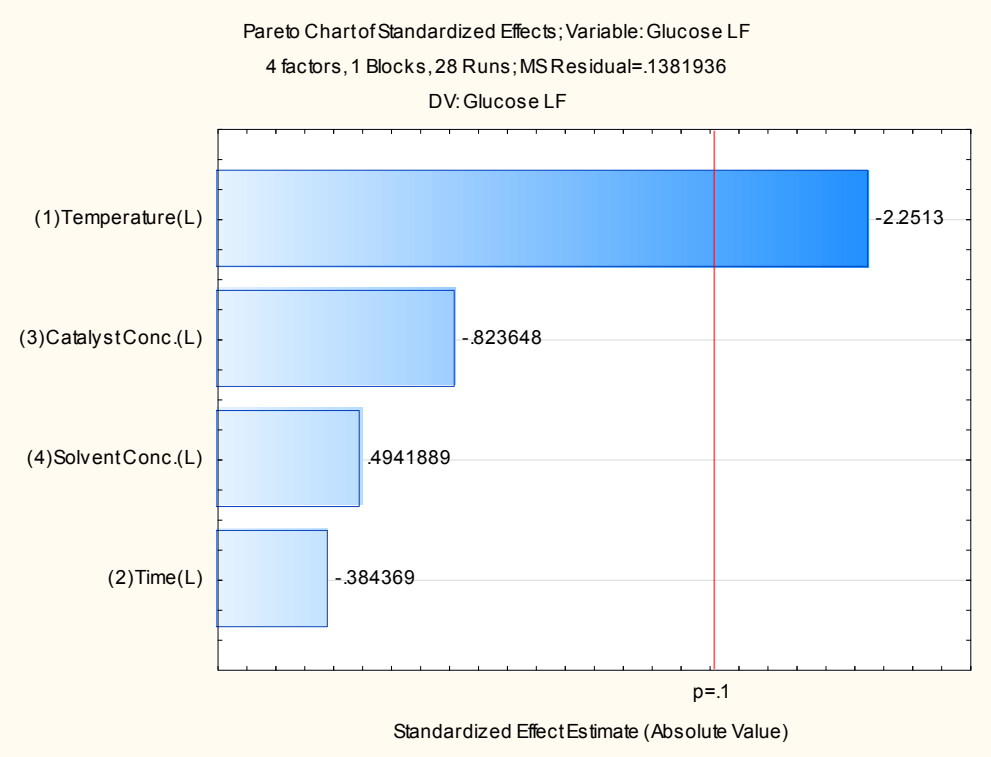


Figure 7: Pareto chart of effects SCB-xylitol fractionation on glucose dissolution

Cellulose preserved in the solid fraction varied across the fractionation runs. Fractionation with xylitol solutions preserved cellulose in the solid residue in the range of 32.80-40.90g/100g and 27.80-38.10g/100g of raw glucose in *E. grandis* and sugarcane bagasse respectively. Ethylene glycol fractionations preserved 27.80-39.30g/100g and 24.60-40.80g/100g of raw glucose in *E. grandis* and sugarcane bagasse respectively. These represents glucose preservation in the residual solid in the range of 69.13-85.39% and 69.09-94.68% of raw glucose in *E. grandis* and sugarcane bagasse under xylitol fractionations, 58.38-82.82% and 61.13-99.9% of raw glucose was preserved in the residual solids of *E. grandis* and sugarcane bagasse under ethylene glycol fractionations. This presents a significant differential between the two solvents in as far as their ability to retain and solubilize cellulose is subject. The minimum preservation of cellulose in the residual solid reported for ethylene glycol fractions are 20% below the minimum preservation generally reported in literature (80% is generally acceptable, Table 5) while the minimum preservation obtained with xylitol fractionations are approximately 10% below generally accepted preservation values as presented earlier in Table 5. However, these ranges cover the target preservation of 80% of initial cellulose in the residual solid and also comparable to values obtained by Zhang, Rackemann, et al., (2013) who fractionated SCB with glycerol at 90°C for 30 minutes obtaining 80% glucose preservation. Others (Castro et al., 2013) *Eucalyptus globulus* subjected to hydrothermolysis at temperatures up to 200°C over different time periods produced solid pulps with almost unaltered cellulose content between 44.2-45.6g, representing above 80% yield of the original cellulose in the raw material.

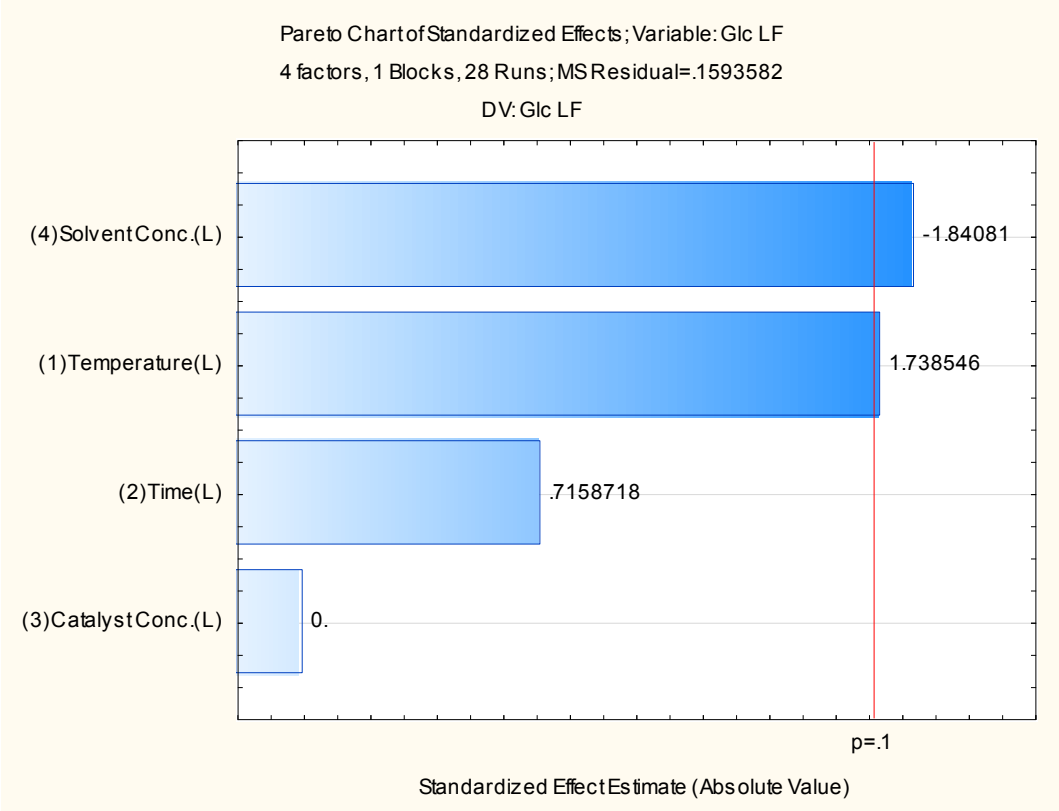


Figure 8: Pareto chart of effects EC-EG on Glucose dissolution

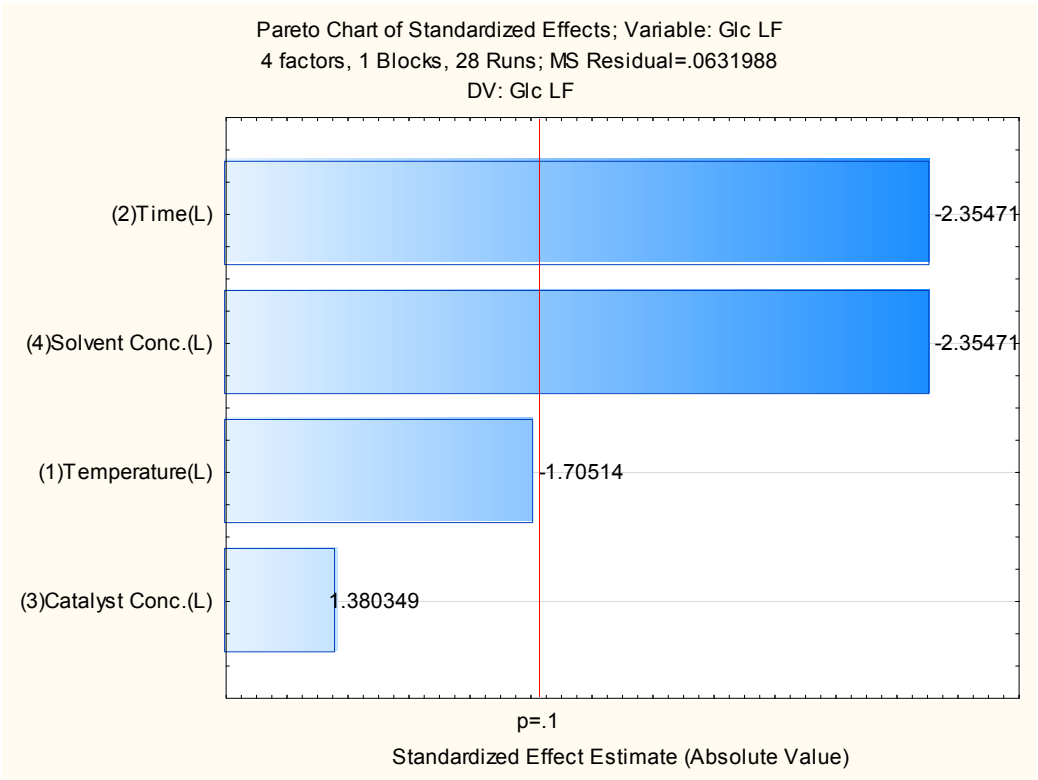


Figure 9: Pareto chart of effects SCB-EG on Glucose dissolution

In conclusion, xylitol fractionations of the two materials achieved the 80% target of keeping cellulose in the solid residue, while ethylene glycol fractionations preserved below 70% of the initial raw material cellulose. This suggests that ethylene glycol is more selective towards cellulose dissolution than xylitol solutions. For xylitol fractionations, this also means minimal destruction of the cellulose component, as compared to ethylene glycol. As discussed earlier, the reactivity of ethylene glycol on cellulose (causing cellulose dissolution) is explained by the small molecular structure of ethylene glycol (62.07g/mol and 2 carbons atoms) as compared to xylitol (152.15g/mol and five carbon atoms), smaller molecules are able to penetrate the pores of the lignocellulose structure to reach enclosed structures such as cellulose easily and also relative to their low viscosities, dissolution of cellulose is eminent (Jian et al., 2013; Singh & Ekhe, 2014). Based on these observations, xylitol is therefore the ideal solvent for preserving cellulose in *E. grandis* and sugarcane bagasse solid residues when compared to ethylene glycol fractionations (Table 17-22).

Table 17: Cellulose mass balance and composition of liquid fraction and solid residue from xylitol fractionation of raw *E. grandis*

Fractionation Conditions						Glucose in liquid fraction (g/100g)						
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Recovered	Dissolved	Glucose Degraded (g/100g)	Glucose in Residual Solid (g/100g)	EH Efficiency (%)	Mass Balance (g/100g)	Recovery (%)
Raw Material Composition									47.45	21.08	47.45	100.00
1	20	1	140	2	79.60	1.00	9.35	8.35	38.10	23.90	39.10	82.40
2	30	1	140	2	81.60	1.10	7.05	5.95	40.40	21.70	41.50	87.46
3	20	2	140	2	80.00	1.00	10.25	9.25	37.20	25.30	38.20	80.51
4	30	2	140	2	78.30	1.20	7.65	6.45	39.80	29.50	41.00	86.41
5	20	1	140	4	83.00	0.90	9.55	8.65	37.90	23.80	38.80	81.77
6	30	1	140	4	81.70	0.90	9.05	8.15	38.40	25.10	39.30	82.82
7	20	2	140	4	79.70	1.10	14.65	13.55	32.80	44.80	33.90	71.44
8	30	2	140	4	76.00	1.40	4.65	3.25	42.80	35.90	44.20	93.15
9	20	1	180	2	66.80	0.90	13.85	12.95	33.60	53.40	34.50	72.71
10	30	1	180	2	66.70	1.10	10.35	9.25	37.10	59.30	38.20	80.51
11	20	2	180	2	57.70	1.30	9.05	7.75	38.40	68.80	39.70	83.67
12	30	2	180	2	59.00	1.40	4.55	3.15	42.90	61.20	44.30	93.36
13	20	1	180	4	64.80	1.00	5.25	4.25	42.20	51.10	43.20	91.04
14	30	1	180	4	47.00	1.40	14.75	13.35	32.70	45.20	34.10	71.87
15	20	2	180	4	52.40	0.20	6.75	6.55	40.70	52.90	40.90	86.20
16	30	2	180	4	54.30	1.10	6.15	5.05	41.30	55.20	42.40	89.36
17	25	1.5	120	3	82.10	1.20	5.15	3.95	42.30	15.00	43.50	91.68
18	25	1.5	200	3	63.90	1.00	9.35	8.35	38.10	62.20	39.10	82.40
19	25	1.5	160	1	81.80	1.10	8.85	7.75	38.60	27.70	39.70	83.67
20	25	1.5	160	5	63.10	0.90	14.65	13.75	32.80	59.60	33.70	71.02
21	25	0.5	160	3	57.60	0.80	13.55	19.75	33.90	51.00	34.70	73.12
22	25	2.5	160	3	62.00	1.40	13.75	12.35	33.70	59.60	35.10	73.97
23	15	1.5	160	3	70.60	1.20	11.95	10.75	35.50	38.40	36.70	77.34
24	35	1.5	160	3	67.40	1.20	14.65	13.45	32.80	58.00	34.00	71.65
25	25	1.5	160	3	68.80	1.00	11.45	10.45	36.00	40.30	37.00	77.98
26	25	1.5	160	3	76.20	1.20	9.35	8.15	38.10	34.70	39.30	82.82
27	25	1.5	160	3	70.10	1.30	14.05	12.75	33.40	32.00	34.70	73.13
28	25	1.5	160	3	72.20	1.30	13.35	12.05	34.10	34.90	35.40	74.60

Table 18: Cellulose mass balance and composition of liquid fraction and solid residue from ethylene glycol fractionation of raw *E. grandis*

Fractionation Conditions						Glucose in liquid fraction (g/100g)		Glucose Degraded (g/100g)	Glucose in Residual Solid (g/100g)	EH Efficiency (%)	Mass Balance (g/100g)	
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Recovered	Dissolved					Recovery (%)
Raw Material Composition									47.45	21.08	47.45	100.00
1	80	1	140	2	77.20	0.70	8.15	7.45	39.30	14.00	40.00	84.30
2	90	1	140	2	68.70	0.30	16.35	16.05	31.10	20.50	31.40	66.17
3	80	2	140	2	61.70	0.40	13.85	13.45	33.60	35.20	34.00	71.65
4	90	2	140	2	69.40	0.10	18.25	18.15	29.20	31.60	29.30	61.75
5	80	1	140	4	55.40	0.10	18.85	18.75	28.60	28.70	28.70	60.48
6	90	1	140	4	62.90	0.10	17.75	17.65	29.70	16.60	29.80	62.80
7	80	2	140	4	66.00	0.50	18.15	17.65	29.30	46.00	29.80	62.80
8	90	2	140	4	63.40	0.10	12.65	12.55	34.80	33.50	34.90	73.55
9	80	1	180	2	53.50	0.50	17.85	17.35	29.60	41.90	30.10	63.44
10	90	1	180	2	64.60	0.20	10.65	10.45	36.80	33.10	37.00	77.98
11	80	2	180	2	52.50	0.30	17.65	17.35	29.80	82.60	30.10	63.44
12	90	2	180	2	57.70	0.20	12.95	12.75	34.50	58.80	34.70	73.13
13	80	1	180	4	55.30	2.10	19.85	17.75	27.60	89.60	29.70	62.59
14	90	1	180	4	58.00	0.80	14.05	13.25	33.40	74.30	34.20	72.08
15	80	2	180	4	46.00	0.80	19.75	18.95	27.70	76.30	28.50	60.06
16	90	2	180	4	43.20	0.80	19.05	18.25	28.40	79.20	29.20	61.54
17	85	1.5	120	3	75.40	0.70	10.85	10.15	36.60	16.90	37.30	78.61
18	85	1.5	200	3	37.00	0.70	15.85	15.15	31.60	54.30	32.30	68.07
19	25	1.5	160	1	69.90	0.90	17.15	16.25	30.30	33.90	31.20	65.75
20	25	1.5	160	5	56.10	0.30	18.85	18.55	28.60	89.10	28.90	60.91
21	25	0.5	160	3	67.90	0.40	12.95	12.55	34.50	26.00	34.90	73.55
22	25	2.5	160	3	68.60	1.20	12.45	11.25	35.00	48.10	36.20	76.29
23	15	1.5	160	3	68.20	0.60	18.35	17.75	29.10	29.80	29.70	62.59
24	35	1.5	160	3	69.50	0.20	15.25	15.05	32.20	28.70	32.40	68.28
25	25	1.5	160	3	64.90	0.90	16.95	16.05	30.50	61.20	31.40	66.17
26	25	1.5	160	3	61.00	0.50	17.65	17.15	29.80	51.80	30.30	63.86
27	25	1.5	160	3	57.70	0.70	18.25	17.55	29.20	56.50	29.90	63.01
28	25	1.5	160	3	61.60	0.50	18.35	17.85	29.10	52.70	29.60	62.38

Table 19: Cellulose mass balance and composition of liquid fraction and solid residue from xylitol fractionation of raw sugarcane bagasse

Fractionation Conditions						Glucose in liquid fraction (g/100g)		Glucose Degraded (g/100g)	Glucose in Residual Solid (g/100g)	EH Efficiency (%)	Mass Balance (g/100g)	
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Recovered	Dissolved					Recovery (%)
Raw Material Composition									40.24	12.76	40.24	100
1	20	1	140	2	77.40	1.30	6.64	5.34	33.60	17.20	34.90	86.73
2	30	1	140	2	82.40	1.10	5.04	3.94	35.20	20.20	36.30	90.21
3	20	2	140	2	76.30	0.90	4.44	3.54	35.80	17.50	36.70	91.20
4	30	2	140	2	73.90	0.50	8.34	7.84	31.90	10.10	32.40	80.52
5	20	1	140	4	73.40	1.10	11.94	10.84	28.30	21.90	29.40	73.06
6	30	1	140	4	71.20	1.20	7.24	6.04	33.00	13.80	34.20	84.99
7	20	2	140	4	58.50	0.80	2.14	1.34	38.10	14.50	38.90	96.67
8	30	2	140	4	73.90	1.10	6.94	5.84	33.30	12.10	34.40	85.49
9	20	1	180	2	64.40	1.00	7.74	6.74	32.50	27.40	33.50	83.25
10	30	1	180	2	63.60	1.00	8.44	7.44	31.80	33.10	32.80	81.51
11	20	2	180	2	54.80	0.30	8.74	8.44	31.50	34.20	31.80	79.03
12	30	2	180	2	59.80	0.80	7.24	6.44	33.00	27.60	33.80	84.00
13	20	1	180	4	62.00	0.10	6.94	6.84	33.30	37.20	33.40	83.00
14	30	1	180	4	63.80	0.50	6.54	6.04	33.70	43.30	34.20	84.99
15	20	2	180	4	56.10	0.50	10.84	10.34	29.40	42.80	29.90	74.30
16	30	2	180	4	59.90	0.90	7.14	6.24	33.10	38.60	34.00	84.49
17	25	1.5	120	3	84.30	0.90	5.04	4.14	35.20	10.70	36.10	89.71
18	25	1.5	200	3	55.00	0.30	12.44	12.14	27.80	22.80	28.10	69.83
19	25	1.5	160	1	83.50	0.80	3.34	2.54	36.90	10.50	37.70	93.69
20	25	1.5	160	5	63.50	0.80	7.04	6.24	33.20	26.30	34.00	84.49
21	25	0.5	160	3	79.50	1.40	3.34	1.94	36.90	12.80	38.30	95.18
22	25	2.5	160	3	73.40	1.40	3.54	2.14	36.70	21.80	38.10	94.68
23	15	1.5	160	3	69.90	1.40	5.54	4.14	34.70	21.00	36.10	89.71
24	35	1.5	160	3	67.20	1.30	7.24	5.94	33.00	17.00	34.30	85.24
25	25	1.5	160	3	64.00	1.40	8.44	7.04	31.80	28.30	33.20	82.50
26	25	1.5	160	3	62.80	1.50	9.84	8.34	30.40	29.00	31.90	79.27
27	25	1.5	160	3	67.70	1.40	9.94	8.54	30.30	24.00	31.70	78.78
28	25	1.5	160	3	64.20	1.20	9.94	8.74	30.30	27.70	31.50	78.28

Table 20: Cellulose mass balance and composition of liquid fraction and solid residue from ethylene glycol fractionation of raw sugarcane bagasse

Fractionation Conditions						Glucose in liquid fraction (g/100g)		Glucose Degraded (g/100g)	Glucose in Residual Solid (g/100g)	EH Efficiency (%)	Mass Balance (g/100g)	
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Recovered	Dissolved					Recovery (%)
Raw Material Composition									40.24	12.76	40.24	100.00
1	80	1	140	2	79.00	0.70	4.94	4.24	35.30	18.90	36.00	89.46
2	90	1	140	2	81.20	0.30	9.14	8.84	31.10	74.90	31.40	78.03
3	80	2	140	2	65.40	0.40	8.14	7.74	32.10	29.80	32.50	80.77
4	90	2	140	2	76.10	0.10	12.54	12.44	27.70	37.00	27.80	69.09
5	80	1	140	4	79.90	0.10	11.04	10.94	29.20	86.10	29.30	72.81
6	90	1	140	4	79.70	0.10	6.84	6.74	33.40	27.00	33.50	83.25
7	80	2	140	4	69.10	0.50	10.74	10.24	29.50	38.90	30.00	74.55
8	90	2	140	4	72.10	0.10	10.84	10.74	29.40	39.20	29.50	73.31
9	80	1	180	2	83.10	0.50	6.54	6.04	33.70	40.50	34.20	84.99
10	90	1	180	2	66.90	0.20	9.74	9.54	30.50	49.00	30.70	76.29
11	80	2	180	2	54.70	0.30	8.84	8.54	31.40	47.90	31.70	78.78
12	90	2	180	2	62.80	0.20	16.24	16.04	24.00	64.40	24.20	60.14
13	80	1	180	4	49.70	2.10	13.44	11.34	26.80	76.80	28.90	71.82
14	90	1	180	4	82.90	0.80	0.34	-0.46	39.90	35.30	40.70	101.14
15	80	2	180	4	68.40	0.80	3.84	3.04	36.40	58.10	37.20	92.45
16	90	2	180	4	55.80	0.80	6.94	6.14	33.30	61.40	34.10	84.74
17	85	1.5	120	3	77.30	0.70	2.34	1.64	37.90	24.60	38.60	95.92
18	85	1.5	200	3	57.40	0.70	10.44	9.74	29.80	68.70	30.50	75.80
19	25	1.5	160	1	79.90	0.90	3.94	3.04	36.30	30.20	37.20	92.45
20	25	1.5	160	5	69.70	0.30	10.24	9.94	30.00	36.40	30.30	75.30
21	25	0.5	160	3	75.60	0.40	11.44	11.04	28.80	23.30	29.20	72.56
22	25	2.5	160	3	69.10	1.20	15.64	14.44	24.60	50.80	25.80	64.12
23	15	1.5	160	3	72.80	0.60	8.14	7.54	32.10	40.50	32.70	81.26
24	35	1.5	160	3	76.90	0.20	6.34	6.14	33.90	65.30	34.10	84.74
25	25	1.5	160	3	68.20	0.90	5.94	5.04	34.30	44.60	35.20	87.48
26	25	1.5	160	3	71.20	0.50	0.14	-0.36	40.30	36.50	40.80	101.39
27	25	1.5	160	3	73.20	0.70	2.44	1.74	37.80	34.90	38.50	95.68
28	25	1.5	160	3	87.00	0.50	1.44	0.94	38.80	38.10	39.30	97.66

Table 21: Cellulose mass balance and composition of liquid fraction and solid residue from hemicellulose pre-extracted *E. grandis* fractionated with ethylene glycol

	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Glucose in liquid fraction (g/100g)		Glucose in residual Solid (g/100g)	Glucose Degraded (g/100g)	Mass balance (g/100g)	Recovery (%)	EH Efficiency (%)
						Dissolved	Recovered					
Initial Raw SCB								47.45	0.00	47.45	100.00	21.08
Hemis Pre-extracted *E. grandis*								42.44	0.00	42.44	100.00	35.39
1	60	1	140	2	88.9	21.74	0.2	20.70	21.54	20.90	49.25	54.10
2	60	2	140	2	83.9	17.74	0.6	24.70	17.14	25.30	59.61	60.60
3	60	1	140	4	84.9	7.84	0.6	34.60	7.24	35.20	82.94	48.10
4	60	2	140	4	75.3	17.54	0.7	24.90	16.84	25.60	60.32	85.50
5	60	1	180	2	70.4	13.74	0.9	28.70	12.84	29.60	69.75	78.40
6	60	2	180	2	44.7	24.64	0.8	17.80	23.84	18.60	43.83	89.80
7	60	1	180	4	63.4	13.24	0.6	29.20	12.64	29.80	70.22	79.60
8	60	2	180	4	55.6	22.44	0.6	20.00	21.84	20.60	48.54	93.20
9	60	1.5	126.4	3	87.1	20.24	0.6	22.20	19.64	22.80	53.72	52.90
10	60	1.5	193.6	3	54.5	19.54	0.6	22.90	18.94	23.50	55.37	72.60
11	60	1.5	160	1.3	74.5	24.54	0.4	17.90	24.14	18.30	43.12	91.40
12	60	1.5	160	4.7	65.9	18.74	0.5	23.70	18.24	24.20	57.02	76.50
13	60	0.7	160	3	78.3	15.24	0.4	27.20	14.84	27.60	65.03	76.00
14	60	2.3	160	3	64.8	18.64	0.2	23.80	18.44	24.00	56.55	85.50
15	60	1.5	160	3	69.6	16.84	0.4	25.60	16.44	26.00	61.26	90.20
16	60	1.5	160	3	68	16.94	0.4	25.50	16.54	25.90	61.03	93.40
17	60	1.5	160	3	70.8	18.24	0.6	24.20	17.64	24.80	58.44	94.50
18	60	1.5	160	3	69.8	15.34	0.5	27.10	14.84	27.60	65.03	93.00

Table 22: Cellulose mass balance and composition of liquid fraction and solid residue from hemicellulose pre-extracted sugarcane bagasse fractionated with ethylene glycol

	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Glucose in liquid fraction (g/100g)		Glucose in residual Solid (g/100g)	Glucose Degraded (g/100g)	Mass balance (g/100g)	Recovery (%)	EH Efficiency (%)
						Dissolved	Recovered					
Initial Raw SCB								40.24	0	40.24	100	12.76
Hemis Pre-extracted SCB								35.39	0	35.39	100	29.52
1	60	1	140	2	60.9	7.29	0.20	28.10	7.09	28.30	79.97	82.20
2	60	2	140	2	56.6	8.59	0.60	26.80	7.99	27.40	77.42	87.90
3	60	1	140	4	56	8.59	0.50	26.80	8.09	27.30	77.14	81.50
4	60	2	140	4	56.4	8.59	0.60	26.80	7.99	27.40	77.42	76.10
5	60	1	180	2	60	7.39	0.70	28.00	6.69	28.70	81.10	76.60
6	60	2	180	2	69.7	0.59	1.20	34.80	-0.61	36.00	101.72	75.70
7	60	1	180	4	74.5	0.19	1.40	35.20	-1.21	36.60	103.42	73.00
8	60	2	180	4	68.6	0.69	2.10	34.70	-1.41	36.80	103.98	33.10
9	60	1.5	126.4	3	77.9	-0.71	1.50	36.10	-2.21	37.60	106.24	72.80
10	60	1.5	193.6	3	68.7	0.59	1.40	34.80	-0.81	36.20	102.29	57.10
11	60	1.5	160	1.3	55.3	9.59	0.60	25.80	8.99	26.40	74.60	76.30
12	60	1.5	160	4.7	74	-0.61	1.20	36.00	-1.81	37.20	105.11	62.60
13	60	0.7	160	3	76.8	2.69	0.80	32.70	1.89	33.50	94.66	40.10
14	60	2.3	160	3	70.4	3.99	0.40	31.40	3.59	31.80	89.86	68.60
15	60	1.5	160	3	74.9	4.29	0.20	31.10	4.09	31.30	88.44	68.10
16	60	1.5	160	3	75.4	3.69	0.30	31.70	3.39	32.00	90.42	74.70
17	60	1.5	160	3	74.7	4.59	0.70	30.80	3.89	31.50	89.01	63.20
18	60	1.5	160	3	74.7	4.89	0.90	30.50	3.99	31.40	88.73	70.90

4.4.3 Hemicelluloses recovery in the liquid fraction

The goal of this study with regard to the hemicellulose component fractionation was to achieve the following specific objectives as highlighted in Chapter 2;

1. Dissolve >80% of hemicelluloses and recover more than 70% of hemicelluloses in the liquid fraction with subsequent extraction with an anti-solvent.

2. Recovered hemicelluloses should also be of polymeric form with minimum molecular weight average of 10 000 gmol-1.

3. To determine and understand the effect of set fractionation parameters such as temperature, catalyst and solvent concentration and time of retention on the fragmentation of eucalyptus into the respective components (lignin, hemicellulose and cellulose) and use these to optimize best fractionation conditions that maximize components' yields and purity.

This section addresses target objectives 1 and 3, whilst objective 2 is further discussed in the following chapter. Recovery and mass balances of xylose are presented in Table 23-28. Xylose is the poorest recovered component of the three as observed in the mass balances data in Tables 23-28. Mass balances for xylitol fractionations are in the range of 42.11-81.82% and 45.66-96.79% for eucalyptus and sugarcane bagasse hemicelluloses respectively, while 50.24-75.60% and 57.39-92.51% added up for eucalyptus and sugarcane bagasse after ethylene glycol fractionations. Sugarcane bagasse reported slightly higher mass balances than eucalyptus with the highest, 96.79% and 92.51% from xylitol and ethylene glycol fractionations respectively. This can be explained by the proportionate amount of more hemicelluloses in sugarcane bagasse (23.35g/100g of raw material) than *E. grandis* (20.90g/100g of raw material). Hemicellulose losses and "disappearance" after fractionation has been widely reported in the literature (da Costa Lopes et al., 2013; Inoue et al., 2008; Katahira et al., 2013; Wetterling, 2012; B. Yang et al., 2011). This is mainly due to excessive hydrolysis of the hemicelluloses and eventual degradation under severe conditions i.e. around run 13-16 were temperatures are elevated to 180°C (see Table 23-28). It is also established that some carbohydrates including hemicelluloses re-combines with dissolved lignin in solution(Winkler, 1981; Xiang, Lee, & Torget, 2004) which may not necessarily be detected in the liquid fraction (Zhang, Rackemann, et al., 2013). In the case of severe fractionation conditions, other reports suggest analyzing products of hemicellulose degradation in order to account for the overall balance (Katahira et al., 2013; Ma et al., 2014).

Table 23: Hemicellulose mass balance and composition of liquid fraction and solid residue from xylitol fractionation of raw *E. grandis*

Fractionation Conditions						Xylose in the liquid fraction (g/100g)		Xylose Degraded (g/100g)	Xylose in Residual Solid (g/100g)	Mass Balance (g/100g)	Recovery (%)
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered				
Raw Material Composition									20.90	20.90	100.00
1	20	1	140	2	79.60	8.70	2.50	6.20	12.20	14.70	70.33
2	30	1	140	2	81.60	7.90	1.70	6.20	13.00	14.70	70.33
3	20	2	140	2	80.00	9.10	2.10	7.00	11.80	13.90	66.51
4	30	2	140	2	78.30	8.60	1.50	7.10	12.30	13.80	66.03
5	20	1	140	4	83.00	8.30	1.90	6.40	12.60	14.50	69.38
6	30	1	140	4	81.70	8.70	1.90	6.80	12.20	14.10	67.46
7	20	2	140	4	79.70	10.70	2.80	7.90	10.20	13.00	62.20
8	30	2	140	4	76.00	12.80	3.00	9.80	8.10	11.10	53.11
9	20	1	180	2	66.80	10.20	4.70	5.50	10.70	15.40	73.68
10	30	1	180	2	66.70	12.70	3.00	9.70	8.20	11.20	53.59
11	20	2	180	2	57.70	12.20	5.20	7.00	8.70	13.90	66.51
12	30	2	180	2	59.00	11.60	5.80	5.80	9.30	15.10	72.25
13	20	1	180	4	64.80	13.90	4.70	9.20	7.00	11.70	55.98
14	30	1	180	4	47.00	13.60	3.80	9.80	7.30	11.10	53.11
15	20	2	180	4	52.40	12.50	3.60	8.90	8.40	12.00	57.42
16	30	2	180	4	54.30	11.70	3.50	8.20	9.20	12.70	60.77
17	25	1.5	120	3	82.10	5.80	1.80	4.00	15.10	16.90	80.86
18	25	1.5	200	3	63.90	14.40	2.30	12.10	6.50	8.80	42.11
19	25	1.5	160	1	81.80	7.00	3.20	3.80	13.90	17.10	81.82
20	25	1.5	160	5	63.10	11.40	4.50	6.90	9.50	14.00	66.99
21	25	0.5	160	3	57.60	14.00	6.40	7.60	6.90	13.30	63.64
22	25	2.5	160	3	62.00	12.30	4.30	8.00	8.60	12.90	61.72
23	15	1.5	160	3	70.60	11.10	5.50	5.60	9.80	15.30	73.21
24	35	1.5	160	3	67.40	10.70	4.20	6.50	10.20	14.40	68.90
25	25	1.5	160	3	68.80	10.90	6.50	4.40	10.00	16.50	78.95
26	25	1.5	160	3	76.20	9.40	4.50	4.90	11.50	16.00	76.56
27	25	1.5	160	3	70.10	12.90	4.40	8.50	8.00	12.40	59.33
28	25	1.5	160	3	72.20	12.80	5.60	7.20	8.10	13.70	65.55

Table 24: Hemicellulose mass balance and composition of liquid fraction and solid residue from ethylene glycol fractionation of raw *E. grandis*

Fractionation Conditions						Xylose in the liquid fraction (g/100g)		Xylose Degraded (g/100g)	Xylose in Residual Solid (g/100g)	Mass Balance (g/100g)	Recovery (%)
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered				
Raw Material Composition									20.90	20.90	100.00
1	80	1	140	2	77.20	9.40	0.40	9.00	11.50	11.90	56.94
2	90	1	140	2	68.70	9.80	0.40	9.40	11.10	11.50	55.02
3	80	2	140	2	61.70	8.50	0.20	8.30	12.40	12.60	60.29
4	90	2	140	2	69.40	10.50	0.10	10.40	10.40	10.50	50.24
5	80	1	140	4	55.40	9.80	0.00	9.80	11.10	11.10	53.11
6	90	1	140	4	62.90	10.20	0.10	10.10	10.70	10.80	51.67
7	80	2	140	4	66.00	10.30	0.50	9.80	10.60	11.10	53.11
8	90	2	140	4	63.40	10.60	0.40	10.20	10.30	10.70	51.20
9	80	1	180	2	53.50	10.40	0.40	10.00	10.50	10.90	52.15
10	90	1	180	2	64.60	9.80	0.10	9.70	11.10	11.20	53.59
11	80	2	180	2	52.50	10.40	0.10	10.30	10.50	10.60	50.72
12	90	2	180	2	57.70	10.00	0.00	10.00	10.90	10.90	52.15
13	80	1	180	4	55.30	11.60	2.10	9.50	9.30	11.40	54.55
14	90	1	180	4	58.00	10.20	2.20	8.00	10.70	12.90	61.72
15	80	2	180	4	46.00	12.30	2.00	10.30	8.60	10.60	50.72
16	90	2	180	4	43.20	11.90	1.80	10.10	9.00	10.80	51.67
17	85	1.5	120	3	75.40	8.00	0.60	7.40	12.90	13.50	64.59
18	85	1.5	200	3	37.00	12.30	2.10	10.20	8.60	10.70	51.20
19	25	1.5	160	1	69.90	9.10	1.20	7.90	11.80	13.00	62.20
20	25	1.5	160	5	56.10	10.50	0.80	9.70	10.40	11.20	53.59
21	25	0.5	160	3	67.90	10.60	1.00	9.60	10.30	11.30	54.07
22	25	2.5	160	3	68.60	11.70	3.40	8.30	9.20	12.60	60.29
23	15	1.5	160	3	68.20	8.80	3.70	5.10	12.10	15.80	75.60
24	35	1.5	160	3	69.50	8.30	0.40	7.90	12.60	13.00	62.20
25	25	1.5	160	3	64.90	8.60	2.40	6.20	12.30	14.70	70.33
26	25	1.5	160	3	61.00	10.20	1.00	9.20	10.70	11.70	55.98
27	25	1.5	160	3	57.70	10.80	1.00	9.80	10.10	11.10	53.11
28	25	1.5	160	3	61.60	9.80	1.30	8.50	11.10	12.40	59.33

Table 25: Hemicellulose mass balance and composition of liquid fraction and solid residue from xylitol fractionation of raw sugarcane bagasse

Fractionation Conditions						Xylose in the liquid fraction (g/100g)		Xylose Degraded (g/100g)	Xylose in Residual Solid (g/100g)	Mass Balance (g/100g)	
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered				Recovery (%)
Raw Material Composition									23.35	23.35	100
1	20	1	140	2	77.40	7.05	4.40	2.65	16.30	20.70	88.65
2	30	1	140	2	82.40	5.85	3.30	2.55	17.50	20.80	89.08
3	20	2	140	2	76.30	9.05	4.50	4.55	14.30	18.80	80.51
4	30	2	140	2	73.90	7.15	2.40	4.75	16.20	18.60	79.66
5	20	1	140	4	73.40	12.45	5.70	6.75	10.90	16.60	71.09
6	30	1	140	4	71.20	10.85	5.50	5.35	12.50	18.00	77.09
7	20	2	140	4	58.50	7.45	6.30	1.15	15.90	22.20	95.07
8	30	2	140	4	73.90	8.05	5.40	2.65	15.30	20.70	88.65
9	20	1	180	2	64.40	8.55	3.00	5.55	14.80	17.80	76.23
10	30	1	180	2	63.60	11.85	3.10	8.75	11.50	14.60	62.53
11	20	2	180	2	54.80	14.05	3.30	10.75	9.30	12.60	53.96
12	30	2	180	2	59.80	13.05	2.60	10.45	10.30	12.90	55.25
13	20	1	180	4	62.00	13.20	1.90	11.30	10.15	12.05	51.61
14	30	1	180	4	63.80	13.21	1.80	11.41	10.14	11.94	51.13
15	20	2	180	4	56.10	13.23	2.00	11.23	10.12	12.12	51.91
16	30	2	180	4	59.90	13.55	3.80	9.75	9.80	13.60	58.24
17	25	1.5	120	3	84.30	6.65	2.60	4.05	16.70	19.30	82.66
18	25	1.5	200	3	55.00	10.95	0.80	10.15	12.40	13.20	56.53
19	25	1.5	160	1	83.50	7.15	5.70	1.45	16.20	21.90	93.79
20	25	1.5	160	5	63.50	14.45	3.10	11.35	8.90	12.00	51.39
21	25	0.5	160	3	79.50	10.75	10.00	0.75	12.60	22.60	96.79
22	25	2.5	160	3	73.40	9.65	7.90	1.75	13.70	21.60	92.51
23	15	1.5	160	3	69.90	11.45	7.20	4.25	11.90	19.10	81.80
24	35	1.5	160	3	67.20	14.15	7.90	6.25	9.20	17.10	73.23
25	25	1.5	160	3	64.00	13.55	9.00	4.55	9.80	18.80	80.51
26	25	1.5	160	3	62.80	13.35	8.50	4.85	10.00	18.50	79.23
27	25	1.5	160	3	67.70	13.15	9.40	3.75	10.20	19.60	83.94
28	25	1.5	160	3	64.20	13.85	7.80	6.05	9.50	17.30	74.09

Table 26: Hemicellulose mass balance and composition of liquid fraction and solid residue from ethylene glycol fractionation of raw sugarcane bagasse

Fractionation Conditions						Xylose in the liquid fraction (g/100g)		Xylose Degraded (g/100g)	Xylose in Residual Solid (g/100g)	Mass Balance (g/100g)	
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered				Recovery (%)
Raw Material composition									23.35	23.35	100.00
1	80	1	140	2	79.00	4.95	1.80	3.15	18.40	20.20	86.51
2	90	1	140	2	81.20	7.15	1.90	5.25	16.20	18.10	77.52
3	80	2	140	2	65.40	6.85	2.60	4.25	16.50	19.10	81.80
4	90	2	140	2	76.10	10.75	2.20	8.55	12.60	14.80	63.38
5	80	1	140	4	79.90	8.65	2.10	6.55	14.70	16.80	71.95
6	90	1	140	4	79.70	5.05	1.80	3.25	18.30	20.10	86.08
7	80	2	140	4	69.10	11.55	3.20	8.35	11.80	15.00	64.24
8	90	2	140	4	72.10	11.45	2.70	8.75	11.90	14.60	62.53
9	80	1	180	2	83.10	9.55	5.60	3.95	13.80	19.40	83.08
10	90	1	180	2	66.90	10.35	4.10	6.25	13.00	17.10	73.23
11	80	2	180	2	54.70	11.45	6.00	5.45	11.90	17.90	76.66
12	90	2	180	2	62.80	9.95	3.10	6.85	13.40	16.50	70.66
13	80	1	180	4	49.70	11.65	2.40	9.25	11.70	14.10	60.39
14	90	1	180	4	82.90	11.95	2.60	9.35	11.40	14.00	59.96
15	80	2	180	4	68.40	10.65	3.00	7.65	12.70	15.70	67.24
16	90	2	180	4	55.80	10.05	3.40	6.65	13.30	16.70	71.52
17	85	1.5	120	3	77.30	4.25	2.50	1.75	19.10	21.60	92.51
18	85	1.5	200	3	57.40	11.75	1.80	9.95	11.60	13.40	57.39
19	25	1.5	160	1	79.90	5.95	3.30	2.65	17.40	20.70	88.65
20	25	1.5	160	5	69.70	10.55	4.80	5.75	12.80	17.60	75.37
21	25	0.5	160	3	75.60	11.85	4.40	7.45	11.50	15.90	68.09
22	25	2.5	160	3	69.10	9.95	4.50	5.45	13.40	17.90	76.66
23	15	1.5	160	3	72.80	13.25	7.20	6.05	10.10	17.30	74.09
24	35	1.5	160	3	76.90	9.55	2.90	6.65	13.80	16.70	71.52
25	25	1.5	160	3	68.20	8.65	4.20	4.45	14.70	18.90	80.94
26	25	1.5	160	3	71.20	6.05	4.30	1.75	17.30	21.60	92.51
27	25	1.5	160	3	73.20	7.25	4.20	3.05	16.10	20.30	86.94
28	25	1.5	160	3	87.00	6.85	4.00	2.85	16.50	20.50	87.79

Table 27: Hemicellulose mass balance and composition of liquid fraction and solid residue from ethylene glycol fractionation of hemicellulose pre-extracted *E. grandis*

	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Xylose in liquid fraction (g/100g)		Xylose in residual Solid (g/100g)	Xylose Degraded (g/100g)	Mass balance (g/100g)	Recovery (%)
						Dissolved	Recovered				
Initial Raw SCB								20.90	0.00	20.90	100.00
Hemis Pre-extracted SCB								9.77	0.00	9.77	100.00
1	60	1	140	2	88.9	4.37	0.4	5.40	3.97	5.80	59.37
2	60	2	140	2	83.9	3.37	0.4	6.40	2.97	6.80	69.60
3	60	1	140	4	84.9	2.77	0.4	7.00	2.37	7.40	75.74
4	60	2	140	4	75.3	3.37	0.9	6.40	2.47	7.30	74.72
5	60	1	180	2	70.4	2.67	1.2	7.10	1.47	8.30	84.95
6	60	2	180	2	44.7	5.47	1.5	4.30	3.97	5.80	59.37
7	60	1	180	4	63.4	2.87	1.3	6.90	1.57	8.20	83.93
8	60	2	180	4	55.6	4.37	1.5	5.40	2.87	6.90	70.62
9	60	1.5	126.4	3	87.1	4.27	0.3	5.50	3.97	5.80	59.37
10	60	1.5	193.6	3	54.5	4.47	1.9	5.30	2.57	7.20	73.69
11	60	1.5	160	1.3	74.5	5.07	0.8	4.70	4.27	5.50	56.29
12	60	1.5	160	4.7	65.9	3.77	1	6.00	2.77	7.00	71.65
13	60	0.7	160	3	78.3	3.07	0.5	6.70	2.57	7.20	73.69
14	60	2.3	160	3	64.8	3.77	0.4	6.00	3.37	6.40	65.51
15	60	1.5	160	3	69.6	3.47	1.2	6.30	2.27	7.50	76.77
16	60	1.5	160	3	68	3.57	1.2	6.20	2.37	7.40	75.74
17	60	1.5	160	3	70.8	4.07	1.6	5.70	2.47	7.30	74.72
18	60	1.5	160	3	69.8	3.67	0.9	6.10	2.77	7.00	71.65

Table 28: Hemicellulose mass balance and composition of liquid fraction and solid residue from ethylene glycol fractionation of hemicellulose pre-extracted sugarcane bagasse

	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Xylose in liquid fraction (g/100g)		Xylose in residual Solid (g/100g)	Xylose Degraded (g/100g)	Mass balance (g/100g)	Recovery (%)
						Dissolved	Recovered				
Initial Raw SCB								23.35	0	23.35	100
Hemis Pre-extracted SCB								4.23	0	4.23	100
1	60	1	140	2	60.9	1.35	1.10	2.88	0.25	3.98	94.09
2	60	2	140	2	56.6	1.82	1.10	2.41	0.72	3.51	82.98
3	60	1	140	4	56	2.40	1.00	1.83	1.40	2.83	66.90
4	60	2	140	4	56.4	3.23	1.10	1.00	2.13	2.10	49.65
5	60	1	180	2	60	2.43	1.60	1.80	0.83	3.40	80.38
6	60	2	180	2	69.7	2.83	2.40	1.40	0.43	3.80	89.83
7	60	1	180	4	74.5	3.23	2.20	1.00	1.03	3.20	75.65
8	60	2	180	4	68.6	3.43	2.80	0.80	0.63	3.60	85.11
9	60	1.5	126.4	3	77.9	2.34	1.40	1.89	0.94	3.29	77.78
10	60	1.5	193.6	3	68.7	3.21	2.90	1.02	0.31	3.92	92.67
11	60	1.5	160	1.3	55.3	2.55	1.60	1.68	0.95	3.28	77.54
12	60	1.5	160	4.7	74	2.49	1.60	1.74	0.89	3.34	78.96
13	60	0.7	160	3	76.8	2.83	1.40	1.40	1.43	2.80	66.19
14	60	2.3	160	3	70.4	3.09	2.30	1.14	0.79	3.44	81.32
15	60	1.5	160	3	74.9	2.13	0.90	2.10	1.23	3.00	70.92
16	60	1.5	160	3	75.4	1.93	0.90	2.30	1.03	3.20	75.65
17	60	1.5	160	3	74.7	2.34	1.90	1.89	0.44	3.79	89.60
18	60	1.5	160	3	74.7	2.49	1.50	1.74	0.99	3.24	76.60

Increase in severity of fractionation conditions (fractionation severity) such as temperature and time is on record to be detrimental to hemicellulose dissolution and recovery in fractionation studies (Diedericks et al., 2012; Emmel et al., 2003; vom Stein et al., 2011). Diedericks et al., (2012) also further established that severity of conditions applied to the fractionation process can be good up to a certain point when peak solubilisation of hemicelluloses is achieved while recovery of solubilized hemicelluloses decline as severity is increased further. This is explained by hydrolysis kinetics of hemicelluloses after solubilisation into the fractionation liquor. The hydrolysis reaction of hemicelluloses usually involves the breakage of one or more ether bonds within the hemicellulose structure resulting in polymers such as xylan or oligomers and monomers. This reaction is pseudo-homogeneous and it is irreversible which follows first-order reaction kinetics (Garrote, Dominguez, et al., 1999). This means depending on the severity of the fractionation (severity factors include temperature, time and activating components such as catalysts) solubilized hemicellulose components (polymers/oligomers/monomers) stays stable in solution until severity is increased beyond their stability after which depolymerisation occurs (breakage of larger molecules into smaller units). For this study, similar behavior in the recovery of hemicelluloses in fractionation liquor was observed as depicted with the illustration of xylitol and EG fractionations in Figures 11 and 12. Xylose content solubilized in the liquid fraction (removed from solid) increased towards more severe conditions as demonstrated in Figure 10. However when temperature is considered as a function of increase in condition severity, xylose recovery in the liquid fraction starts declining after 160°C as simplified in Figure 11-12.

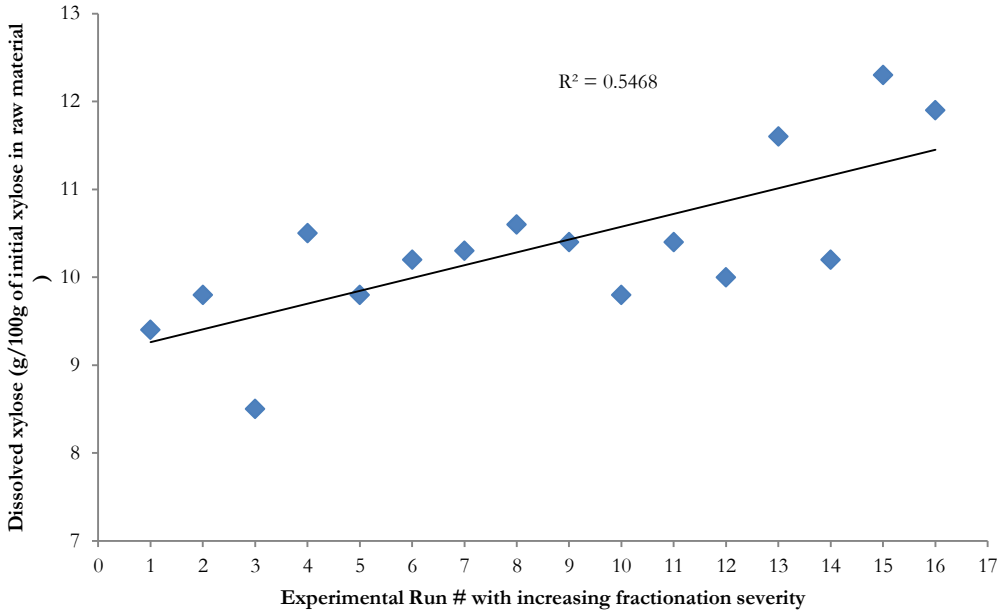


Figure 10: Xylose dissolution in fractionation liquor as a function of increasing fractionation severity (*E. grandis*-xylitol fractionation runs 1-16)

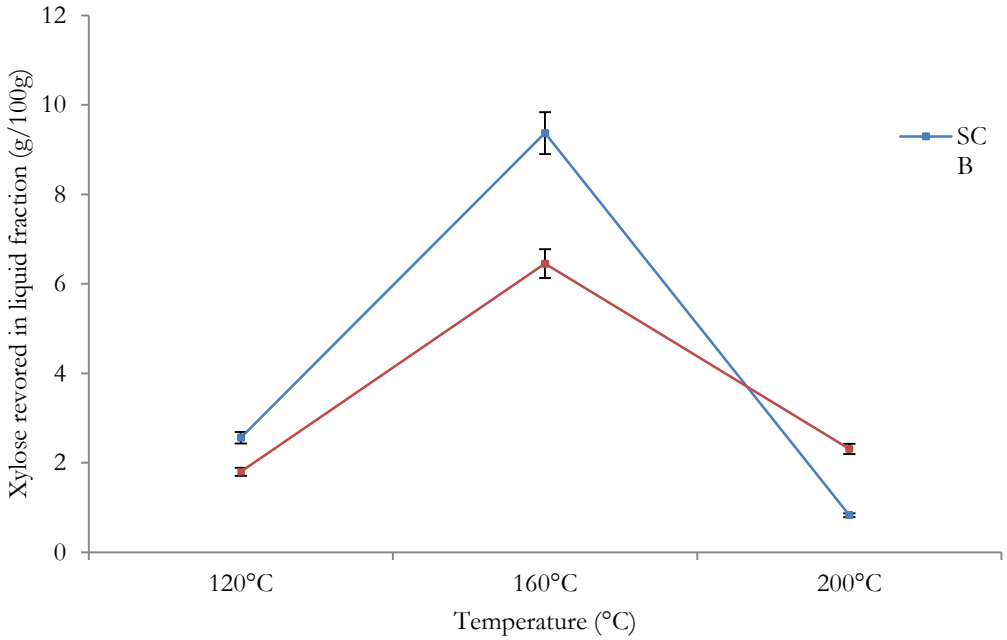


Figure 11: Xylose recovery as a function of increasing fractionation temperatures at median conditions: 25% Xylitol, 1.5% NaOH at 3hours.

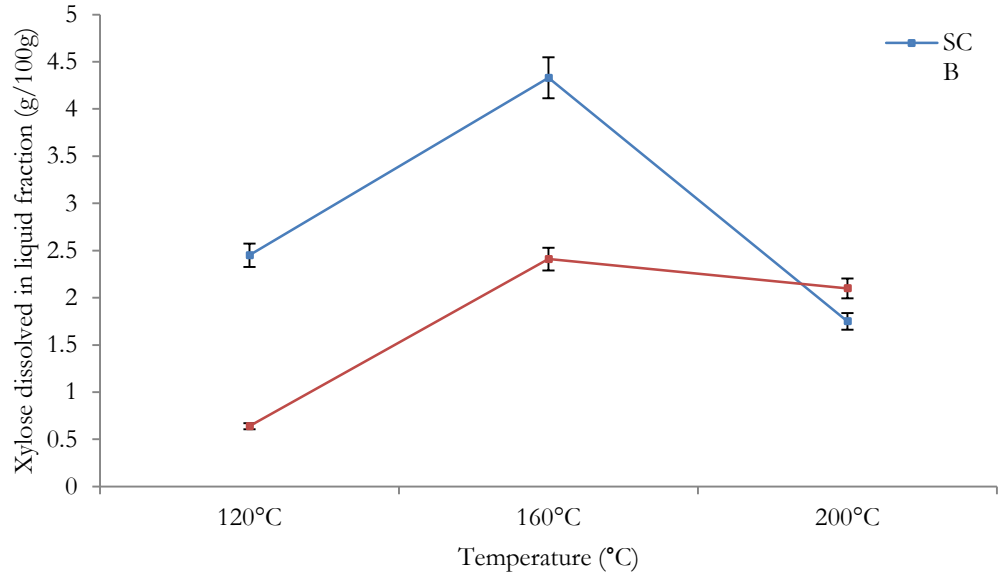
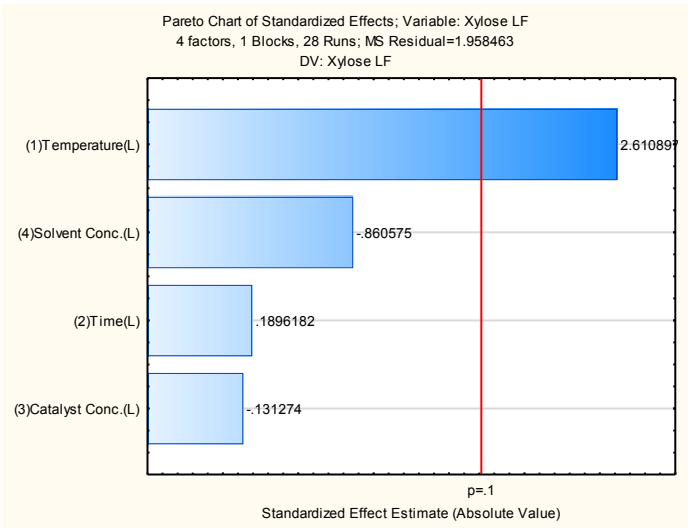


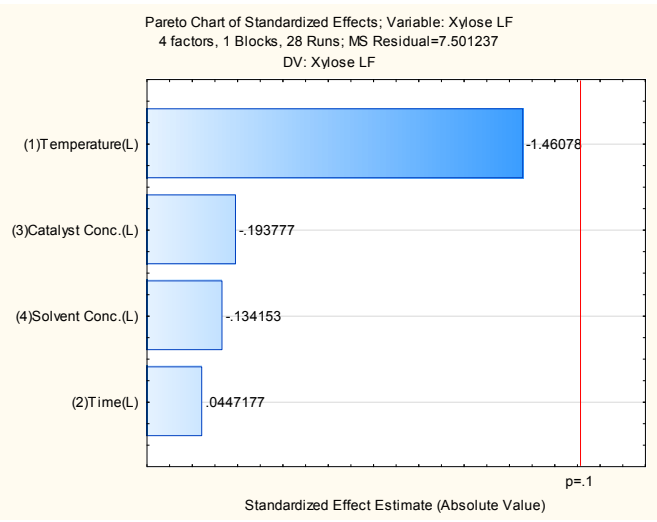
Figure 12: Xylose recovery as a function of increasing fractionation temperatures at median conditions: 60% EG, 1.5% NaOH at 3hours.

Temperature was used to demonstrate the increase in severity argument because paretto charts of the CCD's in Figure 13 which also shows that temperature is the statistically significant ($p<0.1$) factor of all the four independent variables for xylose recovery. Temperature is a determining factor for the amount of hemicelluloses recovered in solution as shown in Figure 14, 15 and 16. This is also demonstrated by the hemicellulose pre-extraction discussion mentioned earlier in section 4.3. Increasing temperature also increases the amount of hemicelluloses released from the solid residue (Hendriks &

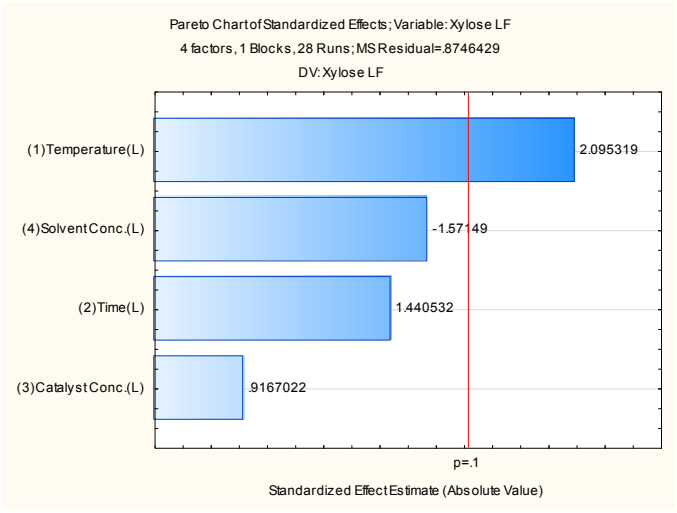
Zeeman, 2009) after which the hemicelluloses will start degrading or their recoveries in the hydrolysate starts declining with temperatures towards 200 °C(Benjamin & Görgens, 2015).



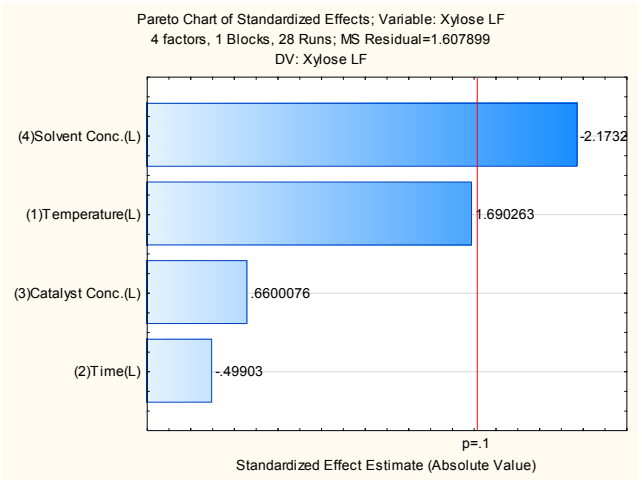
A: EC-Xylitol



B: SCB-Xylitol



C: EC-Ethylene glycol



D: SCB-Ethylene glycol

Figure 13: Pareto chart of independent variable effects on xylose dissolution in fractionation liquor

A further look at the data of runs 1-2, 7-10 and 15-16 of xylitol fractionations of eucalyptus in Table 23-28 and as also the depictions in Figure 13 and 14, indicates that an increase in temperature by 40°C increase xylose recovered in the liquid fraction by >70% after 2hours whilst <30% increase is achieved with additional increase of time to 4 hours. This is explained by the slight increase in severity of fractionation conditions which enables more solubilisation of hemicelluloses (removed from solid). Whilst eucalyptus is used to validate this point, similar behavior with xylitol is also reported for sugarcane bagasse (Appendix D).

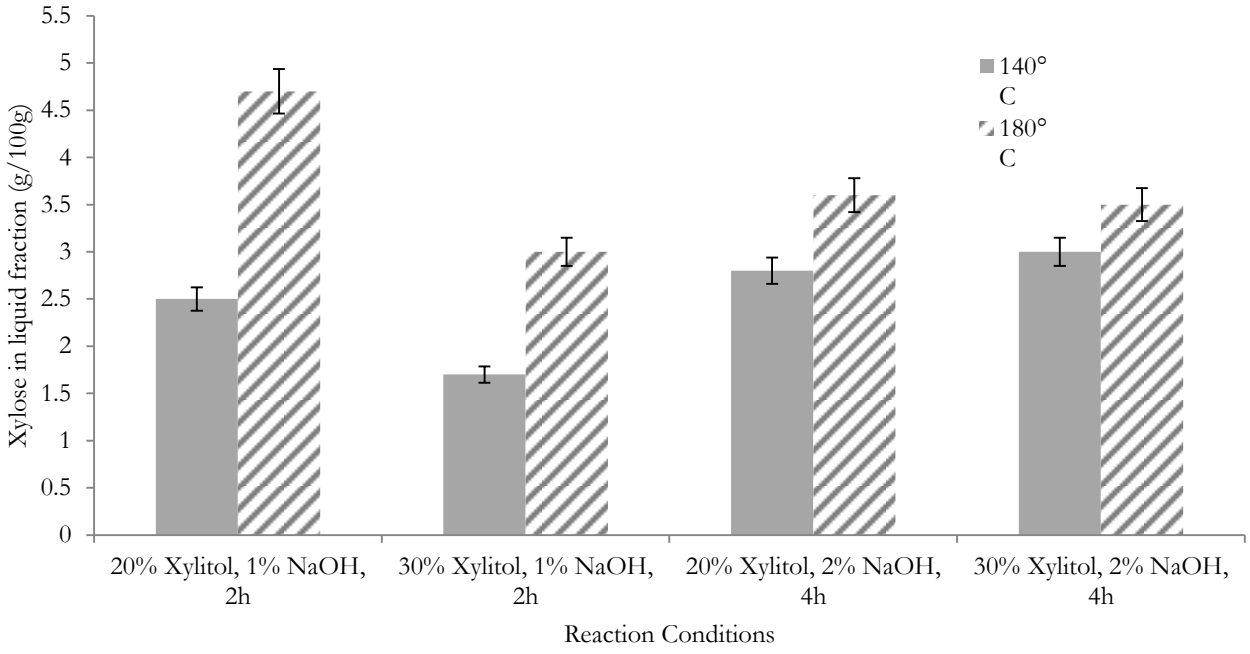


Figure 14: Xylitol eucalyptus fractionations' xylose recovery in solution at different temperatures 140°C (grey bars); 180°C (dashed bars).

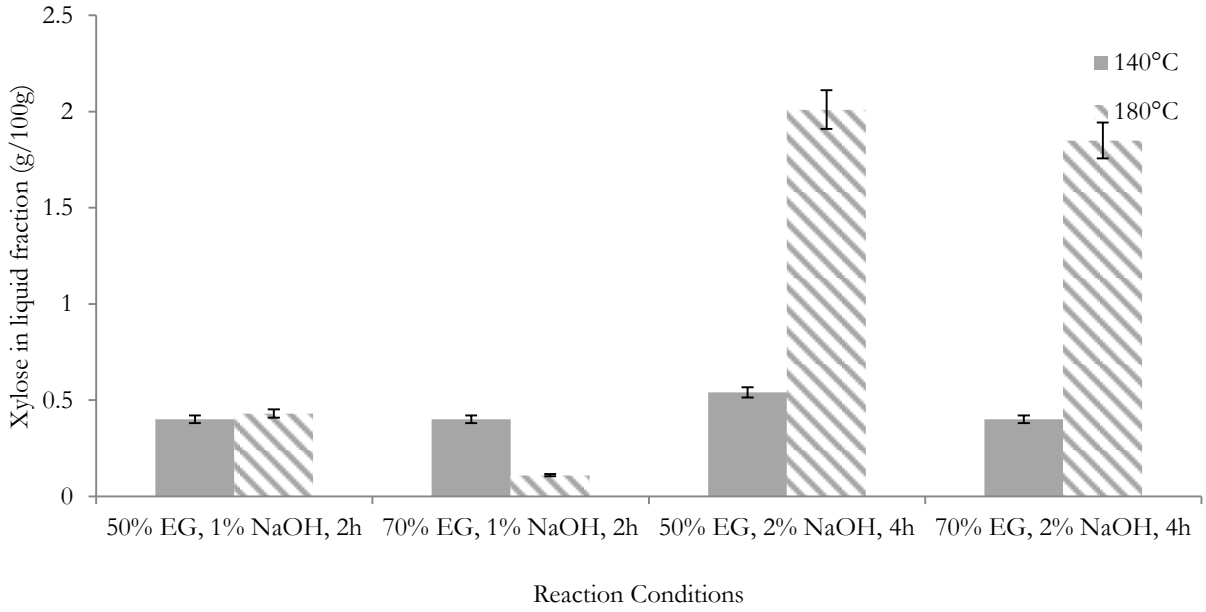


Figure 15: EG eucalyptus fractionations' xylose recovery in solution at different temperatures 140°C (grey bars); 180°C (dashed bars).

On the contrary, all other three factors constant, increasing the concentrations of the two solvents from their minimum level (20% xylitol, w/v and 50% EG, v/v) directly to their maximum concentrations (30% xylitol, w/v and 70% EG, v/v) does not significantly improve recovery of hemicelluloses in the liquid fraction, either a slight decline or increase (Figures 14 and 15).

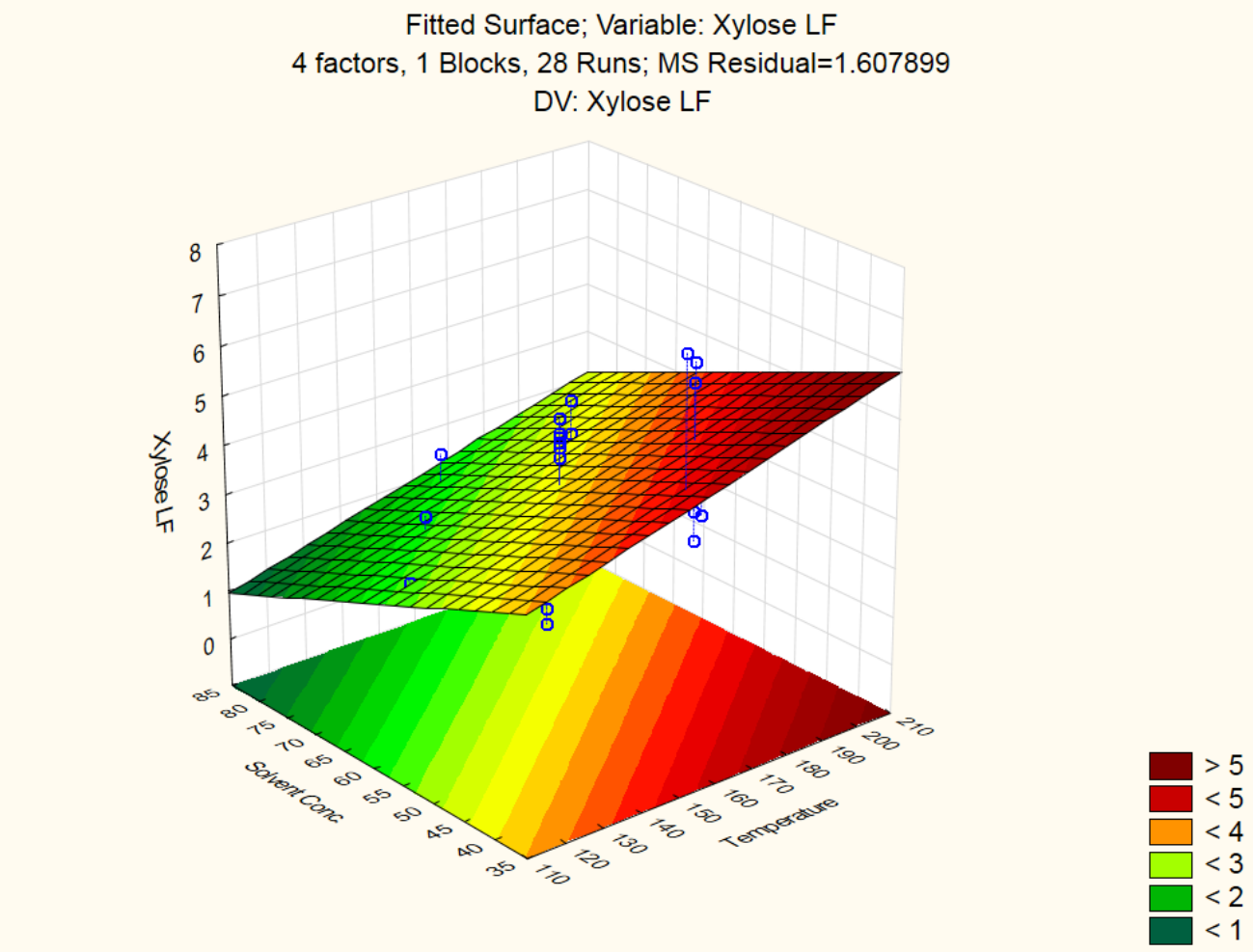


Figure 16: Temperature and Solvent concentration effect of xylose dissolution at midpoint of other variables for SCB-EG fractionation

Hemicellulose solubilisation with xylitol solutions fractionations range from 5.80-14.4g/100g and 5.85-14.45g/100g of raw xylose in *E. grandis* and sugarcane bagasse respectively. Ethylene glycol fractionations reported 8.0-12.30g/100g and 4.25-13.25/100g of raw glucose in *E. grandis* and sugarcane bagasse respectively. These represents dissolution between 27.75-68.89% and 25.05-61.88% of raw xylose in *E. grandis* and sugarcane bagasse under xylitol fractionations, while 38.27-58.85% and 18.20-56.74% of raw glucose was dissolved from *E. grandis* and sugarcane bagasse in ethylene glycol fractionations respectively. Both of these fractionation solvents dissolved far below the anticipated target dissolution of 80% of raw xylose under all fractionation conditions explored. Maximum dissolution was obtained with xylitol fractionations for both raw materials 68.89% and 61.88% of initial xylose from *E. grandis* and sugarcane bagasse respectively. This is explained by the number of hydroxyl groups on the xylitol structure (five hydroxyl groups) than the ethylene glycol structure (two hydroxyl groups), hydroxyl groups makes the structure more reactive towards components in solution (Chen, 2014; Sun, 2009). Like other organic solvents, reactivity of the two polyols (at different reaction rates) initially delignify the lignin macromolecule by breaking the bonds between lignin and hemicelluloses and thereby hydrolysing hemicelluloses (Guo et al., 2012). These dissolution ranges are comparable to xylose dissolved from eucalyptus fractionations studies by Romani et al., 2013 who reported a range of 39-70% at 180−200 °C for 40-90 minutes using 40-80% glycerol water solutions and also falls within the range (50-90%) dissolution of initial cellulose of other various organosolv fractionation solvents including ethanol as reported in Table 5.

Additionally, both fractionation processes achieved hemicellulose dissolutions below values obtained through direct sugarcane hemicellulose pre-extraction with NaOH. As discussed in section 4.3, the pre-extraction step dissolved 19.12g and 11.3g per 100g of initial of xylose in raw sugarcane bagasse and *E. grandis* (81.88% and 53.25% dissolution of initial raw material xylose respectively), whereas maximum xylose solubilized from both processes is below 70% of initial xylose in both substrates (see Table 23-28) owing to the reduced concentration of the hemicellulose hydrolyzing agent NaOH (pre-extraction used 1.5M versus <0.5M used as catalyst in all runs). There is however, a small improvement of <16% (15.64% increase with xylitol and 5.6% with ethylene glycol fractionation) increase in the amount of xylose solubilized from *E. grandis*. This slight increase can be attributed to increase in temperature (NaOH pre-extraction was done below 100°C) and the removal of the lignin component (see Table 23-28) which partly barriers accessibility of hemicelluloses to solvents (Guo et al., 2012). Therefore, pre-extraction should be considered a necessary step prior to this set of fractionations in order to avoid hemicellulose loses, but more especially for ethylene glycol fractionations.

Low recoveries of hemicelluloses in the fractionation liquors are observed in all fractionation processes. Hemicellulose recovered from xylitol fractionation liquors range from 1.53-6.45g/100g and 0.83-9.99g/100g of raw xylose from *E. grandis* and sugarcane bagasse respectively. Hemicelluloses recovered from ethylene glycol fractionation liquors ranged from 0.20-3.68g/100g and 1.75-7.19g/100g of raw xylose from *E. grandis* and sugarcane bagasse respectively. These ranges represent recoveries between 31% and 43% of raw xylose in *E. grandis* and sugarcane bagasse. As ascribed to celluloses losses and poor recoveries in fractionation liquor (section 4.4.2), low recoveries of hemicelluloses as shown in Table 29 from fractionation liquors as compared to recoveries from pure alkaline pre-extraction is attributed to the formation of xylose-glycosides facilitated in polyol solutions (Zhang, Wong, et al., 2013) which means xylose is not picked up by the HPLC instrument as explained (Chemin et al., 2015; Kooiman, 1961; Laine, 2005; Muhammad Safwan et al., 2015; Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013; Zhang, Wong, et al., 2013).

Table 29: Descriptive statistical analysis of hemicellulose recovered in the liquid fraction

	Xylose recovered in liquid fraction (g/100g)					
	EC-xylitol	EC-EG	SCB-xylitol	SCB-EG	Hemicellulose extracted EC-EG	Hemicellulose extracted SCB-EG
Mean	3.75	1.03	4.95	3.44	0.96	1.64
Standard Error	0.28	0.20	0.50	0.26	0.11	0.15
Standard Deviation	1.48	1.04	2.64	1.37	0.49	0.63
Sample Variance	2.20	1.08	6.97	1.87	0.24	0.40
Minimum	1.53	0.00	0.83	1.75	0.27	0.89
Maximum	6.45	3.68	9.99	7.19	1.86	2.86
Count	28	28	28	28	18	18
Confidence Level (95.0%)	0.58	0.40	1.02	0.53	0.24	0.31

Hemicellulose remaining in the solid fraction had a significant effect on enzymatic hydrolysis efficiency as shown in Figure 17. This observation is very significant for xylitol fractionated solid residues, r-square values of 0.4853 and 0.6751 for eucalyptus and sugarcane bagasse respectively and much less significant for EG fractionations with r-squared values of 0.1457 and 0.222 for eucalyptus and sugarcane bagasse respectively. Possible reason for this difference can be aligned to the fact that ethylene glycol treatment removed more xylose from the solid residue although unaccounted for in the liquid as compared to xylitol fractionations mass balances presented in Tables 23 to 28.

Influence of hemicellulose on enzymatic hydrolysis has been a subject of studies for years as reported in literature (Yang et al., 2011). Some authors (Zhao & Zhang, 2012) listed hemicelluloses in addition to other factors like cellulose structure (crystallinity, polymerization) as an indirect factor affecting accessibility of cellulose by enzymes. The significant influence of hemicellulose or lack thereof on enzymatic hydrolysis of cellulose has been a subject of debate (Yang et al., 2011). Nevertheless, our data shows that high enzymatic hydrolysis is achieved with little xylose content in the solid as presented in Figure 17 and when compared to literature reports of Brudecki, Cybulska, & Rosentrater, 2013 and Moxley et al., 2008.

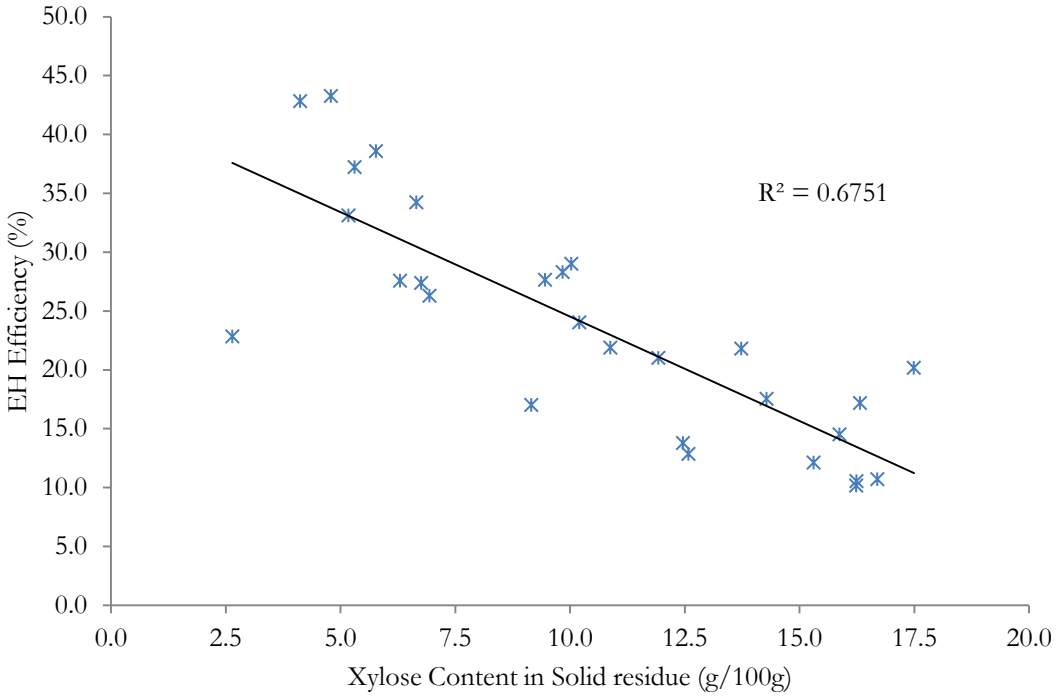


Figure 17: Hemicellulose remaining in the solid fraction has a significant effect on enzymatic hydrolysis efficiency (sugarcane bagasse fractionation residual solids)

In conclusion, three major findings come to light as far as the hemicellulose dissolution and recovery in the fractionation liquor is concerned;

a) Temperature of fractionation plays a significant role in the process of dissolving xylose far more than the solvent concentration and other factors as shown in the paretto charts presented in Figure 13. Highest xylose recovery in solution after dissolution is achieved at around 160°C, the median temperature in the range studied. This is in agreement with thermal degradation of hemicelluloses that begins just right after 160°C (Hendriks & Zeeman,

2009). We could not expect peak xylose concentrates to be dissolved in fractionation liqueur and remain in the liquor for longer times at temperatures close to 180°C, unless other independent factors (time, NaOH concentration, concentration of solvents) are closely monitored.

b) Xylitol fractionations gave higher dissolutions of hemicelluloses in the liquid fraction while still recovering a fair amount of hemicellulose in the residual solid as compared to ethylene glycol as shown in Tables 23-28. This may not be desirable if the solid residue is to be used further in an enzymatic hydrolysis step (Benjamin, García-Aparicio, & Görgens, 2014; Lima et al., 2013; Wang et al., 2012) because of the association of hemicellulose in the solid residue with reduced enzymatic hydrolysis efficiency.

c) The mass balances of hemicelluloses after the two processes were lower when compared to lignin and cellulose (see section 4.4.2-4.4.1), which maybe associated is partly associated with the solvents' behavior of forming glycols ad/or degradation of xylose under the chosen set of conditions. Additionally, dissolution xylose from the substrates with either of the solvents was also below the expected target of 80% of initial xylose in the substrates. For this reason, extracting hemicellulose from biomass before fractionating the remaining two components, cellulose and lignin with these two solvents is a desirable route for sugarcane bagasse which provides for more than 80% dissolution while fractionation of hemicellulose using either of the solvents is preferable over hemicellulose pre-extraction since between 5-15% more of hemicelluloses is extracted from the process when compared to <53% of initial hemicelluloses in raw material is extracted from the NaOH pre-extraction process.

4.4.4 Lignin dissolution and recovery in the liquid fraction

As outlined in Chapter Three, majority of the lignin component in the raw material was expected to be dissolved and recovered in the liquid fraction after the fractionation process. Specific objectives for lignin dissolution and recovery as highlighted in section 1.2;

1. Dissolve and recover more than 70% of lignin from the raw material in the liquid fraction.
2. To determine and understand the effect of set fractionation parameters such as temperature, catalyst and solvent concentration and time of retention on the lignin dissolution and recovery and this information to optimize best fractionation conditions that maximize components' yields and purity.
3. Analyse the quality of lignin obtained at optimum conditions and compare it to literature reports.

This section particularly addresses target objective 1 and 2, whilst objective 3 is further discussed in Chapter Five, particularly in section 5.3.3. Lignin dissolved into the liquid fraction is presented in Tables 30-35. Total mass balance of lignin analysed in the various fractions are also presented in Tables 30-33. Mass balances of the lignin component from majority of the runs are generally well above 70% except four runs, 5 and 11 from SCB fractionations with recoveries of 65.7% and 64.3 with 20% (w/v) xylitol and 50% (v/v) EG respectively. The third was recorded for eucalyptus' run 18, (200°C, 60% v/v EG) with lignin mass balance of 63.4% and the fourth case reported at run 3 (140°C, 50% v/v EG) with a total mass balance of 69.3%. Runs 3, 5 and 11 appear to be outliers as compared to mass balances of other runs with almost similar fractionation conditions. On the other hand, the low mass balance of run 18 can be attributed to the high

temperature used (200°C) which suggest decomposition of lignin (Cãpraru, Popa, Mãlutan, & Lisa, 2009; H. Yang, Yan, Chen, Lee, & Zheng, 2007; Zhou et al., 2011).

Table 30: Lignin mass balance and composition of liquid fraction and solid residue from xylitol fractionation of raw *E. grandis*

Fractionation Conditions						Lignin in the liquid fraction (g/100g)		Lignin Degraded (g/100g)	Lignin in Residual Solid (g/100g)	Mass Balance (g/100g)	
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered				Recovery (%)
Raw Material Composition									25.52	25.52	100.00
1	20	1	140	2	79.60	8.62	9.50	-0.88	16.90	26.40	103.45
2	30	1	140	2	81.60	10.22	12.20	-1.98	15.30	27.50	107.76
3	20	2	140	2	80.00	10.02	6.50	3.52	15.50	22.00	86.21
4	30	2	140	2	78.30	13.82	12.70	1.12	11.70	24.40	95.61
5	20	1	140	4	83.00	7.22	8.90	-1.68	18.30	27.20	106.58
6	30	1	140	4	81.70	4.82	5.20	-0.38	20.70	25.90	101.49
7	20	2	140	4	79.70	9.52	6.60	2.92	16.00	22.60	88.56
8	30	2	140	4	76.00	9.22	8.00	1.22	16.30	24.30	95.22
9	20	1	180	2	66.80	12.42	11.10	1.32	13.10	24.20	94.83
10	30	1	180	2	66.70	10.22	7.10	3.12	15.30	22.40	87.77
11	20	2	180	2	57.70	15.92	10.30	5.62	9.60	19.90	77.98
12	30	2	180	2	59.00	19.52	12.80	6.72	6.00	18.80	73.67
13	20	1	180	4	64.80	16.72	11.70	5.02	8.80	20.50	80.33
14	30	1	180	4	47.00	14.62	11.30	3.32	10.90	22.20	86.99
15	20	2	180	4	52.40	20.52	15.90	4.62	5.00	20.90	81.90
16	30	2	180	4	54.30	18.92	14.50	4.42	6.60	21.10	82.68
17	25	1.5	120	3	82.10	5.42	5.60	-0.18	20.10	25.70	100.71
18	25	1.5	200	3	63.90	13.42	12.90	0.52	12.10	25.00	97.96
19	25	1.5	160	1	81.80	9.22	8.80	0.42	16.30	25.10	98.35
20	25	1.5	160	5	63.10	11.02	10.20	0.82	14.50	24.70	96.79
21	25	0.5	160	3	57.60	10.32	7.80	2.52	15.20	23.00	90.13
22	25	2.5	160	3	62.00	14.42	14.20	0.22	11.10	25.30	99.14
23	15	1.5	160	3	70.60	11.22	12.10	-0.88	14.30	26.40	103.45
24	35	1.5	160	3	67.40	14.52	10.20	4.32	11.00	21.20	83.07
25	25	1.5	160	3	68.80	9.32	8.70	0.62	16.20	24.90	97.57
26	25	1.5	160	3	76.20	8.42	7.80	0.62	17.10	24.90	97.57
27	25	1.5	160	3	70.10	9.02	9.20	-0.18	16.50	25.70	100.71
28	25	1.5	160	3	72.20	7.52	10.40	-2.88	18.00	28.40	111.29

Table 31: Lignin mass balance and composition of liquid fraction and solid residue from ethylene glycol fraction of raw *E. grandis*

Fractionation Conditions						Lignin in the liquid fraction (g/100g)		Lignin Degraded (g/100g)	Lignin in Residual Solid (g/100g)	Mass Balance (g/100g)	Recovery (%)
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered				
Raw Material Composition									25.52	25.52	100.00
1	80	1	140	2	77.20	8.92	5.70	3.22	16.60	22.30	87.38
2	90	1	140	2	68.70	9.42	7.70	1.72	16.10	23.80	93.26
3	80	2	140	2	61.70	13.62	5.70	7.92	11.90	17.60	68.97
4	90	2	140	2	69.40	8.62	10.30	-1.68	16.90	27.20	106.58
5	80	1	140	4	55.40	14.22	8.00	6.22	11.30	19.30	75.63
6	90	1	140	4	62.90	14.42	8.40	6.02	11.10	19.50	76.41
7	80	2	140	4	66.00	12.32	6.60	5.72	13.20	19.80	77.59
8	90	2	140	4	63.40	13.92	12.10	1.82	11.60	23.70	92.87
9	80	1	180	2	53.50	14.62	13.10	1.52	10.90	24.00	94.04
10	90	1	180	2	64.60	11.52	12.00	-0.48	14.00	26.00	101.88
11	80	2	180	2	52.50	18.62	14.90	3.72	6.90	21.80	85.42
12	90	2	180	2	57.70	16.22	13.70	2.52	9.30	23.00	90.13
13	80	1	180	4	55.30	15.72	14.50	1.22	9.80	24.30	95.22
14	90	1	180	4	58.00	18.52	15.80	2.72	7.00	22.80	89.34
15	80	2	180	4	46.00	21.42	19.30	2.12	4.10	23.40	91.69
16	90	2	180	4	43.20	21.22	15.10	6.12	4.30	19.40	76.02
17	85	1.5	120	3	75.40	11.32	6.00	5.32	14.20	20.20	79.15
18	85	1.5	200	3	37.00	22.12	12.80	9.32	3.40	16.20	63.48
19	25	1.5	160	1	69.90	12.52	9.50	3.02	13.00	22.50	88.17
20	25	1.5	160	5	56.10	17.12	14.20	2.92	8.40	22.60	88.56
21	25	0.5	160	3	67.90	9.92	8.40	1.52	15.60	24.00	94.04
22	25	2.5	160	3	68.60	12.02	12.40	-0.38	13.50	25.90	101.49
23	15	1.5	160	3	68.20	9.92	8.60	1.32	15.60	24.20	94.83
24	35	1.5	160	3	69.50	10.42	10.80	-0.38	15.10	25.90	101.49
25	25	1.5	160	3	64.90	11.52	12.00	-0.48	14.00	26.00	101.88
26	25	1.5	160	3	61.00	15.12	11.00	4.12	10.40	21.40	83.86
27	25	1.5	160	3	57.70	17.42	11.00	6.42	8.10	19.10	74.84
28	25	1.5	160	3	61.60	15.22	13.80	1.42	10.30	24.10	94.44

Table 32: Lignin mass balance and composition of liquid fraction and solid residue from xylitol fractionation of raw sugarcane bagasse

Fractionation Conditions						Lignin in the liquid fraction (g/100g)					
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered	Lignin Degraded (g/100g)	Lignin in Residual Solid (g/100g)	Mass Balance (g/100g)	Recovery (%)
Raw Material Composition									22.96	22.96	100
1	20	1	140	2	77.40	7.46	5.80	1.66	15.50	21.30	92.77
2	30	1	140	2	82.40	5.36	4.70	0.66	17.60	22.30	97.13
3	20	2	140	2	76.30	9.06	7.40	1.66	13.90	21.30	92.77
4	30	2	140	2	73.90	8.26	3.80	4.46	14.70	18.50	80.57
5	20	1	140	4	73.40	14.26	6.30	7.96	8.70	15.00	65.33
6	30	1	140	4	71.20	9.26	6.40	2.86	13.70	20.10	87.54
7	20	2	140	4	58.50	9.06	10.20	-1.14	13.90	24.10	104.97
8	30	2	140	4	73.90	10.56	8.80	1.76	12.40	21.20	92.33
9	20	1	180	2	64.40	5.46	5.60	-0.14	17.50	23.10	100.61
10	30	1	180	2	63.60	5.66	6.60	-0.94	17.30	23.90	104.09
11	20	2	180	2	54.80	12.36	7.20	5.16	10.60	17.80	77.53
12	30	2	180	2	59.80	9.36	7.10	2.26	13.60	20.70	90.16
13	20	1	180	4	62.00	5.26	3.90	1.36	17.70	21.60	94.08
14	30	1	180	4	63.80	3.36	4.70	-1.34	19.60	24.30	105.84
15	20	2	180	4	56.10	7.96	7.70	0.26	15.00	22.70	98.87
16	30	2	180	4	59.90	6.26	9.70	-3.44	16.70	26.40	114.98
17	25	1.5	120	3	84.30	1.26	4.70	-3.44	21.70	26.40	114.98
18	25	1.5	200	3	55.00	7.26	1.70	5.56	15.70	17.40	75.78
19	25	1.5	160	1	83.50	4.76	5.60	-0.84	18.20	23.80	103.66
20	25	1.5	160	5	63.50	10.76	5.80	4.96	12.20	18.00	78.40
21	25	0.5	160	3	79.50	2.06	2.90	-0.84	20.90	23.80	103.66
22	25	2.5	160	3	73.40	8.56	9.40	-0.84	14.40	23.80	103.66
23	15	1.5	160	3	69.90	8.56	6.00	2.56	14.40	20.40	88.85
24	35	1.5	160	3	67.20	8.06	5.50	2.56	14.90	20.40	88.85
25	25	1.5	160	3	64.00	7.46	5.40	2.06	15.50	20.90	91.03
26	25	1.5	160	3	62.80	11.56	10.70	0.86	11.40	22.10	96.25
27	25	1.5	160	3	67.70	6.56	7.10	-0.54	16.40	23.50	102.35
28	25	1.5	160	3	64.20	6.96	5.40	1.56	16.00	21.40	93.21

Table 33: Lignin mass balance and composition of liquid fraction and solid residue from ethylene glycol fractionation of raw sugarcane bagasse

Fractionation Conditions						Lignin in the liquid fraction (g/100g)		Lignin Degraded (g/100g)	Lignin in Residual Solid (g/100g)	Mass Balance (g/100g)	
Run	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Dissolved	Recovered				Recovery (%)
Raw Material Composition									22.96	22.96	100.00
1	80	1	140	2	79.00	8.56	7.40	1.16	14.40	21.80	94.95
2	90	1	140	2	81.20	6.76	7.60	-0.84	16.20	23.80	103.66
3	80	2	140	2	65.40	11.36	9.70	1.66	11.60	21.30	92.77
4	90	2	140	2	76.10	8.36	9.60	-1.24	14.60	24.20	105.40
5	80	1	140	4	79.90	5.56	6.40	-0.84	17.40	23.80	103.66
6	90	1	140	4	79.70	5.26	7.00	-1.74	17.70	24.70	107.58
7	80	2	140	4	69.10	14.06	11.00	3.06	8.90	19.90	86.67
8	90	2	140	4	72.10	10.76	9.50	1.26	12.20	21.70	94.51
9	80	1	180	2	83.10	9.16	8.20	0.96	13.80	22.00	95.82
10	90	1	180	2	66.90	9.76	7.00	2.76	13.20	20.20	87.98
11	80	2	180	2	54.70	16.76	10.20	6.56	6.20	16.40	71.43
12	90	2	180	2	62.80	13.46	11.30	2.16	9.50	20.80	90.59
13	80	1	180	4	49.70	14.16	7.00	7.16	8.80	15.80	68.82
14	90	1	180	4	82.90	8.56	8.10	0.46	14.40	22.50	98.00
15	80	2	180	4	68.40	11.66	11.80	-0.14	11.30	23.10	100.61
16	90	2	180	4	55.80	16.46	9.60	6.86	6.50	16.10	70.12
17	85	1.5	120	3	77.30	8.66	6.70	1.96	14.30	21.00	91.46
18	85	1.5	200	3	57.40	10.06	7.40	2.66	12.90	20.30	88.41
19	25	1.5	160	1	79.90	4.86	7.30	-2.44	18.10	25.40	110.63
20	25	1.5	160	5	69.70	10.96	9.70	1.26	12.00	21.70	94.51
21	25	0.5	160	3	75.60	4.96	4.60	0.36	18.00	22.60	98.43
22	25	2.5	160	3	69.10	9.66	11.80	-2.14	13.30	25.10	109.32
23	15	1.5	160	3	72.80	6.06	4.70	1.36	16.90	21.60	94.08
24	35	1.5	160	3	76.90	5.26	5.10	0.16	17.70	22.80	99.30
25	25	1.5	160	3	68.20	14.76	11.30	3.46	8.20	19.50	84.93
26	25	1.5	160	3	71.20	8.06	9.40	-1.34	14.90	24.30	105.84
27	25	1.5	160	3	73.20	10.46	8.90	1.56	12.50	21.40	93.21
28	25	1.5	160	3	87.00	10.46	11.00	-0.54	12.50	23.50	102.35

Table 34: Lignin mass balance and composition of liquid fraction and solid residue from hemicellulose pre-extracted *E. grandis* using ethylene glycol fractionation

	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Lignin in liquid fraction (g/100g)		Lignin in residual Solid (g/100g)	Lignin Degraded (g/100g)	Mass balance (g/100g)	Recovery (%)
						Dissolved	Recovered				
Initial Raw SCB								25.25	0.00	25.25	100.00
Hemis Pre-extracted SCB								17.12	0.00	17.42	100.00
1	60	1	140	2	88.9	9.89	10	7.23	-0.11	17.23	100.64
2	60	2	140	2	83.9	9.98	8.9	7.14	1.08	16.04	93.69
3	60	1	140	4	84.9	9.02	6.9	8.10	2.12	15.00	87.62
4	60	2	140	4	75.3	9.42	9.4	7.70	0.02	17.10	99.88
5	60	1	180	2	70.4	11.12	11	6.00	0.12	17.00	99.30
6	60	2	180	2	44.7	16.62	15.21	0.50	1.41	15.71	91.76
7	60	1	180	4	63.4	13.02	12.1	4.10	0.92	16.20	94.63
8	60	2	180	4	55.6	16.52	15.1	0.60	1.42	15.70	91.71
9	60	1.5	126.4	3	87.1	4.29	4.0	12.83	0.29	16.83	98.31
10	60	1.5	193.6	3	54.5	16.32	15.66	0.80	0.66	16.46	96.14
11	60	1.5	160	1.3	74.5	5.02	4.89	12.10	0.13	16.99	99.24
12	60	1.5	160	4.7	65.9	17.04	18.3	0.08	-1.26	18.38	107.36
13	60	0.7	160	3	78.3	4.52	2.15	12.60	2.37	14.75	86.16
14	60	2.3	160	3	64.8	14.32	13.44	2.80	0.88	16.24	94.86
15	60	1.5	160	3	69.6	11.32	11.1	5.80	0.22	16.90	98.71
16	60	1.5	160	3	68	12.02	11.3	5.10	0.72	16.40	95.79
17	60	1.5	160	3	70.8	11.12	11.8	6.00	-0.68	17.80	103.97
18	60	1.5	160	3	69.8	12.92	11.84	4.20	1.08	16.04	93.69

Table 35: Lignin mass balance and composition of liquid fraction and solid residue from hemicellulose pre-extracted sugarcane bagasse using ethylene glycol fractionation

	Solvent Conc. (%)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Solid Recovery (%)	Lignin in liquid fraction (g/100g)		Lignin in residual Solid (g/100g)	Lignin Degraded (g/100g)	Mass balance (g/100g)	Recovery (%)
						Dissolved	Recovered				
Initial Raw SCB								22.96	0	22.96	100
Hemis Pre-extracted SCB								7.41	0	7.41	100
1	60	1	140	2	60.9	3.48	3.40	3.94	0.08	7.34	99.06
2	60	2	140	2	56.6	0.62	0.00	6.80	0.62	6.80	91.77
3	60	1	140	4	56	4.22	2.90	3.20	1.32	6.10	82.32
4	60	2	140	4	56.4	4.82	3.10	2.60	1.72	5.70	76.92
5	60	1	180	2	60	7.38	7.30	0.04	0.08	7.34	99.06
6	60	2	180	2	69.7	7.42	7.20	0.00	0.22	7.20	97.18
7	60	1	180	4	74.5	7.42	7.80	0.00	-0.38	7.80	105.26
8	60	2	180	4	68.6	7.19	6.80	0.23	0.39	7.03	94.87
9	60	1.5	126.4	3	77.9	3.12	2.00	4.30	1.12	6.30	85.02
10	60	1.5	193.6	3	68.7	3.12	1.10	4.30	2.02	5.40	72.87
11	60	1.5	160	1.3	55.3	4.62	3.10	2.80	1.52	5.90	79.62
12	60	1.5	160	4.7	74	4.62	4.40	2.80	0.22	7.20	97.17
13	60	0.7	160	3	76.8	1.02	1.70	6.40	-0.68	8.10	109.31
14	60	2.3	160	3	70.4	5.82	6.40	1.60	-0.58	8.00	107.96
15	60	1.5	160	3	74.9	4.22	4.50	3.20	-0.28	7.70	103.91
16	60	1.5	160	3	75.4	4.32	3.90	3.10	0.42	7.00	94.47
17	60	1.5	160	3	74.7	3.72	3.90	3.70	-0.18	7.60	102.56
18	60	1.5	160	3	74.7	3.92	3.80	3.50	0.12	7.30	98.52

Whilst more than 70% of lignin from the raw material can be accounted for in the two fractions, other runs accumulated for more than 100% of the initial lignin as the case for runs 1, 2, 5 and 6 as reported in Table 30, reporting mass balances of 103.4, 107.8, 106.58 and 101.49% respectively. Formation of condensation products from lignin decomposition in what is known "alkali promoted self-condensation"(da Costa Lopes et al., 2013) are known to interfere with lignin detection and analysis in the UV/vis(Emmel et al., 2003) which can contribute to the overestimation (Sluiter et al., 2010). Condensation reactions and their products are commonly known to be facilitated by acid catalysts or the presence of *in-situ* levullinic acid or acetic acid molecules (Deng et al., 2014) which in this case may have been produced from the degradation of carbohydrates(Katahira et al., 2013; Winkler, 1981), although we believe this was minimal, particularly if levullinic acid was involved because its in-situ formation from carbohydrate degradation is a four step long process (Deng et al., 2014). However, the presence of an alkali (NaOH) also promotes the re-polymerization (condensation) of solubilised lignin phenol molecules or other lignin monomers in solution with other dissolved molecules in solution i.e. formaldehyde (Sun, 2009).

However Sluiter et al., (2012) in their validated compositional analysis method used in this study indicated there could be high biasness of lignin resulting from interference from degradation products but it should be minimal with careful preparations. Furthermore, Harmsen et al., (2010) argues that, the integration of NaOH in the process provides for a mild environment to reduce lignin condensation; Vena, 2013 disagrees suggesting the mere presence of an alkali initiate lignin condensation reactions and products thereof at high temperatures. We also note that this observation (overestimation), although not recorded for many runs (less than 15) is irregular and not necessarily occurring at severe conditions as reported in the literature (Area et al., 2009; da Costa Lopes et al., 2013; Emmel et al., 2003; Xuezhi Li et al., 2014; Mohammed, 2012; Vena, 2013; Winkler, 1981; Xiang et al., 2004; Zhang, Wong, et al., 2013).

For raw materials fed directly into the fractionation process, xylitol fractionations generally solubilized lesser lignin (18.0-80.0% and 5.49-62.10% of initial lignin in raw *E. grandis* and sugarcane bagasse respectively) as compared to ethylene glycol fractionations (33.77-86.67% and 21.16-72.99% of initial lignin in raw *E. grandis* and sugarcane bagasse respectively) (Table 30-33). For recovery of lignin in fractionation liquors, for xylitol fractionations recovered 10.08g/100g and 6.29g/100g of initial lignin in raw eucalyptus and sugarcane bagasse respectively, while ethylene glycol fractionation recovery was 11.19g/100g and 8.54g/100g of initial lignin in raw eucalyptus and sugarcane bagasse respectively. However, the difference in all cases is very minimal, <2.25g/100g of raw lignin, meaning recovery of lignin from either solvent liquor is comparable. This trend is also same at optimum conditions as discussed in section 4.4.5. Additionally, ethylene glycol fractionations also dissolved the highest amount of lignin, 22.12g/100g of raw material. This was achieved with EG fractionation of the raw *E. grandis* at

run 18 (Table 31), with 85% EG, 1.5%NaOH, 200°C and 3 hours run time. This represents approximately 86.68% dissolution of the initial lignin in raw eucalyptus. The highest dissolution of sugarcane bagasse was 17.26g achieved at 80% EG, 2.0%NaOH, 180°C and 2 hours run time representing dissolution of 72.99% of the initial lignin in raw sugarcane bagasse. These findings are lower but comparable to Lima et al., (2013)'s findings in which a two-step fractionation of two eucalyptus varieties' achieved lignin dissolution of 84% and 79% for *E. grandis* and *E.grandis x urophylla* respectively using 1% HCl followed by 4% NaOH. Our results were also lower but comparable to 84% dissolution achieved by Zhang, Rackemann, et al., (2013) who used glycerol carbonate to fractionate sugarcane bagasse at much lower reaction conditions (90°C, 30 minutes and an acid catalyst) but 54% dissolution was achieved with EG under the same conditions.

The hemicellulose pre-extraction step was not only beneficial for extracting and preserving hemicelluloses as discussed earlier in section 4.4.3, but it also proved beneficial for improving dissolution of lignin from the solid structure with almost complete delignification with ethylene glycol in most runs after fractionation of the hemicellulose pre-extracted residues (Table 34-35). Since 65.98% and up of raw material lignin was already dissolved from NaOH hemicellulose pre-extraction of sugarcane bagasse (see section 4.3), fractionation of hemicellulose pre-extracted *E. grandis* solid residues with Ethylene glycol seem more ideal due to improvement in lignin dissolution, dissolution of 33.26% of initial lignin in raw material with NaOH pre-extraction versus up to 67.49% dissolution of initial raw lignin from fractionation of pre-extracted residues. The high lignin dissolution from lignin from eucalyptus as compared to sugarcane bagasse can be associated with the amount of lignin in the pre-extracted materials, i.e. 17.12g/100g and 7.41g/100g of hemicelluloses pre-extracted sugarcane bagasse and *E. grandis* respectively and also due to the removal of hemicelluloses from the lignocellulose matrix during the hemicellulose-extraction processes.

For direct raw material fractionations, the spread of lignin from fractionation liquor appears to be inconsistent across the runs as shown in Figure 18 where majority of lignin dissolved is ranging between 5g/100g to 12.5g/100g of raw material lignin, unlike trends observed for hemicellulose and cellulose which tend to be influenced by the combined influence of increase in fractionation severity. For lignin, the influence of temperature on its removal from the lignocellulose structures is not to be ignored. With increase in temperature, more lignin is liberated from the lignocellulose structure as observed in Figures 18-19. Others (vom Stein et al., 2011) also reported that fractionating beech wood in a one step process using 2-Methyltetrahydrofuran (MTHF) with increasing temperature from 85 to 150°C increased recovery of lignin in fractionation liquor from 4g to 11.5g/100g of raw material lignin.

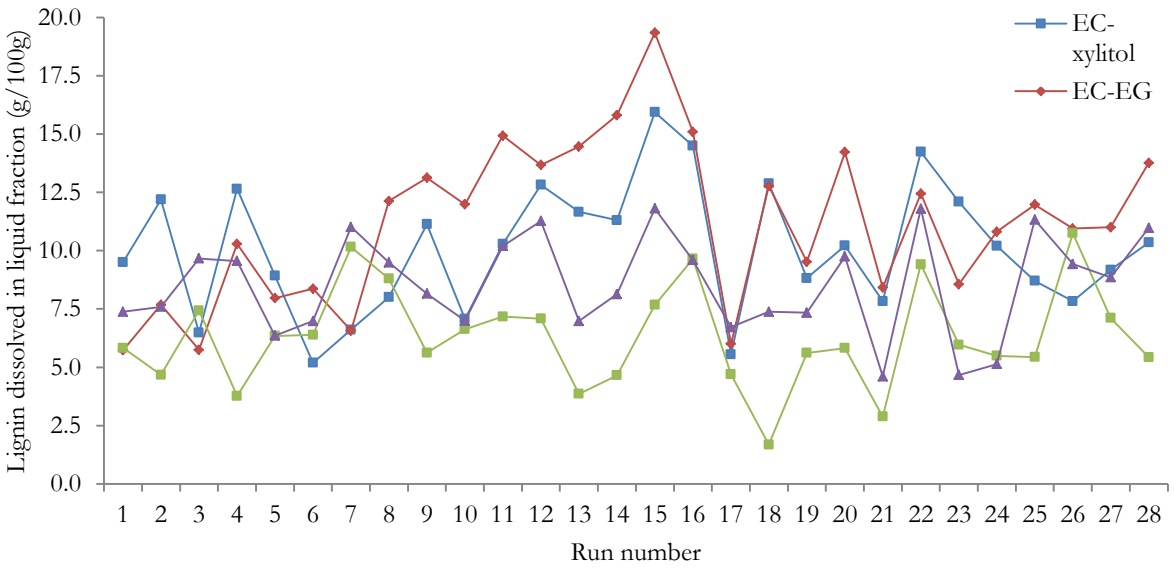


Figure 18: Lignin distribution across the fractionation runs

Recovery of lignin from solution is related to solubilized lignin, the more lignin in solution (solubilized), the higher the recovery as observed in the trends presented in Tables 30 to 35. According to da Costa Lopes et al., (2013) the lignin polymer is efficiently solubilized near its glass transition temperature around 165°C, although it may differ slightly with lignin composition. Palonen, (2004) reported that degradation of native lignin happens at temperatures above 200°C while dissolution of its monomer units begins at temperatures as low as 100°C. From the factor standardized effect analysis of lignin dissolved in solution, temperature is the most statistically significant factor ($p<0.1$) as observed in Figure 23 to 25. The relationship between temperature and lignin dissolution is also demonstrated with scatter plots in Figures 19 to 20 which shows that lignin dissolution increases with temperature increase. The correlation between the two is clearly demonstrated with R^2 values of 0.5996 and 0.6518 for eucalyptus fractionations with ethylene glycol and xylitol respectively.

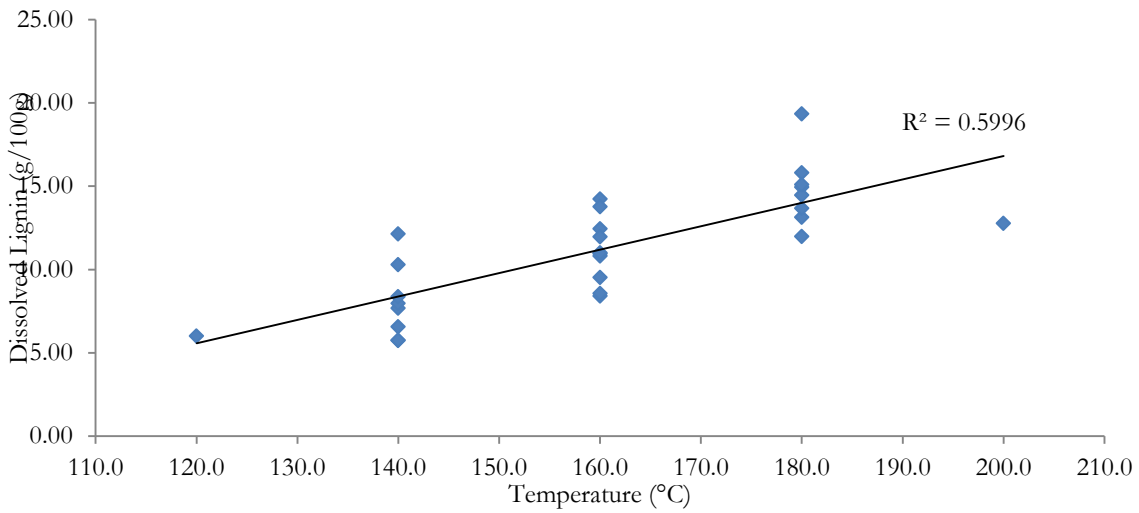


Figure 19: Eucalyptus-EG fractionations' Temperature-and dissolved lignin scatterplot

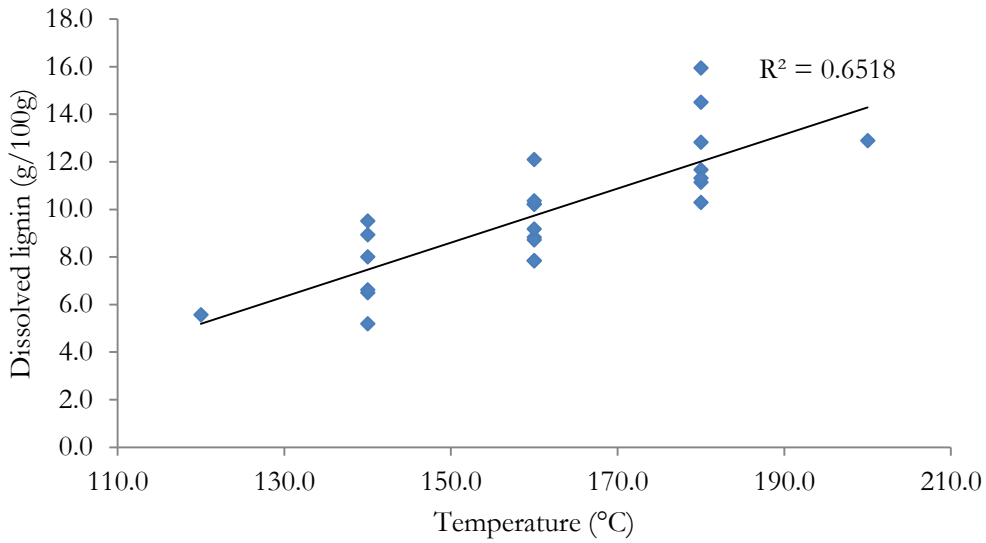


Figure 20: Eucalyptus- xylitol fractionations' -Temperature-and dissolved lignin scatterplot

On the contrary, sugarcane bagasse' lignin dissolution is influenced more by increase in catalyst concentration than temperature as shown in paretto charts in Figure 23 and 26. The linear dependency between the two is plotted in Figure 21 and 22. Gradual adjustment in the concentration of NaOH catalyst has shown to not only preserve cellulose and dissolve hemicellulose (Diedericks et al., 2012; Rabetafika et al., 2014) but it is also a good delignifying agent as demonstrated in section 4.3 and as also reported by others (Li, 2011; Peng et al., 2012). Increasing NaOH concentration in fractionating eucalyptus from 0.25% to 4%, at constant time and temperature (90°C and 30minutes) Lima et al., 2013, demonstrated that they could dissolve lignin increasingly up to 84.1% and 78.5% of the initial raw material lignin in *E. grandis* and *E. grandis x urophylla* respectively. Their lignin concentration was twofold

the range used in this study, 0.5-2.5%,however, our maximum lignin dissolved with direct raw SCB fractionation with either of the two solvents was below 12g/100g of initial raw material lignin, which is approximately 52% of the raw SCB lignin content, which falls in the range 34 to 77% from eucalyptus fractionation done by Romani et al., 2013at almost similar conditions 180−200 °C, 40−90 minutes using a polyol, 40−80% glycerol.

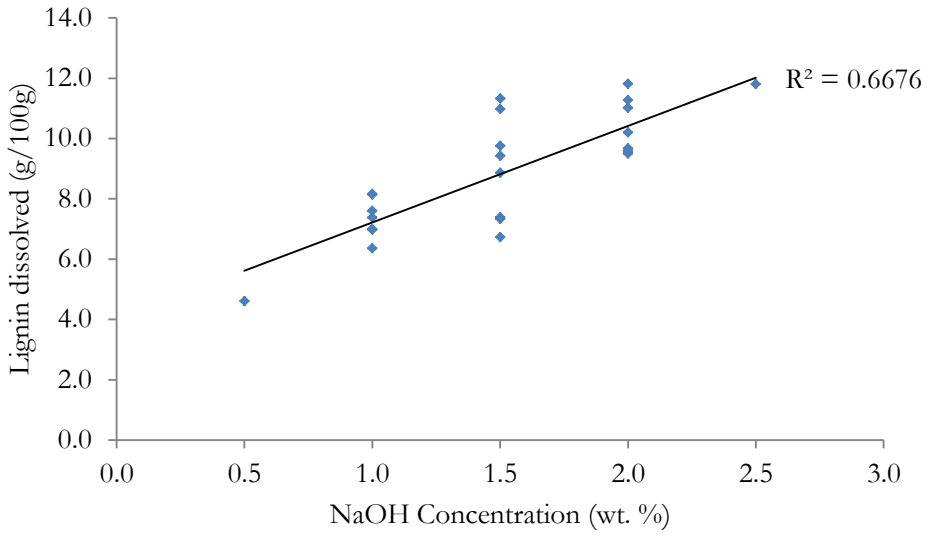


Figure 21: SCB EG fractionations' –NaOH Concentration and dissolved lignin scatterplot

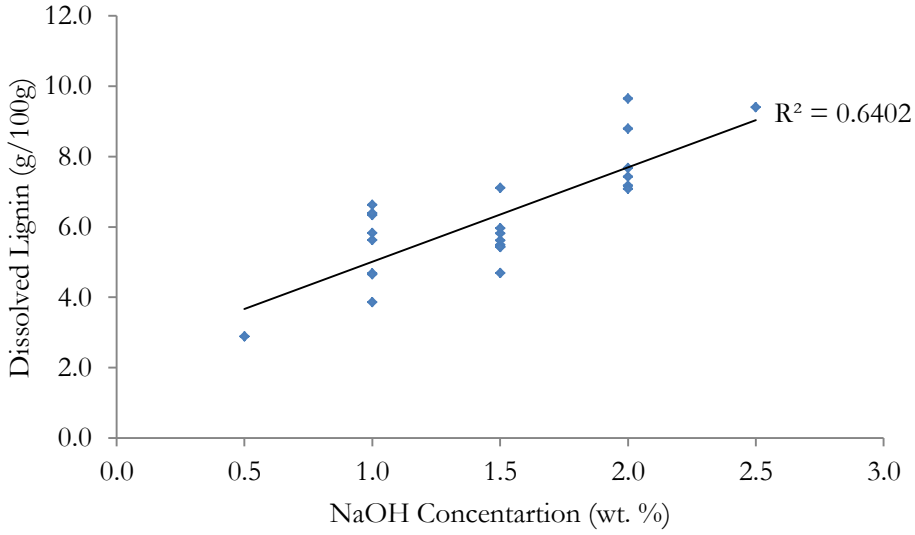


Figure 22: SCB xylitol fractionations'–NaOH Concentration and dissolved lignin scatterplot

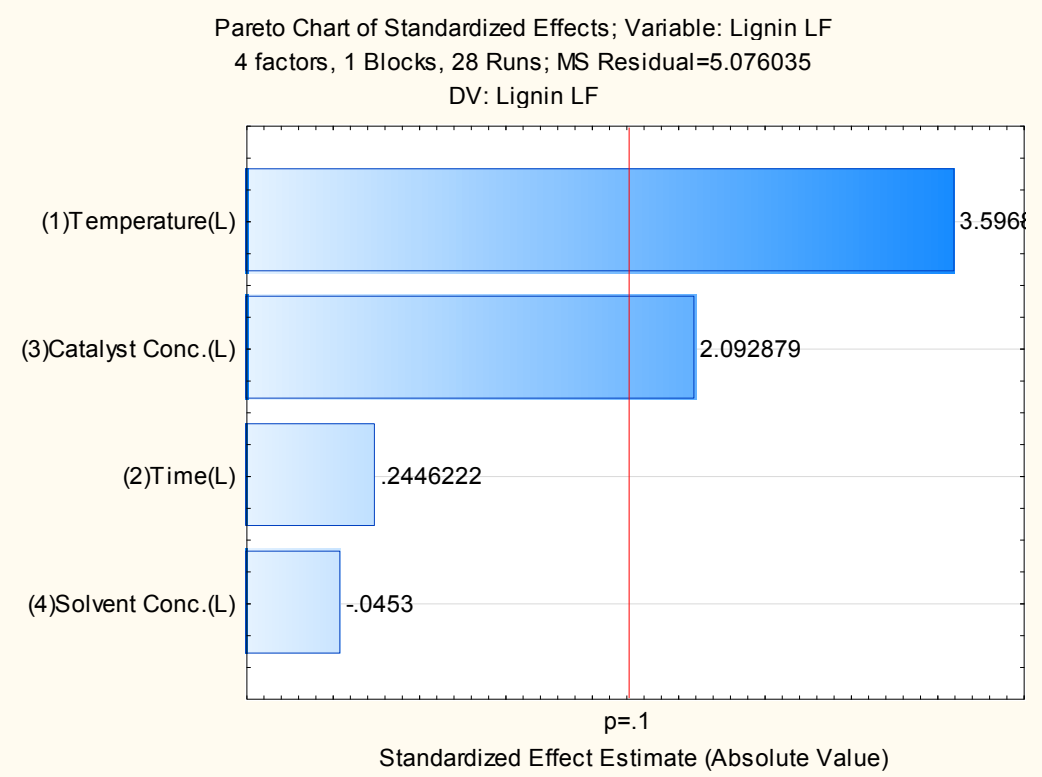


Figure 23: EC-xylitol Pareto chart of effects on lignin dissolution

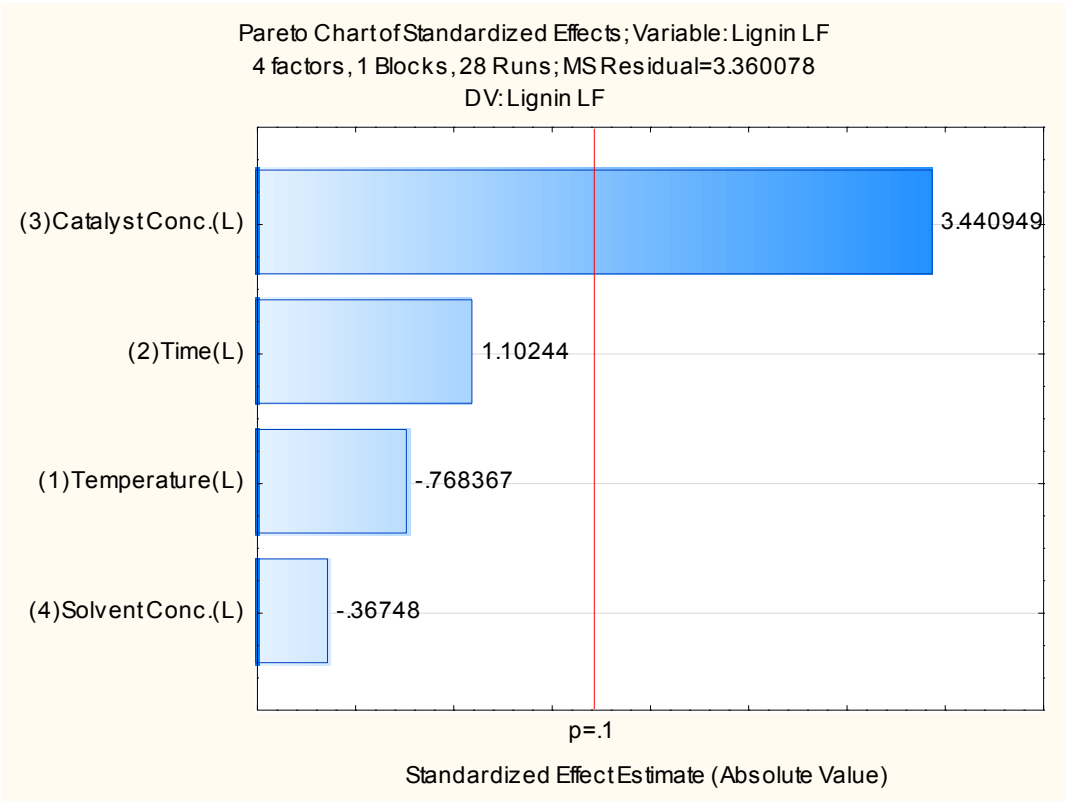


Figure 24: SCB- xylitol Pareto chart of effects on lignin dissolution

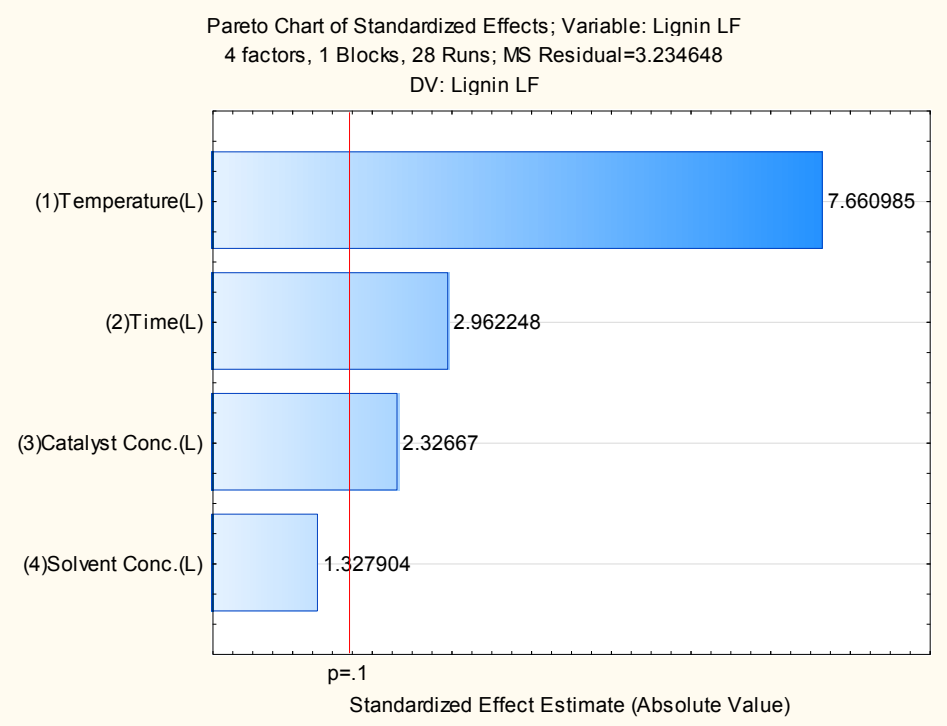


Figure 25: *E. grandis* EG Pareto chart of effects on liquid fraction lignin

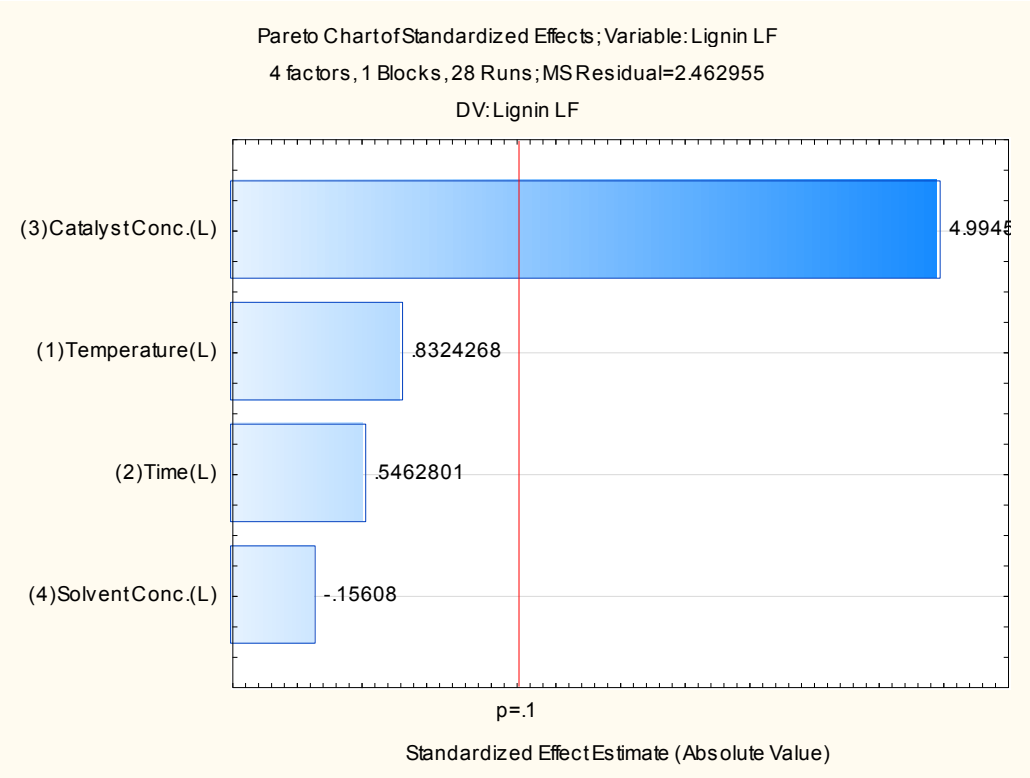


Figure 26: SCB-EG Pareto chart of effects on liquid fraction lignin

Surprisingly, the solvent alone does not have influence on dissolution of lignin as a response variable. The paretto charts shows the solvent as the least dissolution impacting factor and is most efficient when coupled with other variables. This is also supported by the effect estimate analysis in Table 36. This behavior is explained by the reaction mechanisms of lignin dissolution which requires activators such as temperature to effect dissolution (Garrote, Dominguez, et al., 1999; Katahira et al., 2013) as demonstrated in surface response model in Figure 27.

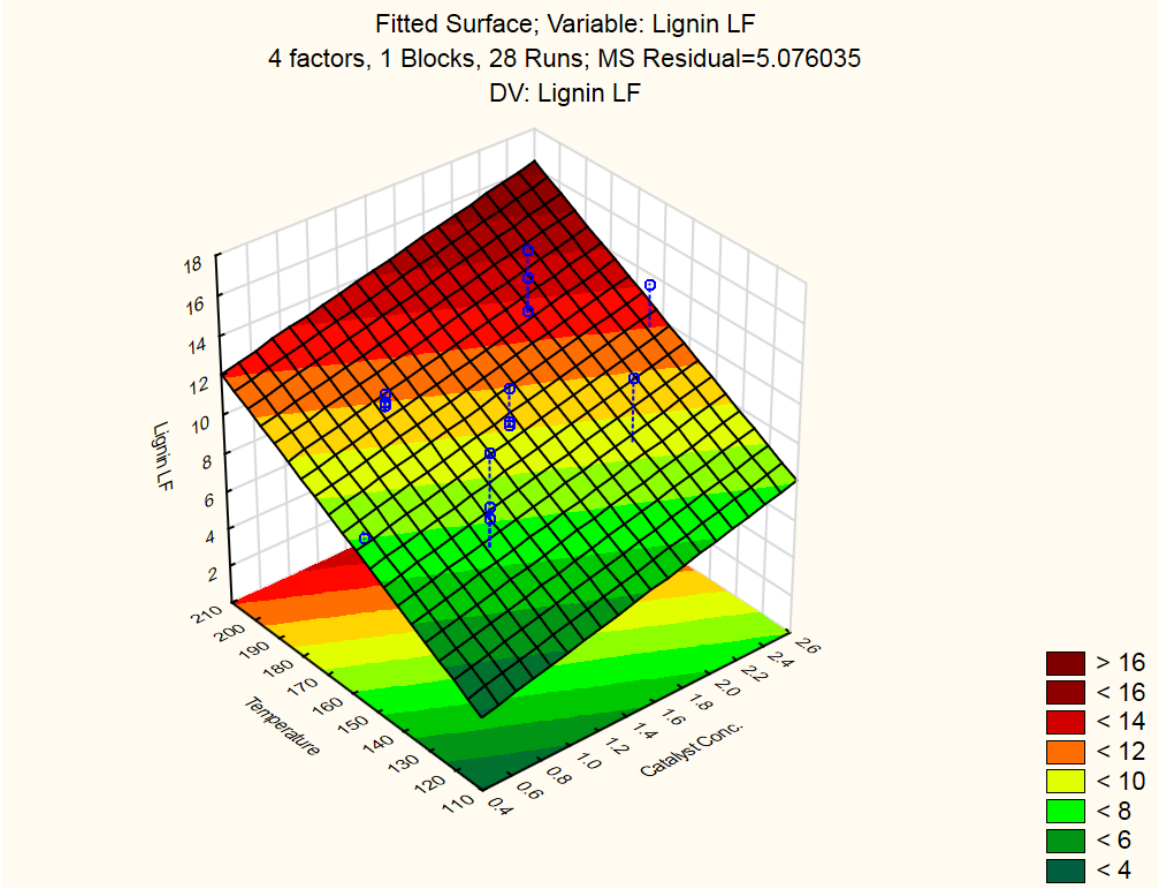


Figure 27: Temperature and catalyst concentration effect on lignin dissolution (*E. grandis*-Xylitol)

Table 36: Effect estimate analysis of Xylitol's SCB fractionation on lignin dissolution

Factor	Effect Estimates; Var.:Xylose LF; R-sqr=.65029; Adj:.57368 (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in Eucalyptus Glycol Analysis.stw) 4 factors, 1 Blocks, 28 Runs; MS Residual=1.352308 DV: Xylose LF									
	Effect	Std.Err.	t(13)	p	-90.% Cnf.Limt	+90.% Cnf.Limt	Coeff.	Std.Err. Coeff.	-90.% Cnf.Limt	+90.% Cnf.Limt
Mean/Interc.	4.17	0.581444	7.18040	0.000007	3.14530	5.204698	4.175000	0.581444	3.14530	5.204698
(1)Temperature(L)	0.87	0.474747	1.84309	0.088229	0.03425	1.715745	0.437500	0.237373	0.01713	0.857873
Temperature(Q)	-1.31	0.474747	-2.76024	0.016217	-2.15116	-0.469672	-0.655208	0.237373	-1.07558	-0.234836
(2)Time (L)	-0.25	0.474747	-0.54415	0.595549	-1.09908	0.582412	-0.129167	0.237373	-0.54954	0.291206
Time (Q)	-0.36	0.474747	-0.75918	0.461292	-1.20116	0.480328	-0.180208	0.237373	-0.60058	0.240164
(3)Catalyst Conc.(L)	0.34	0.474747	0.71968	0.484459	-0.49908	1.182412	0.170833	0.237373	-0.24954	0.591206
Catalyst Conc.(Q)	-0.16	0.474747	-0.33790	0.740833	-1.00116	0.680328	-0.080208	0.237373	-0.50058	0.340164
(4)SolventConc.(L)	-1.12	0.474747	-2.36968	0.033956	-1.96575	-0.284255	-0.562500	0.237373	-0.98287	-0.142127
Solvent Conc. (Q)	0.13958	0.474747	0.29402	0.773387	-0.70116	0.980328	0.069792	0.237373	-0.35058	0.490164
1L by 2L	-1.08	0.581444	-1.87034	0.084117	-2.11720	-0.057802	-0.543750	0.290722	-1.05860	-0.028901
1L by 3L	-0.28	0.581444	-0.49446	0.629233	-1.31720	0.742198	-0.143750	0.290722	-0.65860	0.371099
1L by 4L	-0.33	0.581444	-0.58045	0.571535	-1.36720	0.692198	-0.168750	0.290722	-0.68360	0.346099
2L by 3L	0.36	0.581444	0.62345	0.543774	-0.66720	1.392198	0.181250	0.290722	-0.33360	0.696099
2L by 4L	0.56	0.581444	0.96742	0.350989	-0.46720	1.592198	0.281250	0.290722	-0.23360	0.796099
3L by 4L	-0.23	0.581444	-0.40847	0.689580	-1.26720	0.792198	-0.118750	0.290722	-0.63360	0.396099

Finally, the analysis of lignin data after fractionation of the materials and with the respective solvents and other conditions discussed above have provided invaluable information on the behavior of the two solvents, which is summarized as follows;

1. More lignin was dissolved from raw materials with ethylene glycol fractionations as compared to xylitol fractionations. Xylitol fractionations generally solubilized 18.0-80.0% and 5.49-62.10% of initial lignin in raw *E. grandis* and sugarcane bagasse respectively as compared to ethylene glycol fractionations which solubilized 33.77-86.67% and 21.16-72.99% of initial lignin in raw *E. grandis* and sugarcane bagasse respectively) (Table 30-33). This suggests that ethylene glycol effectively breaks the lignin-carbohydrate bonds far better than xylitol solutions. This explains the small molecular structure of ethylene glycol (62.07g/mol and 2 carbons atoms) as compared to xylitol (152.15g/mol and five carbon atoms), smaller molecules are able to penetrate the pores of the lignocellulose structure to reach intermolecular bonds between carbohydrates and lignin also relative to its low viscosity, dissolution of lignin is eminent (Jian et al., 2013; Singh & Ekhe, 2014)

2. The hemicellulose pre-extraction step necessary for improving dissolution of lignin in the subsequent fractionation step with ethylene glycol. Dissolution of lignin from raw fractionation of raw material achieved up to 86.67% of raw material lignin, whilst up near complete dissolution 99.53% of initial lignin in the material was dissolved. This improvement is due to removal of the hemicellulose component, some lignin and cellulose with the NaOH pre-extraction step. Partial removal of these components create large pores for solvent penetration and renders some of the bonds between carbohydrates and lignin weaker which makes it easier for the solvent to break intermolecular bonds and therefore dissolve the lignin polymer.

3. Temperature is one of the critical factors as presented in Figures 22 to 27, for lignin dissolution because it supports reactions which break the lignin-carbohydrate bonds through activation. Increase in temperature increases dissolution of the lignin macromolecule (Agrawal et al., 2014) by weakening the covalent ether bonds between lignin and carbohydrates effecting dissolution of lignin.

4. NaOH also came out as one of the critical factors responsible for lignin dissolution, second after temperature (Figure 22-25). NaOH is responsible for cleavage of α- and β-aryl ether bonds (Bujanovic, Ralph, Reiner, Hirth, & Atalla, 2010) effecting lignin dissolution.

5. Polyols, although widely known to be excellent delignifying agents as reported in literature (Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013) have in this study revealed that they require other variable conditions such as temperature and a catalyst to effectively dissolve lignin as shown in results presented in Tables 30-35. Literature also reported the use of polyols combined with catalysts, mostly acids to delignify lignin lignocellulose (Moghaddam et al., 2014; Muhammad Safwan et al., 2015).

4.4.5 Component recovery at optimum conditions and conclusions

Based on recorded data from the multiple fractionation conditions, the models were used to fit for optimum fractionation conditions. Determined optimum conditions were then run to get actual data, validate the optimum estimation and also to obtain materials for qualitative assessment tests as presented in Chapter Five. The models were fitted to give the best response possible, given the tediousness of the process steps involved. The accuracy of results and data interpretations of each CCD were measured based on the R-square and R-square adjusted reported in Appendix D.

The desirability (expressed as a value from 0 as least desirable to 1 as most desirable) for optimum conditions was computed using Statistica 12.6 as explained by Kuhnt & Rudak, 2013. This was done by predicting responses of dependent variables (carbohydrate contents, dissolved lignin, residual solid lignin

and enzymatic hydrolysis), or Y variables, by fitting the actual values of dependent variables using regression equations based on levels of independent variables (temperature, time, catalyst and solvent concentration) or X variables. This was then used to predict levels of the X variables that concurrently generate a prediction of the most desirable responses of Y variables.

Once the CCD was fitted satisfactorily for linear model with R adjusted >0.4, with alpha value and Confidence Interval kept at 0.1 and 90% respectively, the desirability for each of the Y variables were set at maximum or most desirable (desirability = 1) and/or to minimum or undesirable (desirability = 0) to meet the goals of this study. As mentioned earlier, dependent variables considered were; glucose (g/100g), xylose (g/100g), solvent soluble lignin (g/100g), acid insoluble lignin (g/100g) and enzymatic hydrolysis efficiency (%). Although a quality parameter, enzymatic hydrolysis was optimized together with yield factors for three reasons (1) enzymatic hydrolysis efficiency reveals the extension of cellulose independence from lignin and hemicelluloses enclosure (2) enzymatic hydrolysis is related to the amount of lignin dissolved or released from the solid residue after fractionation (3) extension of hemicellulose removal from the solid residue is related to enzymatic hydrolysis. All these are in-line with the desired fractionation route (residual cellulose rich solid and dissolution of hemicelluloses and lignin). Additionally, this is information which could not be directly deduced and interpreted from all other quality parameters measured in this study.

A summary of desirability weight allocation is presented in Table 37. These allocations were based on our hypotheses and the actual reaction mechanisms of polyols as discussed in sections 1.4.2-1.4.4; glucose was expected to concentrate in the solid fraction hence the desirability for glucose is set at maximum in the solid fraction while set at minimum in the liquid fraction i.e. 0. Similarly, majority of hemicelluloses and lignin were expected to be dissolved into the liquid fraction hence maximum desirability is desired for the two components in the liquid fraction i.e. desirability = 1. Ultimately and in line with the aims of the research, poor performance of the fractionations could only be acceptable with desirability of 0.5 or more as show in Table 37. And finally, high desirability was always set for enzymatic hydrolysis of residual solids.

Table 37: Desirability weight allocation for dependent variables

(a-Glucose (g/100g), b-Xylose (g/100g), c- Solvent soluble lignin, d- Acid insoluble lignin e-Enzymatic hydrolysis efficiency)

	Dependent (Y) variables						
	Liquid Fraction			Solid Fraction			Enzymatic hydrolysis (%)
Desirability	[a]Glc	[b]Xyl	[c]SSL	Glc	Xyl	[d]AIL	
High	0	1	1	1	0	0	1
Medium	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Low	1	0	0	0	1	1	0

Predictions and desirability profiling analysis results are presented in Appendix D together with factor levels and predicted responses. Selected desirability surface contours are presented in Appendix D. As observed from the desirability values presented; it was challenging to achieve desirability of over 0.9 because of the variability and number of dependent factors considered. These desirability values obtained represent the highest compromise for each of the components and gives the best overall optimum conditions for fractionation; 0.71221 and 0.64871 for EC and SCB with xylitol as a solvent and 0.60819, 0.81191 for EC and SCB with EG respectively, the most possible conditions at which hemicelluloses, lignin and cellulose can be fractionated in reasonable yield and quality. Desirability for fractionation of hemicellulose pre-extracted materials using 60% ethylene glycol gave desirability of 0.57549 and 0.81785 for EC and SCB respectively.

Predicted results at optimum conditions from the models and the actual run assays processed from predicted conditions are presented in Tables 38-41 alongside their respective fractionation conditions. Overall, the models used fitted quite well, with desirability values ranging between 0.58 to 0.82. Looking at the data that was generated after actual runs, there is minimal variability between the predicted and actual results (less than 10% based on 95% confidence interval).

Table 38: Cellulose preservation and dissolution predicted at optimum raw material fractionation conditions from model fit, 90% CI and alpha value at 0.1 versus runs from actual experiment runs

					Statistical estimated desirable composition							
Fractionation	Temp (°C)	Time (Hours)	Catalyst Conc. (wt. %)	Solvent Conc. (%)	Glucose dissolved in the Liquid Fraction (g/100g)	Glucose recovered in the Liquid Fraction (g/100g)	Estimated degraded Glucose (g/100g)	Glucose remaining in Solid Fraction (g/100g)	Mass Balance (g/100g)	Recovery (%)	EH[e]	Desir-ability
Xylitol Sugarcane bagasse	160	4	2	20	6.37	1.02	5.35	33.87	34.89	86.70	30.17	0.65
Xylitol *E. grandis*	180	4	1	15	7.03	0.70	6.33	40.42	41.12	86.66	56.16	0.71
EG. SCB	200	5	1.5	40	1.64	1.47	0.17	38.60	40.07	99.58	64.20	0.81
EG. *E. grandis*	180	4	2.5	80	24.65	0.45	24.20	25.80	26.25	55.32	81.10	0.61
					Actual composition							
Raw sugarcane bagasse								40.24	40.24	100.00	12.76	-
Raw *E. grandis*								47.45	47.45	100.00	21.08	-
Xylitol Sugarcane bagasse	160	4	2	20	6.24	1.00	5.24	33.00	34.00	84.49	28.40	-
Xylitol *E. grandis*	180	4	1	15	7.45	1.10	6.35	38.90	40.00	84.30	57.50	-
EG. SCB	200	5	1.5	40	8.44	1.50	6.94	31.80	33.30	82.75	59.76	-
EG. *E. grandis*	180	4	2.5	80	18.25	0.60	17.65	29.20	29.80	62.80	76.77	-

Table 39: Hemicellulose dissolution and recovery predicted at optimum raw material fractionation conditions from model fit, 90% CI and alpha value at 0.1 versus runs from actual experiment runs

					Statistical estimated desirable composition						
Fractionation	Temp (°C)	Time (Hours)	Catalyst Conc. (wt. %)	Solvent Conc. (%)	Xylose dissolved in the Liquid Fraction (g/100g)	Xylose recovered in the Liquid Fraction (g/100g)	Estimated degraded Xylose (g/100g)	Xylose remaining in Solid Fraction (g/100g)	Mass Balance (g/100g)	Recovery (%)	Desir-ability
Xylitol Sugarcane bagasse	160	4	2	20	13.12	6.39	6.73	10.23	16.62	71.18	0.65
Xylitol *E. grandis*	180	4	1	15	12.72	5.60	7.12	8.18	13.78	65.93	0.71
EG. SCB	200	5	1.5	40	18.46	5.53	12.93	4.89	10.42	44.63	0.81
EG. *E. grandis*	180	4	2.5	80	14.60	1.50	13.10	6.30	7.80	37.32	0.61
					Actual composition						
Raw sugarcane bagasse								23.35	23.35	100.00	-
Raw *E. grandis*								20.90	20.90	100.00	-
Xylitol Sugarcane bagasse	160	4	2	20	12.85	7.30	5.55	10.50	17.80	76.23	-
Xylitol *E. grandis*	180	4	1	15	11.08	6.70	4.38	9.82	16.52	79.04	-
EG. SCB	200	5	1.5	40	16.97	4.40	12.57	6.38	10.78	46.17	-
EG. *E. grandis*	180	4	2.5	80	13.87	1.10	12.77	8.03	9.13	43.68	-

Table 40: Lignin dissolution and recovery in fractionation liquor predicted at raw material optimum fractionation conditions from model fit, 90% CI and alpha value at 0.1 versus runs from actual experiment runs

					Statistical estimated desirable composition						
Fractionation	Temp (°C)	Time (Hours)	Catalyst Conc. (wt. %)	Solvent Conc. (%)	Lignin dissolved in the Liquid Fraction (g/100g)	Lignin recovered in the Liquid Fraction (g/100g)	Estimated degraded Lignin (g/100g)	Lignin remaining in Solid Fraction (g/100g)	Mass Balance (g/100g)	Recovery (%)	Desir-ability
Xylitol Sugarcane bagasse	160	4	2	20	9.60	9.41	0.19	13.36	22.77	99.17	0.65
Xylitol *E. grandis*	180	4	1	15	21.74	17.43	4.31	3.78	21.21	83.11	0.71
EG. SCB	200	5	1.5	40	18.53	12.73	5.80	4.43	17.16	74.74	0.81
EG. *E. grandis*	180	4	2.5	80	20.04	17.80	2.24	5.48	23.28	91.22	0.61
					Actual composition						
Raw sugarcane bagasse								22.96	22.96	100.00	-
Raw *E. grandis*								25.52	25.52	100.00	-
Xylitol Sugarcane bagasse	160	4	2	20	13.82	7.30	6.52	9.14	16.44	71.60	-
Xylitol *E. grandis*	180	4	1	15	19.61	15.10	4.51	5.91	21.01	82.33	-
EG. SCB	200	5	1.5	40	17.86	10.50	7.36	5.10	15.60	67.94	-
EG. *E. grandis*	180	4	2.5	80	21.22	14.20	7.02	4.30	18.50	72.49	-

Table 41: Optimum fractionation conditions for the fractionation of hemicellulose pre-extracted solid residues with ethylene glycol from model fit, 90% CI and alpha value at 0.1 versus runs from actual experiment runs

					Statistical estimated desirable composition												
					Components dissolved in the Liquid Fraction (g/100g)			Components recovered in the Liquid Fraction (g/100g)			Estimated degraded Components (g/100g)			Solid Fraction			EH[e]	Desir-ability
Fractionation	Temp (°C)	Time (Hours)	Catalyst Conc. (wt. %)	Solvent Conc. (%)	[a]Glc	[b]Xyl	[c]SSL	[a]Glc	[b]Xyl	[c]SSL	[a]Glc	[b]Xyl	[c]SSL	Glc (g/100g)	Xyl (g/100g)	[d]AIL (g/100g)		
Raw sugarcane bagasse														40.24	23.35	22.96	12.76	-
Raw *E. grandis*														47.45	20.90	25.52	21.08	-
Hemis extracted SCB					4.85	19.12	15.55	2.01	16.09	10.55	2.84	3.03	5.00	35.39	4.23	7.41	29.52	-
Hemis extracted *E. grandis*					5.01	11.13	8.40	1.03	7.55	6.32	3.98	3.58	2.08	42.44	9.77	17.12	35.39	-
EG. Pre-extracted SCB	193	1.32	0.66	60	4.63	3.04	4.16	0.77	2.05	3.99	3.86	0.99	0.17	30.76	1.19	3.25	66.28	0.65
EG. Pre-extracted EC	176.82	3.84	1.5	60	16.82	8.54	15.56	0.55	1.30	15.95	16.27	7.24	0.39	25.62	1.23	1.56	86.28	0.71
					Actual Composition													
EG. Pre-extracted SCB	193	1.32	0.66	60	6.96	2.35	6.88	0.43	1.26	6.00	6.53	1.09	0.88	28.43	1.88	5.68	77.18	0.81
EG. Pre-Extracted EC	176.82	3.84	1.5	60	16.42	8.44	16.03	0.50	0.75	15.72	15.92	7.69	0.31	26.02	1.33	7.14	71.30	0.61

As discussed in the earlier sections, 4.4.3, hemicellulose recovery from fractionation liquor was reported to start declining at temperatures above 160°C. However, models predicted optimum dissolution to be achieved at temperatures above 160°C. Xylitol fractionations particularly required temperatures between 160°C to 180°C for optimization which is attributed to the low concentration of xylitol (15-20wt %). On the contrary, ethylene glycol fractionations required relatively higher temperatures for optimum fractionation results, between 180°C and 200°C, high temperatures are required to dissolve more hemicelluloses. High temperature fractionations are a concern, especially when easily degraded components such as hemicelluloses (Xiang et al., 2004) are priority for oligosaccharide recovery. But for polyols, high temperatures have been tested to give good responses as the case with Romani et al., 2013's fractionation of *E.globulus* 180 -200 °C, using a range of 40−80%glycerol and 40−90 minutes achieving preservation of up to 77% of cellulose, 84% dissolution of hemicelluloses and approximately 67.74% lignin delignification. As compared to our conditions, shorter reaction conditions were used 40-90 minutes versus this study's 4-5 hours. In the same study, up to 98% enzymatic saccharification was reported, whereas under the set of optimum conditions in this study, enzymatic saccharification of solids from ethylene glycol fractionations reported, 59.76% for SCB and 76.77% for EC. Enzymatic hydrolysis increased after fractionation of hemicellulose pre-extracted solids with 60% EG and reduced temperature (176.82°C), enabling up to 83.18% and 81.30% saccharification efficiency for SCB and EC respectively.

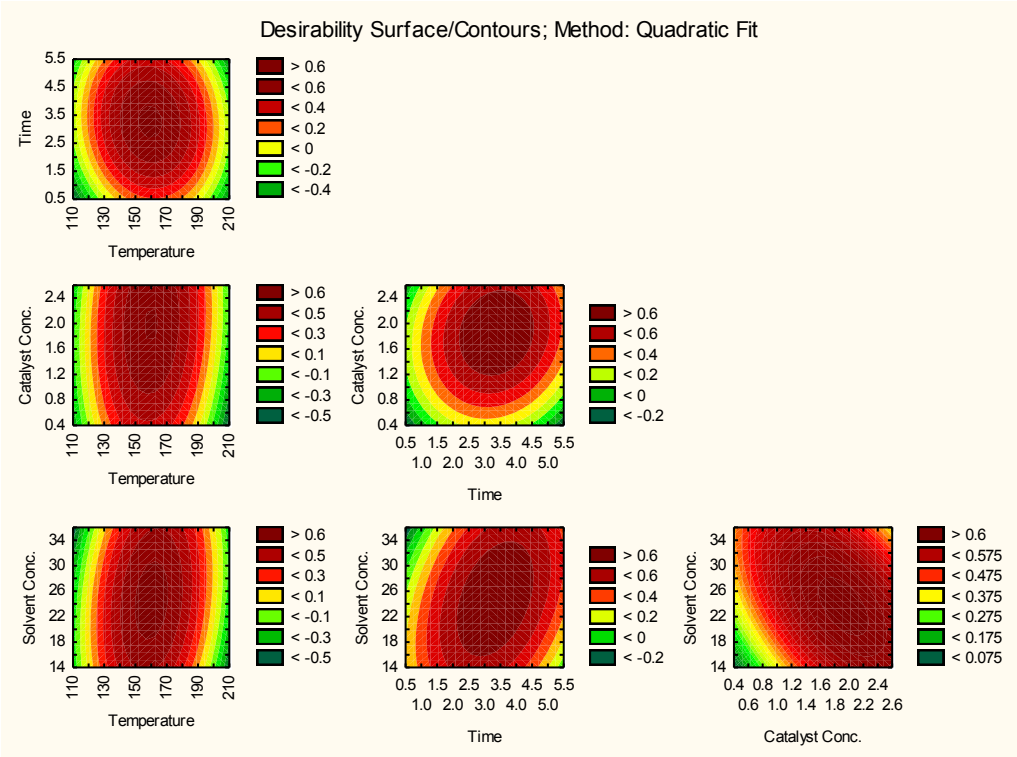


Figure 28: Desirability surface contours for SCB-xylitol fractionations

Generally, increasing severity of some conditions such as temperature and concentration of catalyst enhances optimization towards higher desirability, i.e. the desirability surface plot of SCB-xylitol fractionations as shown in the surface contours in Figure 27, demonstrating that increasing temperature and time while operating at moderate catalyst concentration and solvent concentration is the ideal optimum setting to achieve the targets of this study. Additionally, it appears that to achieve optimum results, temperature and solvent concentration has to work inversely, i.e. high solvent concentration and low temperature as it is with xylitol fractionations i.e. for sugarcane bagasse, optimum conditions were achieved at 160°C, 20% xylitol at 4 hours and NaOH concentration of 2.0%, while eucalyptus optimization was done at 180°C, 15% xylitol at 4 hours and NaOH concentration of 1.0%. The same concept applies with ethylene glycol fractionations; optimization for sugarcane bagasse fractionation was achieved at 200°C, 40% ethylene glycol at 5 hours and 1.5% NaOH, while eucalyptus fractionation optimization estimated at 180°C, 80% ethylene glycol for 4 hours and NaOH concentration of 2.5%. Similar observation between temperature and concentration of NaOH is also noticed between the two fractionations setups. Depending on the variable that is high between temperature and solvent concentration, the fractionation activation is determined by either component which is high (limiting factor), explaining this inverse proportionality.

Under the predicted optimum conditions and based on general fractionation runs reported in sections 4.4.2-4.4.4, the following are hereby noted:

- Enzymatic hydrolysis efficiency of solids from xylitol' fractionations are lower than ethylene glycol fractionations, i.e. at optimum conditions about 28.40% and 57.50% versus 59.76% and 76.77% efficiency was achieved for sugarcane bagasse and eucalyptus respectively. This suggests ethylene glycol to be able to expose cellulose from the lignin-hemicellulose enclosure much better than xylitol. This is due to the fact that ethylene glycol fractionations removed more lignin from the solid residue as compared to xylitol fractionations (section 4.4.4). Lignin is one of the factors affecting cellulose accessibility by enzymes, hence higher lignin content is associated with lower digestibility (Table 38)

- For the range of conditions tested with all solvents and substrates more hemicellulose is dissolved with xylitol fractionations as compared to ethylene glycols' and this is also observed at optimum fractionation conditions i.e. 7.3g/100g of initial raw material xylose was dissolved from SCB using xylitol, while still keeping 10.5g/100g of initial xylose in the solid, whereas 4.4g/100g of initial xylose was dissolved from SCB with ethylene glycol fractionations while only 6.38g/100g of initial xylose in the raw material remained in the solid residue. This demonstrated the ability of xylitol to effectively break the hemicellulose-cellulose and hemicellulose-lignin bridges to dissolve hemicelluloses better than ethylene glycol which is explained in terms of number of active hydroxyls on the xylitol molecule

which are responsible for hemicellulose hydrolysis. Ethylene glycol fractionations are also accompanied by greater loses of hemicelluloses in solution, which can be aligned to one or two of the factors discussed in section 4.4.2-4.4.3, i.e. formation of xylosides formed between xylose molecules and the glycol present in solution or degradation of hemicelluloses due to higher temperatures used in the fractionations (Deng et al., 2014; Moghaddam et al., 2014; Zhang, O'Hara, et al., 2013)

- At optimum conditions, more lignin is dissolved with ethylene glycol as compared to xylitol fractionations. This is could be due to the high temperatures optimized for ethylene glycol fractionations which in turn favors lignin dissolution (Adler, 1977; Brienzo et al., 2009; Jian et al., 2013; Kirk, 1983; Kline, Hayes, Womac, & Labb, 2010; Lapierre, 2008; Lima et al., 2013; vom Stein et al., 2011) and destruction of other components at such hemicelluloses (Xiang et al., 2004). This trend is also observed throughout the range of conditions tested.

- While xylitol fractionations dissolved more hemicelluloses than ethylene glycol fractionations. Their dissolution was still lower than what was achieved from hemicellulose pre-extraction with NaOH owing to the reduced concentration of the hemicellulose hydrolyzing agent NaOH (pre-extraction used 1.5M versus <0.5M used as catalyst in all runs). In addition, xylitol fractionation of residual solids also contained more lignin as compared its counterpart i.e. at optimum fractionation conditions about 9.14g/100g and 5.91g/100g of raw material lignin remained in residual solid of SCB and EC respectively while 5.10g/100g and 4.30g/100g of raw material lignin remained in ethylene glycol fractionation residual solids, suggesting ethylene glycol to be more reactive towards covalent C-C bonds than xylitol. Lignin in residual solid is among some of the hindrances towards enhanced enzymatic hydrolysis of the solid (Katahira et al., 2013; Palonen, 2004), which is associated with poor digestibilities of xylitol fractionation residual solids.

- Xylitol fractionations of the two materials achieved the 80% target of keeping cellulose in the solid residue, while ethylene glycol fractionations preserved below 70% of the initial raw material cellulose (Table 39), suggesting that ethylene glycol is more selective towards cellulose dissolution than xylitol solutions. For xylitol fractionations, this also means minimal destruction of the cellulose component, as compared to ethylene glycol. As discussed earlier the reactivity of ethylene glycol on cellulose (causing cellulose dissolution) is explained by the small molecular structure of ethylene glycol (62.07g/mol and 2 carbons atoms) as compared to xylitol (152.15g/mol and five carbon atoms), smaller molecules are able to penetrate the pores of the lignocellulose structure to reach enclosed structures of cellulose easily due to their low viscosities, dissolution of cellulose is eminent (Jian et al., 2013; Singh & Ekhe, 2014). Xylitol is therefore the ideal solvent for preserving cellulose in *E.*

grandis and sugarcane bagasse solid residues when compared to ethylene glycol fractionations as demonstrated in Tables 18-21.

Chapter Five

5 QUANTITATIVE ASSESSMENT OF THE QUALITY OF PRODUCTS FROM *EUCALYPTUS GRANDIS* AND SUGARCANE BAGASSE FRACTIONATIONS

5.1 Introduction

In addition to the yields of the respective fractions from the fractionation process, desirability and effectiveness of solvents and the fraction process setup were assessed by subjecting the product streams to various wet chemical and analytical test methods. This allowed further insight into the usefulness of the products in terms of their quality and suitability for post processing into value added materials and products with specific focus on the following quality targets:

- Crystallinity of the cellulose rich residue of >50% and enzymatic digestibility of more than 80% efficiency as applicable for efficient conversion of cellulose into biofuels.
- Recovery of hemicellulose polymers and biopolymers with Molecular weight average of 10 000 $gmol^{-1}$ or more for applications in production of foams, biopolymeric films plastics and bio-composites.
- Lignin carbon content of >30% and a syringyl-guaicyl (S/G) ratio of >1.52 and 3.06 for sugarcane bagasse and *E. grandis* respectively.

Functional groups are the core determinants of the types of chemical structures in the products. The Fourier Transform Infrared Resonance (FTIR) method was used for this purpose; all three streams were analysed for functional groups. Chemical structures of the components revealed specific information regarding the reactions that occurred between the components, solvents and other reactions conditions such as temperature, reaction time and NaOH as a catalyst.

Because cellulose is the main raw material source for biobased products, its quality is assessed in terms of its digestibility by enzymes; the higher the digestibility, the easier it is to convert cellulose to fermentable sugars so that eventually it gets converted to biobased products. A combination of two enzymes is normally used to act on the different components in cellulose, cellulases for polymeric cellulose breakdown and endoglucanase for glucan units. Additionally, accessibility of enzymes is also said to be partly attributed to its crystallinity (Hendriks & Zeeman, 2009; Hou, Smith, Li, & Zong, 2012; Terinte et al., 2011; Xu et al., 2012), hence the crystallinity index (CI) of the cellulose stream is to be measured.

Furthermore, in order to confirm that indeed the fractionation processes generated hemicellulose biopolymers and/or oligomers, the molecular weight analysis of the hemicellulose stream was analysed. Size exclusion chromatography approach was considered for this purpose. High molecular weights are associated with longer hemicellulose chains; polymers and oligomers.

Last but not least, structural composition of lignin expressed in terms of its main building blocks, the monomeric units, syringil and guacyl were also assessed. This data provides useful information on the process itself and the effect it has on virgin lignin. Additionally, the purity of lignin was also assessed by a proximate analysis.

5.2 Results and Discussions

5.2.1 Quantitative assessment of the quality of residual cellulose rich solid fraction

a) Functional group identification

Characterization of virgin raw materials, SCB and EC, with FTIR spectroscopy revealed changes in the functional groups composition between the raw materials and fractionated components which are also directly linked to the yields reported in Chapter 4. Summary of findings are presented in Table 42. Figure 31 shows the spectrum of the virgin raw materials. Although there are differences in intensities of the peaks, the substrates revealed similar trends for main bands, characteristic of their compositions. Table 42 presents a summary of the higher and main bands observed in the feedstock. Broad bands visible in both raw materials at 3310-3340 cm^{-1} was due to O-H stretching vibration, and the band at 2930 cm^{-1} was characteristic of various types of C-H bonds.

FTIR spectras of the residual solids from the fractionation and pre-extraction processes are presented in Figure 30 and 31, while raw material spectras are shown in Figure 29. As confirmed by the sugar compositional analysis of residual solids, they are enriched with cellulose; hence we expect the FTIR spectra of these solids to reflect a defined structure of cellulose. Raw materials and commercial Avicel was used for this comparison. However, since other components, i.e. lignin and hemicellulose were not completely removed from the solid through the fractionation or hemicellulose pre-extraction processes, their functional groups bands were still observed in the residual solid spectras. We further observe that the peak appearing in the regions 1700 to 1756cm^{-1} of untreated raw materials is absent in most of the treated samples or observed albeit very low intensity. This disappearance of the band that occurs at about 1730 cm^{-1} (the carbonyl stretching region of hemicelluloses) reveals that chemical treatment of the raw materials results in the cleavage of ester bands of hemicelluloses (Hou et al., 2013), such as acetyl and uronic ester groups (Sun et al., 2014), this could not be verified from the compositional analysis of the

feedstock. Lima et al., 2013 further suggests that our bands in the raw materials at 1731cm^{-1} and 1730cm^{-1} for EC and SCB respectively, which comes close to their finding at 1738/1734cm^{-1} are characteristic of hemicelluloses C=O conjugates in xylans(Yoo, 2012). The absence of this band in treated solids further reiterates that hemicelluloses were being removed from the raw materials when subjected to xylitol/EG fractionation or NaOH extraction.

Table 42: Assignment of major infrared bands for raw materials

Wavenumber (cm^{-1}) (EC/SCB)	Vibration	Contributing source	Reference
3364/3375	O-H linked shearing	Polysaccharides	(S. N. Sun et al., 2014)
2918/2908	C-H symmetrical stretching	Polysaccharides	(Sun et al., 2014)
1731/1732	C=O unconjugated stretching	Xylans	(Lima et al., 2013)
1614/1593	C-O aromatic ring	Lignin	(Rodrigues, Meier, Faix, & Pereira, 1999b)
1423/1422	C-H deformation	Lignin	(Bodîrlău & Teacă, 2009a)
1030/1031	C-O stretch	Polysaccharides	(Rodrigues et al., 1999b)
895/896	C-H deformation	Cellulose	(Rodrigues et al., 1999b)
	β-glycosidic linkages	Polysaccharides	(Zhang, Rackemann, et al., 2013)

As compared to commercial cellulose; Avicel, our cellulose rich solids from both materials do not possess two strong peaks within the region 1100 to 1160cm^{-1}. Some residual solids display a single peak in this region while absent in others, although two strong peaks are present in the commercial cellulose (Avicel) as observed in Figure 30, at 1104m^{-1} and 1157cm^{-1} respectively. The two peaks are known C-O stretches contributed by the polysaccharides(Hou et al., 2013; Watkins, Nuruddin, Hosur, Tcherbi-Narteh, & Jeelani, 2014; Zhang, O'Hara, et al., 2013).

In addition to some of the major bands summarized Table 42, generally bands observed in cellulose rich solids near the regions 1000-895cm^{-1} proves the presence of polysaccharides(Xuezhi Li et al., 2014; Postma, 2012; Watkins et al., 2014; Yao et al., 2015), 2400-2500cm^{-1} region bands are assigned to lignin(J.

Li, 2011) while those in the region 2500-2600cm^{-1} associated with aromatic rings present in lignin monomers, i.e. syringil and guacyl (Cãpraru et al., 2009, 2009; H. Yang et al., 2007; Zhou et al., 2011). Furthermore, the removal of amorphous cellulose from the raw materials with the fractionation treatment or hemicellulose extraction results in the exposure of the crystalline cellulose (Guo et al., 2012; Kumar et al., 2009b; Wyman et al., 2005; H. Yang et al., 2007) exposes functional groups of cellulose bands, for instance the bands from cellulose contributions such as the O-H linked shearing and C-H symmetrical stretching found in the regions 2600-3600cm^{-1} increase in intensity (Sun et al., 2014; H. Yang et al., 2007). An opposite observation when some bands gets reduced in intensity as compared to the raw material bands also suggest that the particular component contributing to that band was either partially removed or completely removed from the raw material by the treatment process. This is in agreement with the HPLC data presented in Table 17-22 were hemicelluloses and lignin was removed from the raw material leaving a cellulose enriched solid residue A similar trend was reported by Bodîrlău & Teacă, (2009b) with reduction of the hydroxyl group band intensity at 3456 cm^{-1} after fractionation due to hydrolysis. This observation also supports the cellulose losses reported in section 4.4.2.

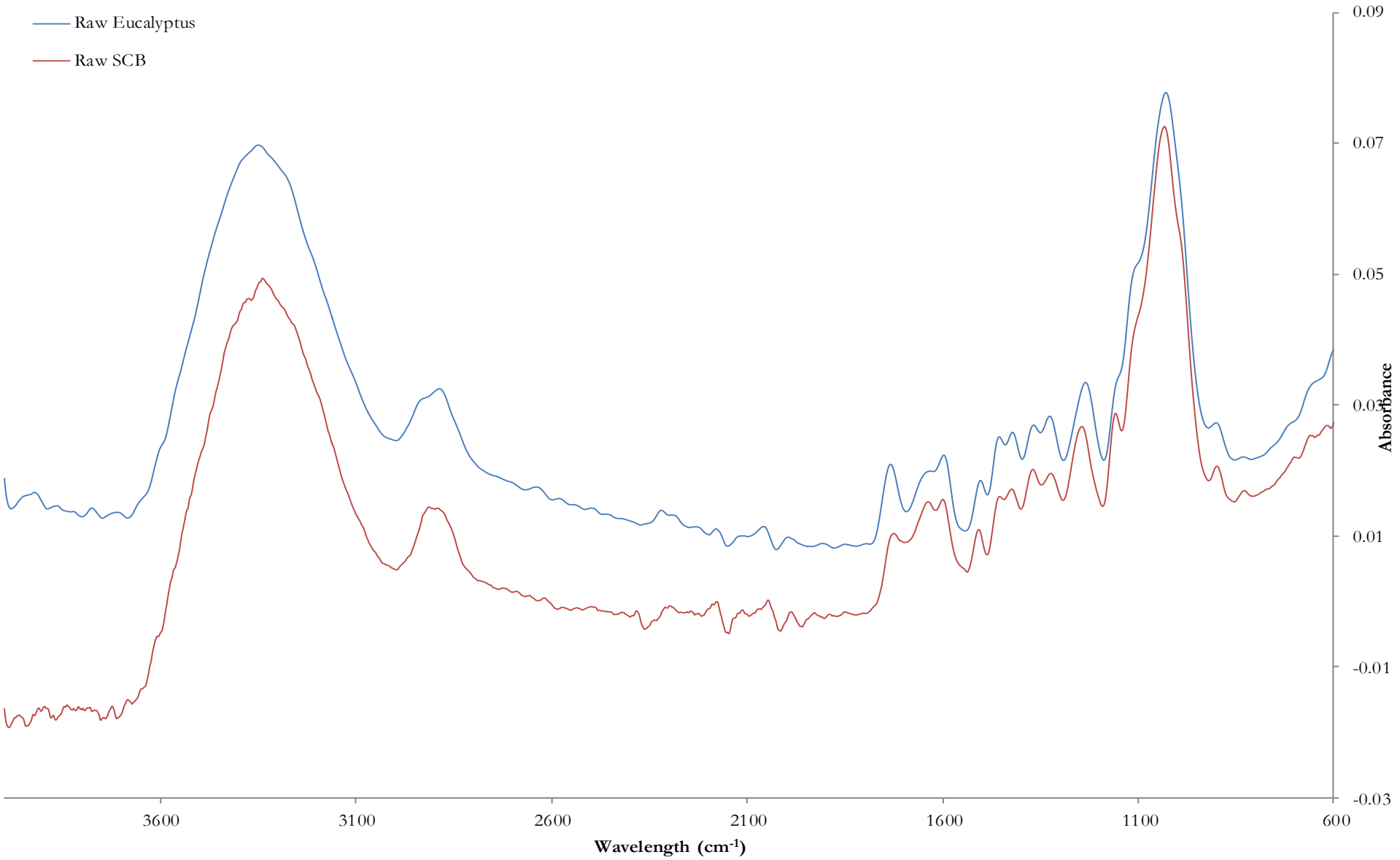


Figure 29: FTIR Spectra of virgin raw materials

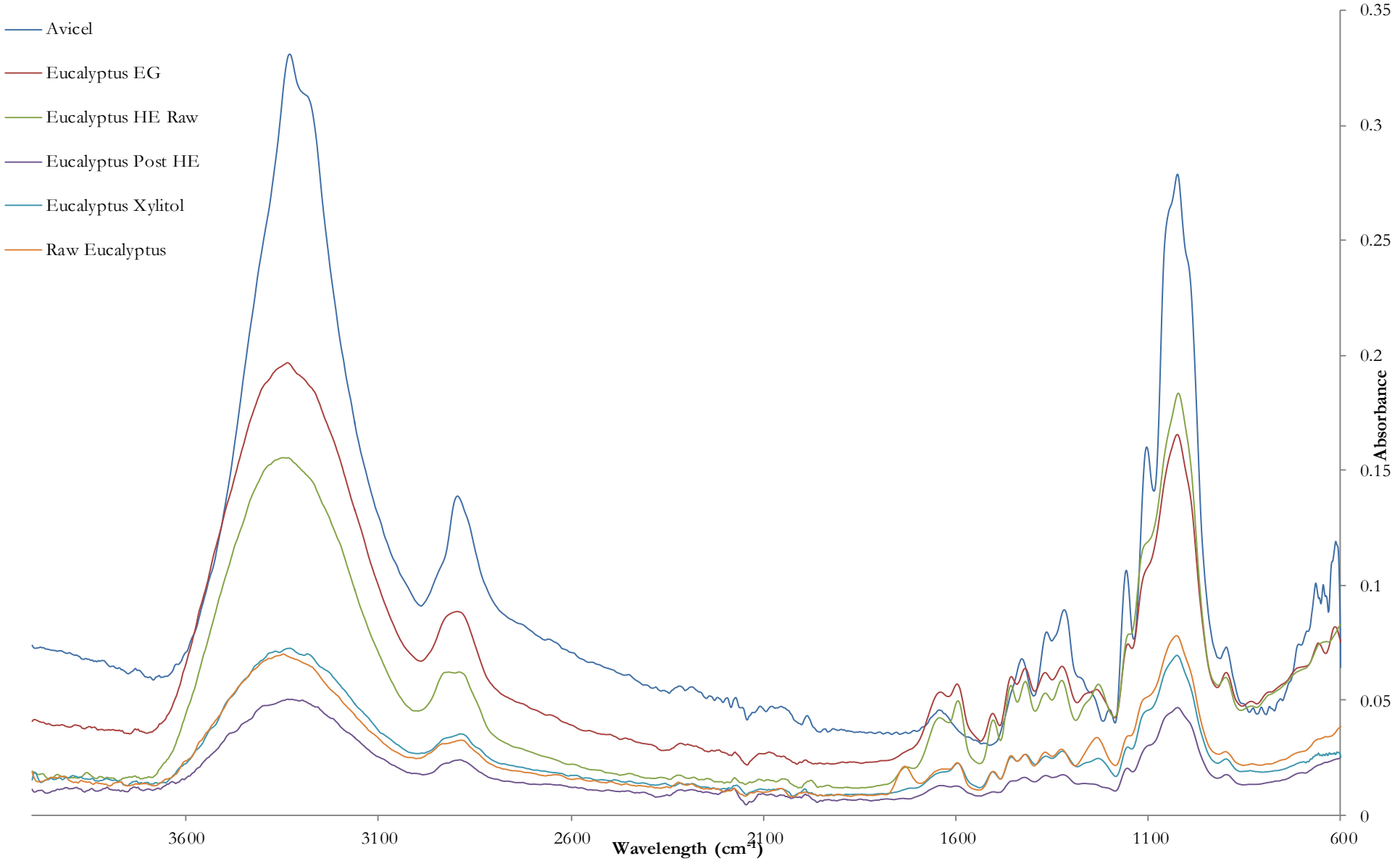


Figure 30: FTIR Spectra of treated EC residual solids

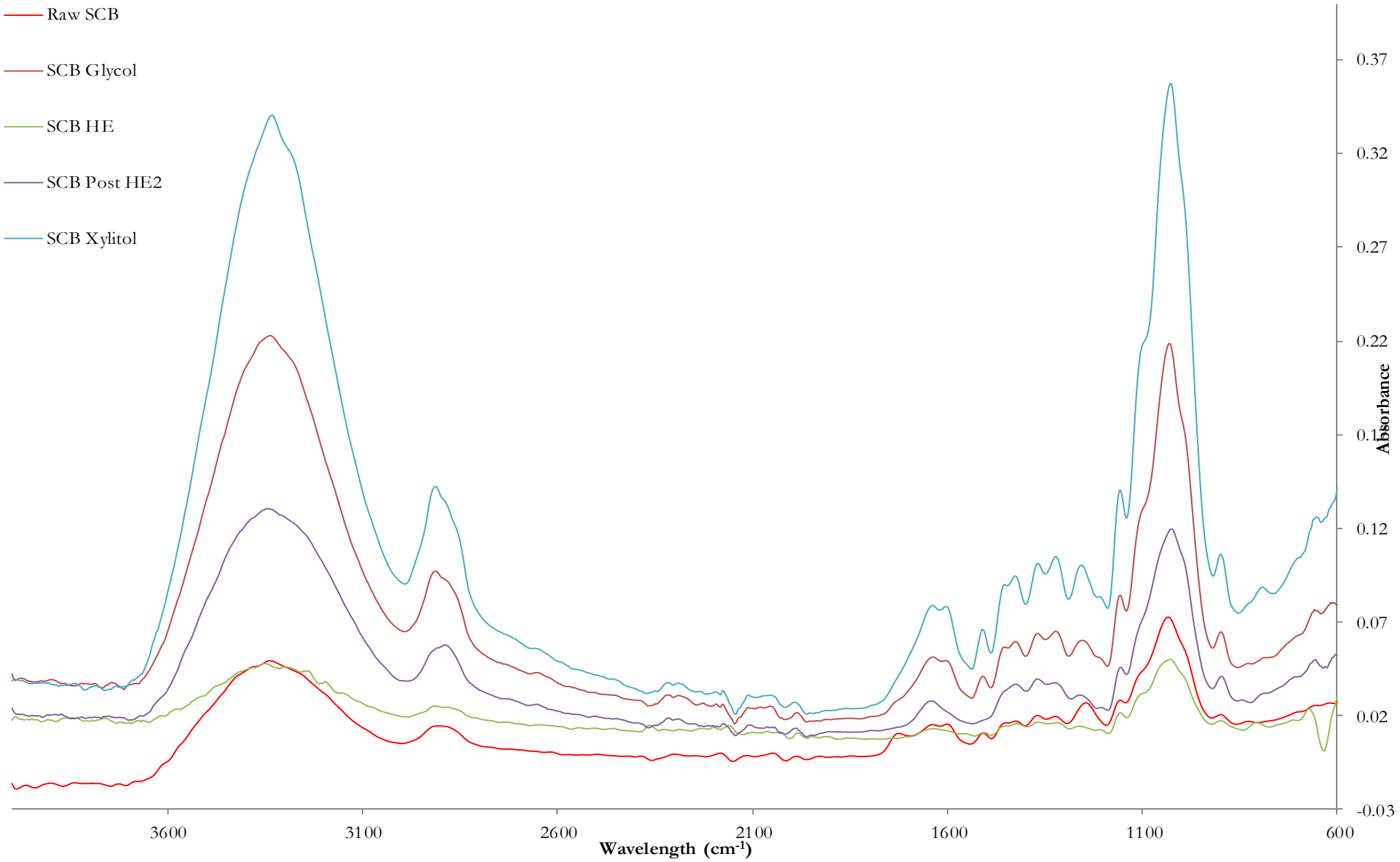


Figure 31: FTIR Spectra of treated SCB residual solids

b) Cellulose Crystallinity Index

Principal I_{002} and I_{am} peaks were taken at 18.0129° and 22.0057° for consistency, additionally, peak points of principal peaks of all samples are near these respective angles. The crystallinity of the residual solids are reported in Table 43. CIs of the raw materials are 10.3% apart, SCB 29.7%, while that of EC is 40.0%. This variation can be attributed to the difference in the compositions of the raw materials (Park et al., 2010). From the raw material compositional analysis data presented in Chapter Four, EC has high cellulose to lignin and hemicellulose ratio, as compared to that of SCB. This implies that there could be more crystalline component in EC than in SCB. Additionally, Lima et al., 2013 reported a linear relationship between CI and the glucose content which is in agreement with the amount of cellulose and the CI we recorded for the raw materials reported in section 4.2.

Furthermore, SCB CI (29.7%) obtained in this study as shown in Table 43 is lower than previously reported findings, 44.4% with a holo-cellulose content of 66.8% (Sakdaronnarong & Jonglertjunya, 2012), while (Zhang, Rackemann, et al., 2013) reported even higher CI, 68% for bagasse with 43.8% glucan. Meanwhile, others also reported higher CIs of their raw EC 64.5% with a glucose amount of 41.5% (M. a Lima et al., 2013), 83.83% with 42.0% glucan (Wang et al., 2012) as compared to what we obtained in this study (40.0%). This variation can be attributed to the several factors affecting the CI determination, such as the particle size of the samples, chemical composition, method used and or the calculation method employed (Park et al., 2010).

To confirm CI results obtained in this study, commercial cellulose (Avicel) was analysed to have CI of 74.6 ±0.2%. Park et al., 2010, reported Avicel CI values between 60.6-91.7% using various XRD methods, and also summarising Avicel CI values from their literature studies to be between 70-92% using the XRD peak height method similar to one used in evaluating our results. Lima et al., 2013 reported 85.3% for Avicel. It appears there is no consistency or defined range of CI values expected for commercial Avicel either. Our finding, 74.6% is somewhat in the median range of expected values and also given the low deviation, our method and findings are reasonable and in agreement with the trend in literature.

As expected, there was an increase in crystallinity index after raw material fractionation (Muhammad Safwan et al., 2015; Palonen, 2004). This is explained by the removal of amorphous components hemicellulose and lignin as discussed through sections 4.4.1-4.4.5 and some non-crystalline cellulose (Bernardinelli, Lima, Rezende, Polikarpov, & deAzevedo, 2015; Park et al., 2009). As summarized in Table 43, residual solids from EG treatments displayed higher crystallinity (49.1% EC, 50.1% SCB) as

compared to the ones from xylitol processes (32.3% EC, 40.2% SCB). Additionally, residual solids from the NaOH hemicellulose pre-extraction step also had high crystallinity (55.2% EC and 52.9% SCB) than both xylitol process and EG derived residues; crystallinity follows the order NaOH Pre-extraction residues> EG residues> Xylitol residues> Raw materials. This can be reasoned with an earlier observation reported in section 4.4.3-4.4.5 that EG removed more lignin, hemicelluloses and some proportions of cellulose from both materials as compared to the xylitol process.

Table 43: Cellulose Crystallinity

Cellulose Source	I_{002} peak intensity (Ir)	I_{am}peak intensity (Ir)	Crystallinity Index (%)
Avicel	563	2216	74.6
Raw Eucalyptus	1476	2461	40.0
Hemicellulose Extracted Eucalyptus	683	1526	55.2
Glycol HE residual Eucalyptus	745	1339	44.4
Xylitol residual Eucalyptus	1165	1720	32.3
Glycol residual Eucalyptus	627	1232	49.1
Raw SCB	758	1078	29.7
Hemicellulose Extracted Bagasse	1753	3721	52.9
Glycol HE residual SCB	1299	2145	39.4
Xylitol residual SCB	796	1330	40.2
Glycol residual SCB	607	1216	50.1

* Hemicellulose Pre-extracted

There is however a decline in crystallinity on materials treated with EG after hemicellulose pre-extraction, although slight for EC as shown in Table 43. The residual solids after hemicellulose extraction had a crystallinity of 55.2% and 52.9% and reduced to 41.60% and 39.4% for EC and SCB respectively. A

portion of cellulose was removed with the majority of hemicelluloses in the pre-extraction step, additional treatment with EG enabled more cellulose to be removed from the solid, reducing the amount of not only amorphous components but the crystalline cellulose as well, and this is in agreement with the linear relationship between the CI and the amount of cellulose in the material (Lima et al., 2013).

As reported elsewhere (Chikouche et al., 2015) the intensities of the main crystalline peak (I_{002}) increase after solvent treatment, while others (Sathitsuksanoh, Zhu, Wi, & Percival Zhang, 2011) have observed a decline in intensities in addition to decreasing CI. We have observed peak intensity decline, but with an increase on overall CI, as shown in Figure 32 and 33. However, to the best of our understanding, it appears that the peak intensities have no bearing on the CI value, possibly because the equation originally derived by Segal et al., 1959, is expressed as a ratio and does not account for the broadness or width of the peaks (Park et al., 2010) as accounted for in Ruland-Vonk and Hermans- Weidinger XRD methods (Terinte et al., 2011). Regardless of peak intensities, our CIs increased after raw material treatment. This is a common trend as reported elsewhere (Bernardinelli et al., 2015).

It is to our understanding that research on CI determination methods is ongoing. The CI is also said to be method dependent as demonstrated in literature (Bernardinelli et al., 2015; Park et al., 2010, 2009), thus method choice is entirely dependent on use and interpretation of CI data generated. For this study, relative height to minimum method was used to get approximate and empirical measure of relative crystallinity in the cellulose fraction and to support conclusions from cellulose recovery and enzymatic hydrolysis data.

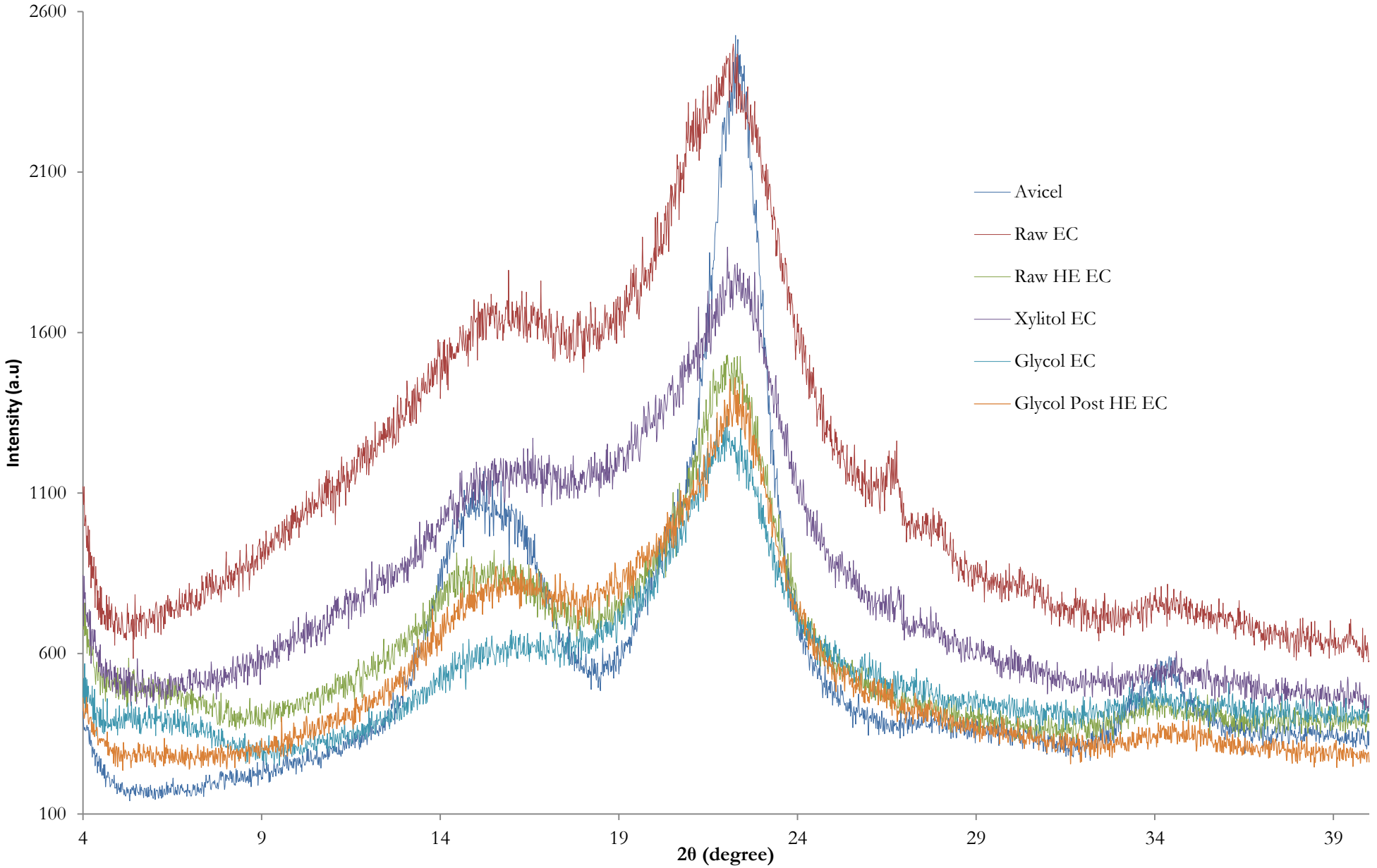


Figure 32: X-Ray diffractogram of eucalyptus substrates

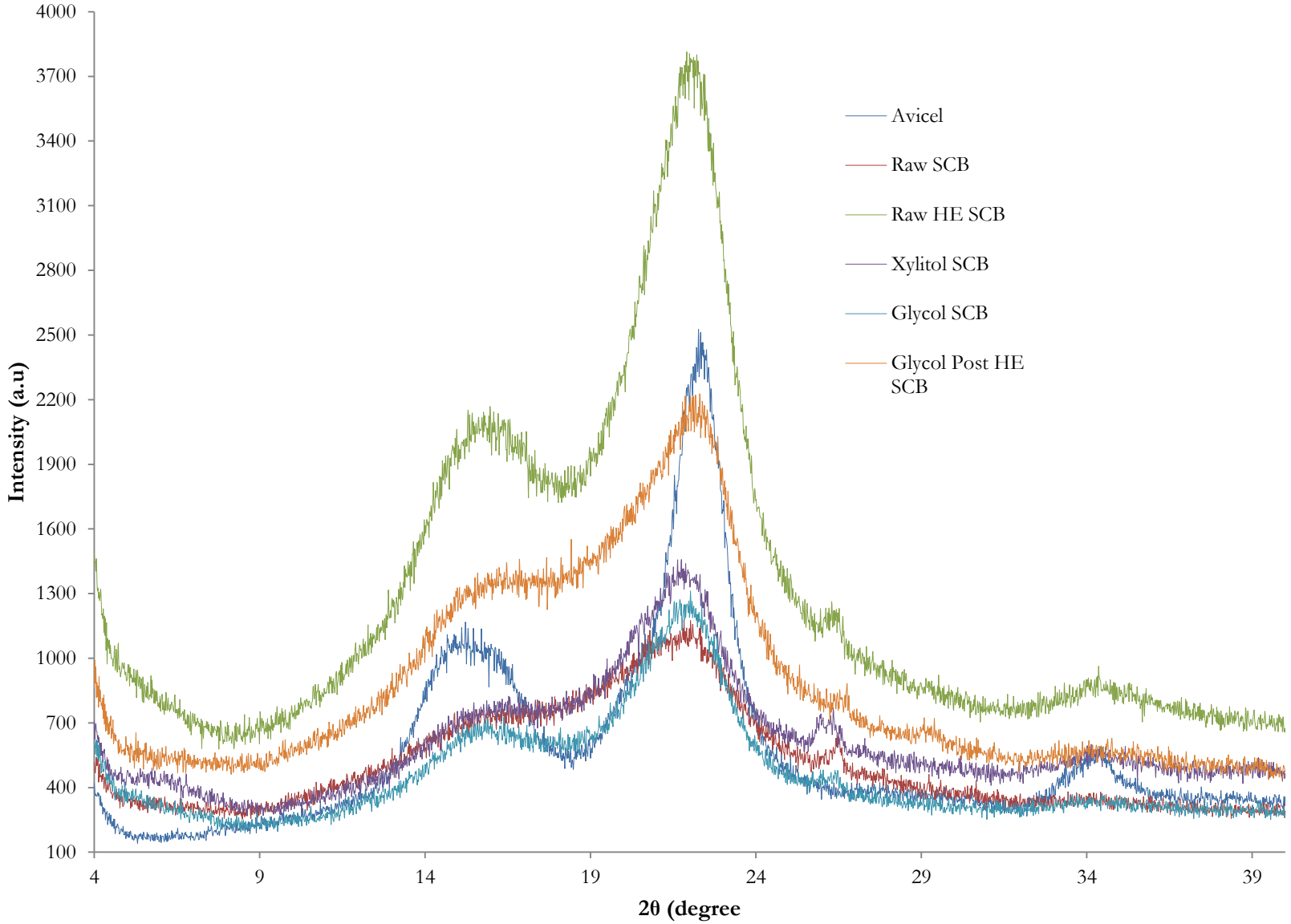


Figure 33: X-Ray diffractogram of sugarcane bagasse substrates

c) Cellulose digestibility

Full enzymatic hydrolysis results for all CCD's are summarized in Tables 44 to 45. For optimum conditions, cellulose digestibility is shown in Table 46. The enzyme efficiency was calculated based on a procedure (Resch et al., 2015) in which the amount of glucose that is regenerated after enzyme hydrolysis is compared to the initial glucose content in the original substrate and expressed as a percentage.

Table 44: Summary of enzymatic hydrolysis efficiency of the solid residues before optimization (without hemicellulose pre-extracted)

					Enzymatic Hydrolysis Efficiency (%)			
					Raw E. grandis		Raw SCB	
					21.08		12.76	
Run	Solvent Conc. (%) (Xylitol/EG)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	EC/Xylitol	EC/EG	SCB/Xylitol	SCB/EG
1	20/50	1.0	140.0	2.0	23.93	14.04	17.2	18.9
2	30/70	1.0	140.0	2.0	21.68	20.53	20.2	74.9
3	20/50	2.0	140.0	2.0	25.33	35.21	17.5	29.8
4	30/70	2.0	140.0	2.0	29.52	31.62	10.1	37.0
5	20/50	1.0	140.0	4.0	23.78	28.67	21.9	86.1
6	30/70	1.0	140.0	4.0	25.13	16.60	13.8	27.0
7	20/50	2.0	140.0	4.0	44.80	46.01	14.5	38.9
8	30/70	2.0	140.0	4.0	35.88	33.46	12.1	39.2
9	20/50	1.0	180.0	2.0	53.36	41.90	27.4	40.5
10	30/70	1.0	180.0	2.0	59.27	33.14	33.1	49.0
11	20/50	2.0	180.0	2.0	68.85	82.62	34.2	47.9
12	30/70	2.0	180.0	2.0	61.17	58.77	27.6	64.4
13	20/50	1.0	180.0	4.0	51.13	89.61	37.2	76.8
14	30/70	1.0	180.0	4.0	45.24	74.30	43.3	35.3
15	20/50	2.0	180.0	4.0	52.87	76.29	42.8	58.1
16	30/70	2.0	180.0	4.0	55.22	79.17	38.6	61.4
17	25/60	1.5	120.0	3.0	15.04	16.89	10.7	24.6
18	25/60	1.5	200.0	3.0	62.15	54.29	22.8	68.7
19	25/60	1.5	160.0	1.0	27.67	33.87	10.5	30.2
20	25/60	1.5	160.0	5.0	59.63	89.06	26.3	36.4
21	25/60	0.5	160.0	3.0	51.05	26.00	12.8	23.3
22	25/60	2.5	160.0	3.0	59.59	48.11	21.8	50.8
23	15/40	1.5	160.0	3.0	38.40	29.76	21.0	40.5
24	35/80	1.5	160.0	3.0	58.03	28.65	17.0	65.3
25[a]	25/60	1.5	160.0	3.0	40.29	61.24	28.3	44.6
26[a]	25/60	1.5	160.0	3.0	34.65	51.78	29.0	36.5
27[a]	25/60	1.5	160.0	3.0	32.04	56.53	24.0	34.9
28[a]	25/60[5]	1.5	160.0	3.0	34.93	52.70	27.7	38.1

[5] [a] replicates at center points in the experimental design

Table 45: Enzymatic Hydrolysis Efficiency of hemicellulose pre-extracted solid residues fractionated with EG before optimization

Run	Ethylene Glycol Conc. (%, v/v)	Catalyst Conc. (wt. %)	Temp (°C)	Time (Hours)	Enzymatic Hydrolysis Efficiency-EC (%)	Enzymatic Hydrolysis Efficiency-SCB (%)
1	60	1	140	2	54.15	82.19
2	60	2	140	2	60.62	87.89
3	60	1	140	4	48.06	81.53
4	60	2	140	4	85.53	76.05
5	60	1	180	2	78.39	76.62
6	60	2	180	2	89.77	75.67
7	60	1	180	4	79.63	72.98
8	60	2	180	4	93.17	33.08
9	60	1.5	126.4	3	52.88	72.78
10	60	1.5	193.6	3	72.64	57.08
11	60	1.5	160	1.3	91.36	76.33
12	60	1.5	160	4.7	76.46	62.59
13	60	0.7	160	3	75.98	40.12
14	60	2.3	160	3	85.52	68.59
15	60	1.5	160	3	90.19	68.06
16	60	1.5	160	3	93.39	74.66
17	60	1.5	160	3	94.52	63.25
18	60	1.5	160	3	93.02	70.95

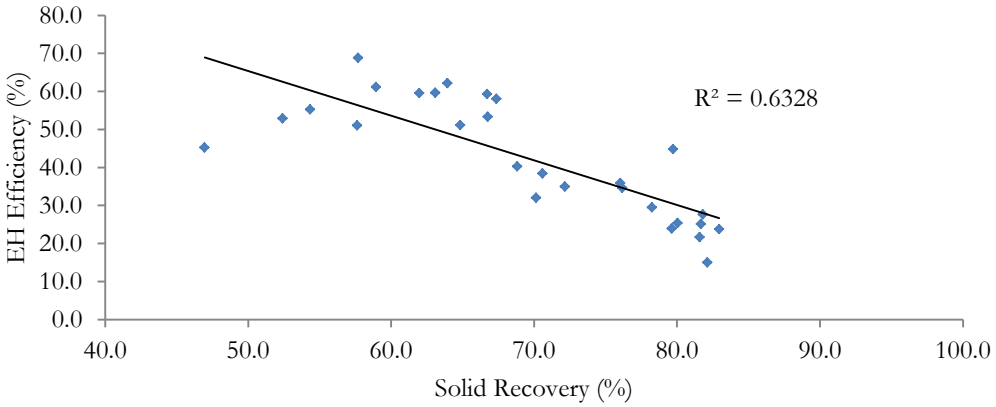
It is be observed in Table 44 and 45 that there is a general increase in enzymatic hydrolysis of solid residues that were fractionated with EG and also a hemicellulose pre-extraction step as compared to digestibilities of non-extracted hemicellulose solid residues fractionated with the same solvent. The processes of hemicellulose pre-extraction expose cellulose in the residual solid with removal or delocalization of hemicelluloses and some portions of the lignin from the surface of cellulose; these two components hinder accessibility of cellulose by enzymes. Additionally, hemicellulose pre-extraction with NaOH (an alkali) causes swelling of amorphous cellulose fibres which improves pore size and

subsequently enzymatic hydrolysis efficiency (Wen et al., 2015). Further fractionation of an already treated substrate (hemicellulose extracted) further removes lignin and hemicellulose from the macrostructure of the lignocellulose complex, further enhances exposure of cellulose to enzymes. In their enzymatic hydrolysis study of eucalyptus wood Wen et al., (2015) reported that pre-swelling the substrate (4% NaOH, 25 °C, 24 h) before enzymatic hydrolysis greatly improved efficiency. This was attributed to (1) change in crystallization morphology of the solid residue (2) transformation of natural cellulose I to its polymorph cellulose II which is more amorphous and (3) change in the surface morphology of the raw material (compact and rigid) to the more loosened and rough surface which is attractive for reaction with enzyme active sites (Wen et al., 2015).

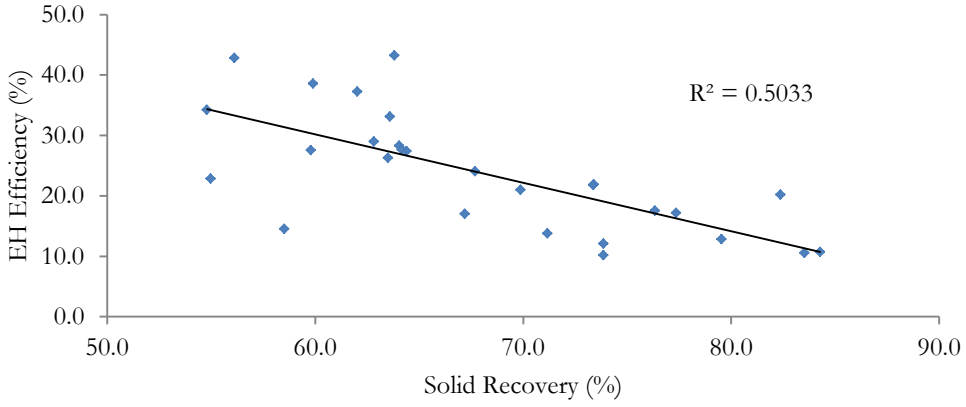
Table 46: Enzymatic hydrolysis values at optimum fractionation conditions

					Content of hydrolyzed residue			
Fractionation	Temp	Time	Catalyst Conc.	Solvent Conc.	Glc %	Xyl %	[d]AIL	EH[e] (%)
Xylitol Sugarcane bagasse	160	4	2.0	20	33.0	10.5	9.12	28.4
Xylitol *E. grandis*	180	4	1.0	15	38.9	9.82	5.91	57.5
Ethylene G. SCB	200	5	1.5	40	31.8	6.38	5.10	59.7
EG. *E. grandis*	180	4	2.5	80	27.2	7.03	4.30	76.7
EG. Pre-extracted SCB	176.82	1.32	0.66	60	28.43	5.36	1.68	83.18
EG. Pre-Extracted EC	176.82	3.84	1.5	60	26.02	6.17	2.14	81.30

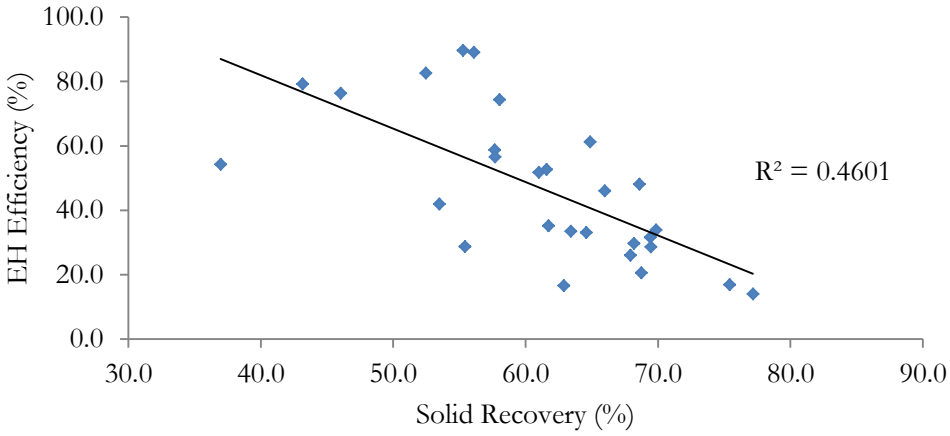
There is a notably direct relationship between the solid recovery and the hydrolysis efficiency. As shown in Figure 34, and similar observation for all CCD's, the high the solid yield the lower the efficiency. Higher solid yield means that the severity of the fractionation was not that high or effective to fractionate components. This also means that the solid remains recalcitrant. However, as the severity of the treatment increases i.e. increase in temperature as shown in Figures 35 to 36, time or catalyst concentration, residual solid yield decrease and fractionation efficiency increases as well. With increase in severity, more cellulose is freed of lignin and hemicellulose, and is more accessible to enzymes. In addition to solid yield, other factors also have an influence on enzymatic hydrolysis such as the cellulose characteristics before or after fractionation (e.g., Polymerization degree, its accessible surface area and crystallinity) and also other biomass components such as lignin and hemicelluloses.



A



B



C

Figure 34: Relationship between solid recovery and material enzymatic hydrolysis efficiency (A: EC-xylitol, B: SCB-xylitol, C: EC-EG)

Overall, ethylene glycol fractionated celluloses gave higher enzymatic digestibility as compared to xylitol celluloses as presented in Tables 44-46. This is due to the fact that ethylene glycol fractionations removed relatively more lignin from the solids as reported in section 4.4.3. Higher lignin in the solid residue is an indication that a proportion of cellulose is still recalcitrant (Xu et al., 2012). The higher the lignin in the solids, the lower the enzymatic hydrolysis trend as observed for all CCD's as shown in the scatter plots in Figures 37-39 (only eucalyptus was used for this emphasis). This trend is irrespective of solvent used because enzymatic hydrolysis efficiency is partly influenced by the amount of lignin remaining in the solid (Harmsen et al., 2010; Hou et al., 2013; Katahira et al., 2013; Yoo, 2012). Lignin is said to be a hindrance for enzymatic hydrolysis(Katahira et al., 2013). This is believed to be the case because lignin binds cellulose in a composite enclosure which in-turn minimize accessibility of cellulose to microorganisms facilitating digestibility (Yang et al., 2011). In summary and based on results presented in this section, the following factors contribute to reduced enzymatic hydrolysis:

- Lignin content in the solid residue has an impact on enzymatic hydrolysis as demonstrated in Figure 37 to 39. When compared to raw materials, fractionated solid residues have higher enzymatic digestibility which is attributed to the recalcitrance of the raw material and the amount of lignin enclosure which reduces accessibility of cellulose polymers to enzymes. The more lignin there is in the material, the lower the digestibility of the material. This observation is similar for both materials irrespective of fractionation solvent used.

- Residual hemicelluloses in the solid residue influence enzymatic hydrolysis. High hemicellulose content in the solid is one of the contributing factors to reduced enzymatic hydrolysis (Agrawal et al., 2014). Hemicelluloses enables absorption and adsorption of enzymes on their structures which act as a physical barrier for accessibility of cellulose polymers by enzymes, this reduces the digestibility efficiency (Zhao, Zhang, 2012).

- Crystallinity of the residual solid is linked to enzymatic hydrolysis (Figure 37-39, lignin contributes to crystallinity of lignocellulose). Lower crystallinity of the residual solid is associated with lower enzymatic hydrolysis (Mesa et al., 2011). As discussed in section 5.3.1c, lower crystallinity is in large attributed to the presence of amounts of amorphous components in the solid residue and these are mainly hemicellulose and lignin polymers.

- Other factors affecting hydrolysis efficiency and which were not investigated in this study include the amount of cell wall proteins and physical barriers such as accessible surface area (Kumar et al., 2009b; Menon & Rao, 2012), pore volume, particle size and cellulose degree of polymerization (Zhao, Zhang, 2012).

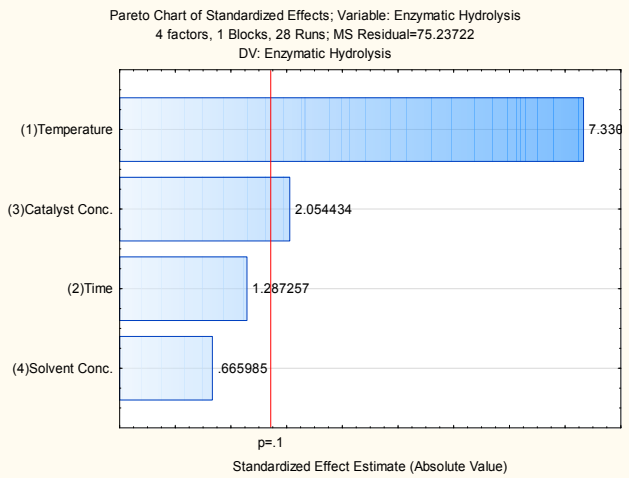


Figure 35: Pareto chart of Effect: EC-Xylitol

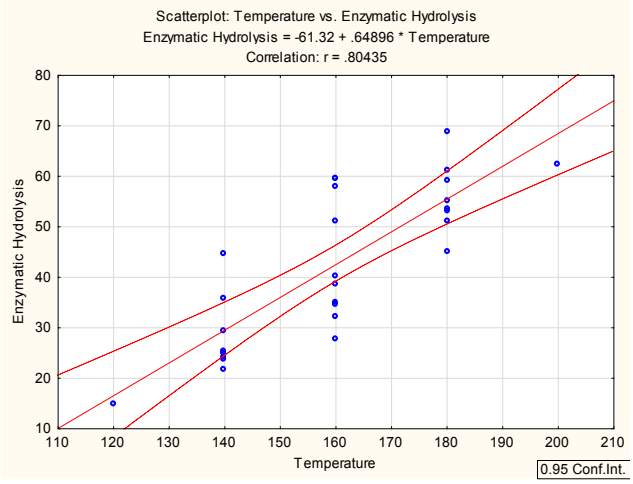


Figure 36: Scatter plot-Enzymatic hydrolysis and temperature of fractionation (EC-Xylitol)

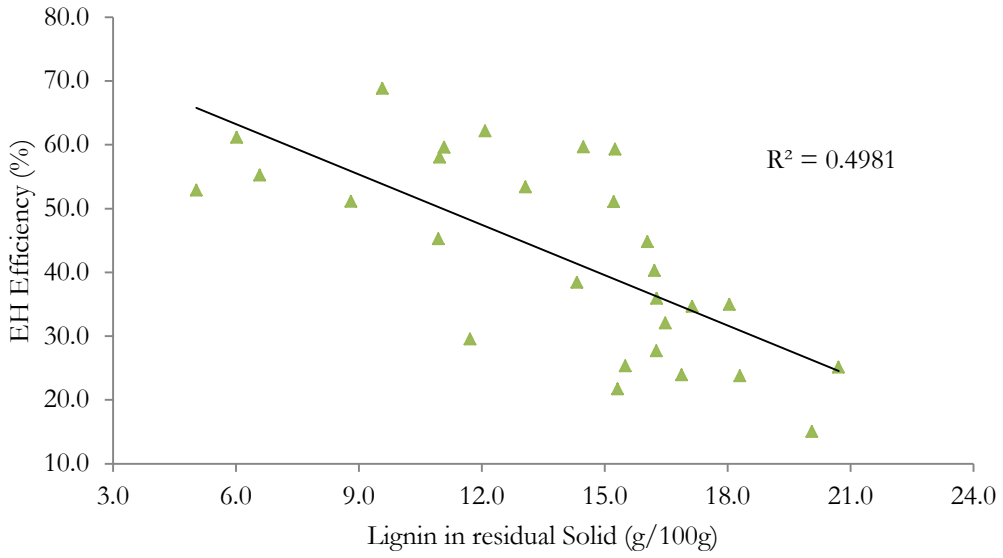


Figure 37: Influence of lignin content on EH efficiency, EC-Xylitol fractionation

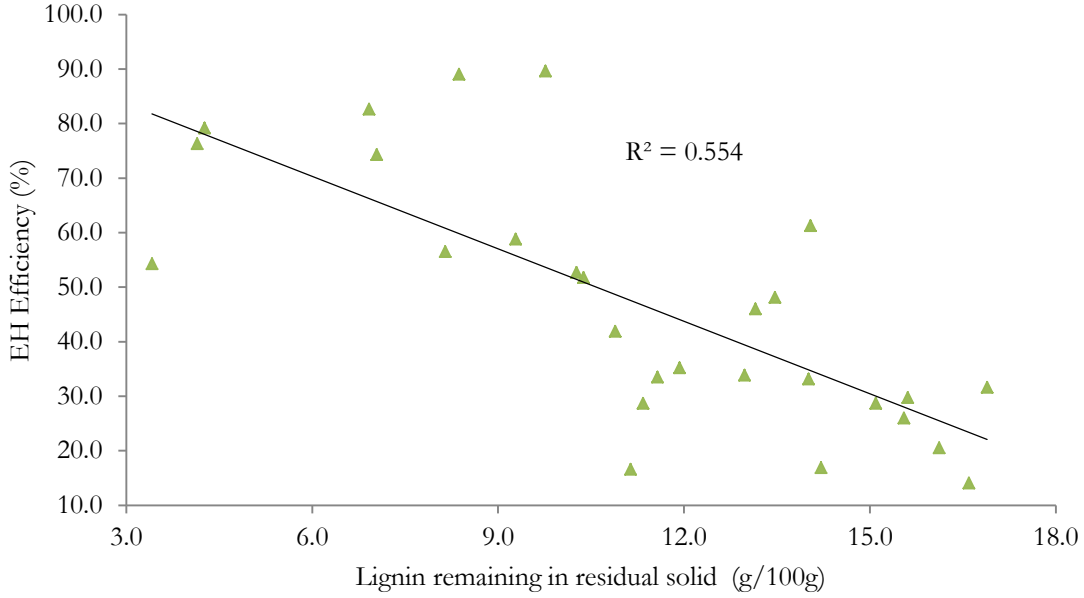


Figure 38: Influence of lignin content on EH efficiency, EC-EG fractionation

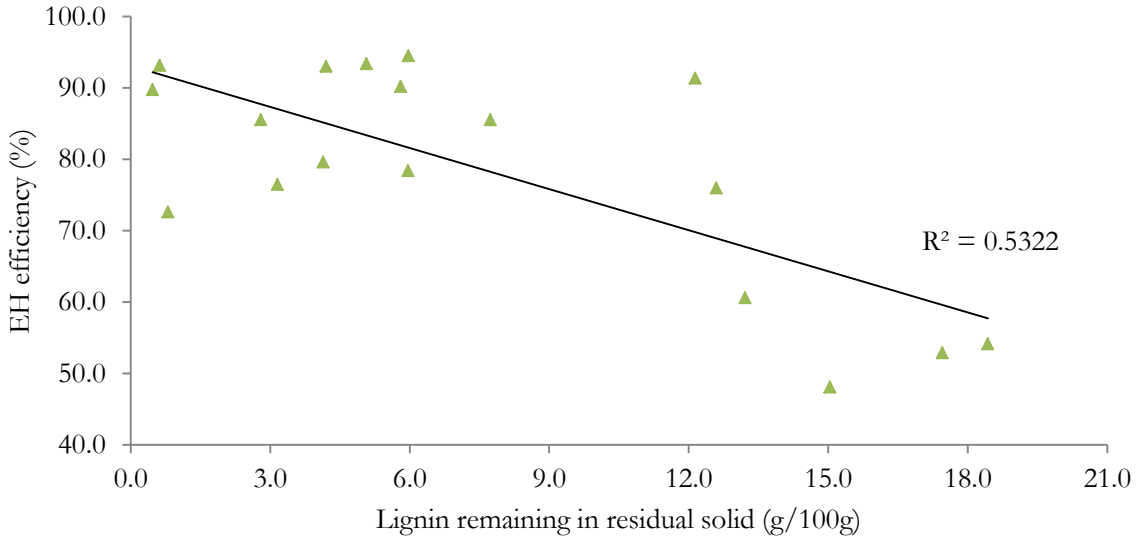


Figure 39: Influence of lignin content on EH efficiency, Hemicellulose Pre-extracted EC-EG Fractionation

5.2.2 Quantitative assessment of the quality of isolated hemicelluloses

a) Gravimetric Analysis of acetone-precipitated hemicelluloses

Acetone isolated hemicelluloses from the fractionation processes at optimum conditions were analysed gravimetrically to determine the amount of lignin still attached to their structures. These results are presented in Table 47. No acid soluble lignin was detected by UV-vis spectrometer. Additionally,

commercial xylan and D-xylose did not have detectable lignin, confirming their purity. More acid soluble lignin was reported for hemicelluloses derived from the NaOH pre-extraction step. This can be argued by the observation from previous reports (Chimphango, 2010; Postma, 2012; Vena, 2013), which suggested pre-extracted hemicelluloses from the NaOH process are mostly oligomers and likely to still maintain a lignin-carbohydrate linkage (Rabetafika et al., 2014).

However, our results are almost similar to 2.88-7.76% of the initial klason lignin in the raw material analysed in hemicelluloses extracted from SCB by treatment with 3% NaOH at 55°C for a period of 3hours and latter precipitated from solution with 60% ethanol (Peng et al., 2009), except for higher lignin content in NaOH extracted hemicelluloses, 15.03% and 17.83% of the initial lignin in the raw material for SCB and EC hemicelluloses respectively. Furthermore, hemicelluloses from ethylene glycol fractionations recorded high concentration of lignin as compared to xylitol fractionations, i.e. 8.44% and 6.36% of initial Klason lignin in the raw material, using ethylene glycol for eucalyptus and sugarcane bagasse respectively, versus 1.11% and 3.01% of initial Klason lignin in the raw material using xylitol for eucalyptus and sugarcane bagasse respectively. Although there is a slight difference in the lignin concentrations remaining in the hemicellulose macromolecules of the respective materials, eucalyptus maintained higher lignin concentration as compared to sugarcane bagasse. This is true for NaOH pre-extracted hemicelluloses and ethylene glycol fractionations, but the opposite for xylitol treatment, i.e. lignin content in eucalyptus extracted hemicelluloses is 2.7 times that in sugarcane bagasse hemicelluloses.

Lignin is said to re-combine with carbohydrates when both are in solution (Luo, Fang, & Smith, 2014; Xiang et al., 2004) which gives reason to the presence of lignin in our hemicellulose samples. However, naturally, lignin is bonded to hemicellulose through covalent type bonding including amongst other bonds, two major bonds either an ether linkage or ester linkage between the two molecules (Peng et al., 2009), making the separation rather difficult. This then mean, in order for complete fractionation of lignin and hemicellulose to produce a pure hemicellulose fraction, ethylene glycol and xylitol molecules assisted by the catalyst NaOH and other reaction conditions, the reactions of these molecules needed to engage in breaking the ester bond of uronic acid between the carboxylic acid group on hemicellulose and phenolic hydroxyl group on lignin (Zhang, Rackemann, et al., 2013), ester bond of uronic acid between lignin's hydroxycinnamic acid and hemicellulose' hydroxyl group of its arabinofuranose unit(Bobleter, 1994; Deutschmann & Dekker, 2012; Doering, Lathe, & Persson, 2012; Pawar, Koutaniemi, Tenkanen, & Mellerowicz, 2013; Peng et al., 2012) or the ester to ether bridge formed between lignin and hemicellulose by ferulic acid (Peng et al., 2012).

However, as this may seem difficult to achieve others (Chimphango, 2010; Deutschmann & Dekker, 2012; Peng et al., 2012; Yao et al., 2015) concluded that the solvent precipitating hemicelluloses from the solution plays an important role, not only in the recovery yield but also in the amount of lignin remaining in the hemicellulose macromolecule. Peng et al., 2012, reported that while precipitating hemicelluloses from the liquid fraction, increasing ethanol concentration from 15% to 60% assisted in lowering lignin content in their hemicelluloses from 6.1 to 2.9% of initial lignin content in the raw material from SCB treatment with 3% NaOH at 55°C for a period of 3 hours. Hemicellulose free lignin is important for further value addition and thus lignin should be lowered as much as possible.

Table 47: Chemical composition of hemicellulose extracted from the liquid fraction at optimum conditions

Hemicellulose Source	Ash Content (g/100g)	Acid Insoluble Lignin (g/100g)	Acid Soluble lignin (g/100g)	Percentage of Acid Insoluble Lignin
SCB-NaOH Pre-extraction	0.0001	0.0053	u.d[6]	15.03%
EC-NaOH-Pre-extraction	0.0002	0.0068	u.d	17.83%
SCB Xylitol	0.0001	0.0008	u.d	3.01%
EC Xylitol	0.0001	0.0006	u.d	1.11%
SCB-Ethylene Glycol	0.0002	0.0013	u.d	6.36%
EC-Ethylene Glycol	0.0002	0.0028	u.d	8.44%
Post Ext SCB	0.0001	0.0001	u.d	0.06%
Post Ext EC	0.0003	0.0005	u.d	0.08%
Xylan-Beechwood	0.0000	0.000	u.d	n.d
D-xylose	0.0000	0.000	u.d	n.d

[6] n.d for undetectable by UV-Vis

a) Functional group determination

Figure 40 and 41 shows the FTIR spectras of hemicelluloses extracted from EC and SCB fractionations respectively. Generally, signature bands for this group of polysaccharides occur dominantly in the region 800-1200cm^{-1} (Sun et al., 2014). The absorption band at around 3400cm^{-1}on both hemicellulose samples is confirming the stretching of –OH groups(Chemin et al., 2015; Laine, 2005; Ma et al., 2014; Moghaddam et al., 2014; Sedlmeyer, 2011). The broad and high intensity peaks stretched in the region 2800-3100cm^{-1}which Cao et al., 2012 argues to belong to C-H stretching vibrations. Because the hemicellulose extracted were not completely dry or free of water, a band around 1600 cm^{-1} confirms the bending mode of water molecules(Harmsen et al., 2010; Muhammad Safwan et al., 2015; Peng et al., 2009; Rabetafika et al., 2014).

Other bands in the region 1000-1200cm^{-1} in all hemicelluloses are attributed to vibrations of glycosidic bonds and C-OH stretching vibrations(Ma et al., 2014; Peng et al., 2009; Vena, 2013) in arabinoxylans (Peng et al., 2012) confirmed by an arabinosyl shoulder around 900cm^{-1} (Brienzo et al., 2009; Rabetafika et al., 2014).Arabinosyl is a pectin (Scheller & Ulvskov, 2010) found to be feruloylated on side chains directly linked to the backbone of some hemicellulose oligosaccharidessuch as xylan (Doering et al., 2012).This further affirms findings in the later section 5.3.2c that oligomeric hemicelluloses are produced in these processes. However, these bands are particularly intense in xylitol fractionations, followed by hemicellulose pre-extracted hemicelluloses and lastly in ethylene glycol fractionations.

All spectras have a small band extension around 1730cm^{-1}. This should only be present in hemicellulose profile if there are acetyl, uronic or ester groups still attached to it (Lima et al., 2013). The band around the region of 1700cm^{-1} is normally associated with lignin monomers(Bodîrlău & Teacă, 2009b; Kruger, 2013; Watkins et al., 2014). The presence of this band in hemicellulose spectras is confirmed by Hou, Li, & Zong, 2013 to belong to lignin attached to hemicelluloses as confirmed by the presence of lingins in hemicellulose samples discussed earlier in section 5.3.2a, while others (Lima et al., 2013) suggest it to belong to hemicellulose C=O conjugate in xylans. Komiyama et al. 2009, attribute it to either acetyl groups or ester linkages of carboxylic stretching groups of ferulic acid. The absence or low intensity of this peak in other hemicellulose spectras, particularly eucalyptus fractionations implies the two polyol solvents in combination with NaOH catalyst have completely cleaved the ester bonds from hemicelluloses (Peng et al., 2012).

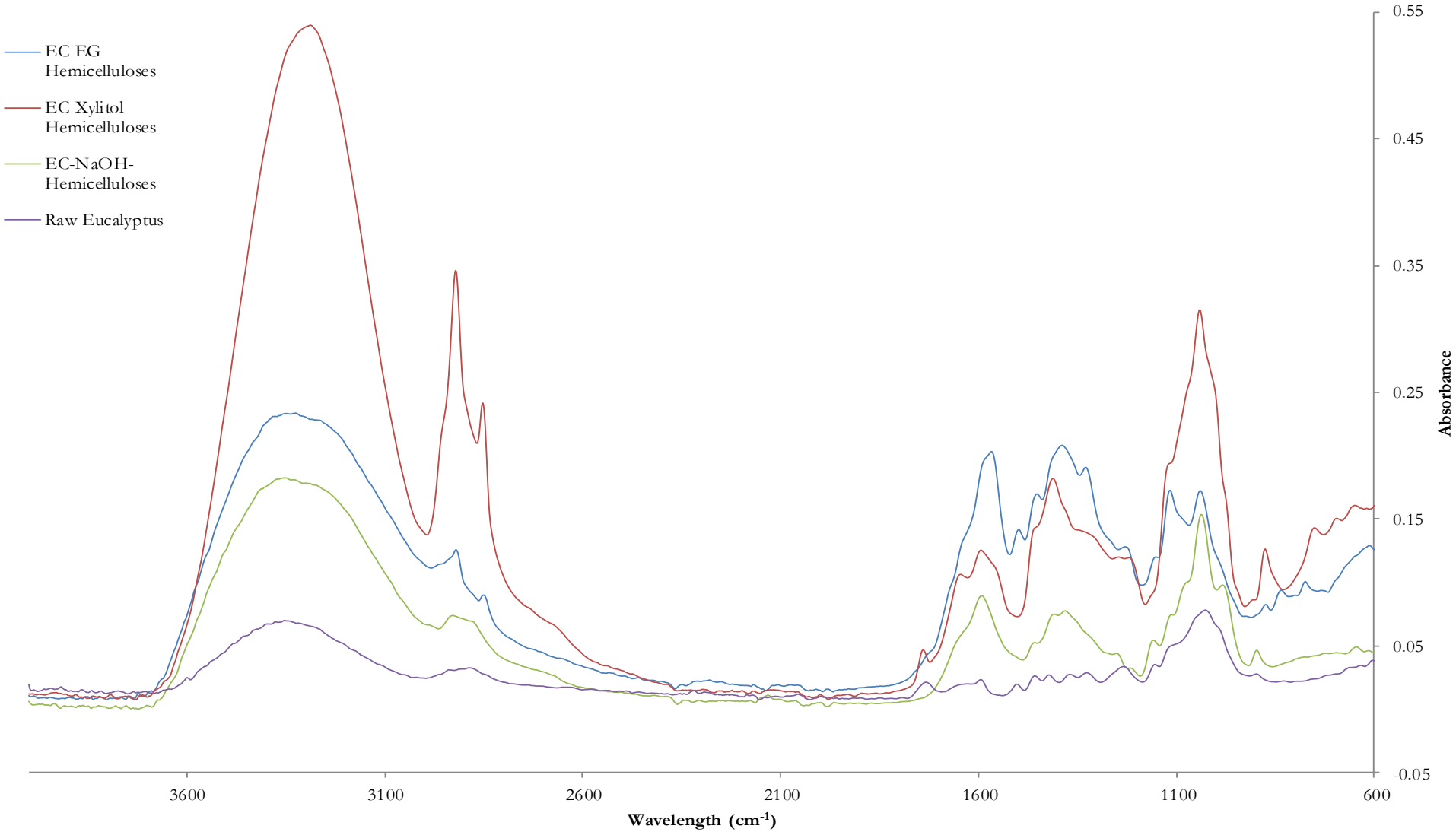


Figure 40: EC Hemicelluloses FTIR Spectra

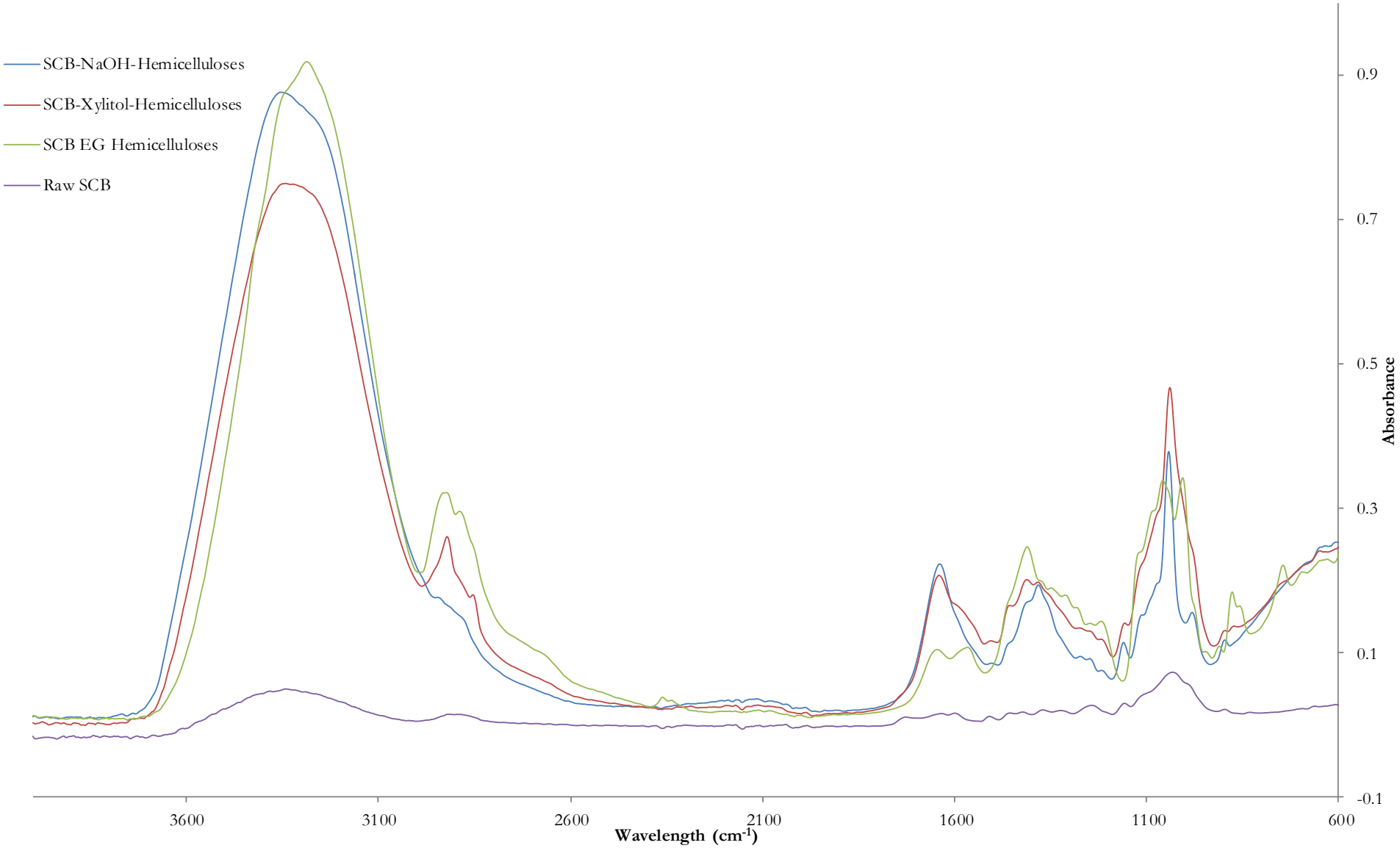


Figure 41: SCB Hemicelluloses FTIR Spectra

b) Size exclusion chromatography (SEC) for molecular weight determination

Information from SEC can be utilized to provide hemicelluloses molecular weights and also to evaluate their homogeneity (Rabetafika et al., 2014). By using weight distribution results of hemicelluloses it is possible to determine if isolated hemicelluloses are comprised of monomers, oligomers or polymers (Rabetafika et al., 2014) and thereby answering whether target 2 "Recovered hemicelluloses should be of polymeric/oligomeric form" mentioned in section 4.4.3 is met. Hemicellulose molecular weight information can also provide insight on possibility of contaminants such as lignin (Rabetafika et al., 2014). The SEC results from the analysis of hemicelluloses isolated at optimum conditions determined from section 4.4.5 are presented in Table 48, which shows that our hemicelluloses M_w ranged between 270gmol^{-1} reported for monomeric xylose, also analysed as a control and 61 644gmol^{-1} reported for *E. grandis* hemicelluloses from NaOH pre-extraction.

Table 48: The weight-average (M_w), number-average (M_n) molecular weight in gmol^{-1}, and the polydispersity index (DPI) as (M_w/M_n), and weight-average degree of polymerization (DP_w) of the hemicellulose streams.

Hemicellulose Source	M_w	M_n	DP_w	PDI
SCB-NaOH Pre-extraction	33638	22835	224	1.47
EC-NaOH-Pre-extraction	61644	45134	411	1.37
SCB Xylitol	22377	10658	149	2.10
EC Xylitol	18400	17200	123	1.07
SCB-Ethylene Glycol	20866	6218	139	3.36
EC-Ethylene Glycol	20185	1492	14	1.40
Post Ext SCB	300	200	2	1.50
Post Ext EC	200	200	1	1.00
Xylan-Beechwood	16882	11407	113	1.48
D-xylose	270	250	2	1.08

Raw material pre-extracted with NaOH produced hemicelluloses with the highest weight-average molecular weights, 33638 and 61644 gmol^{-1} for sugarcane bagasse and *E. grandis* respectively, both of

which are relatively higher than weight average molecular weights of hemicelluloses isolated by Rabetafika et al., 2014, from pear pomace using a three solvents, sodium hydroxide, alkaline hydrogen peroxide and a two-step sodium chlorite/sodium hydroxide all between 60-70°C giving M_ws' of 21300, 22 400 and 17 300gmol^{-1} respectively. Pre-extracted hemicelluloses weight averages also surpass commercial Beech wood xylans at a weight average molecular weight of 16882gmol^{-1}. This confirms that these hemicelluloses are of oligomeric form, which is also further reiterated by weigh-average degree of polymerization above 25, widely accepted for insoluble hemicellulose polymers (Ma et al., 2014). However, sugarcane bagasse pre-extracted hemicelluloses *Mw's* were lower than those of *E. grandis*. This can be explained by the higher content of lignin remaining in hemicelluloses (as shown in Figure 42) of eucalyptus as discussed earlier in section 5.3.2a. The lignin-carbohydrate bond between hemicelluloses and lignin is believed to contribute to elevated weight average molecular weights for hemicellulose (Carà et al., 2013; Laine, 2005; Peng et al., 2012; Rabetafika et al., 2014).

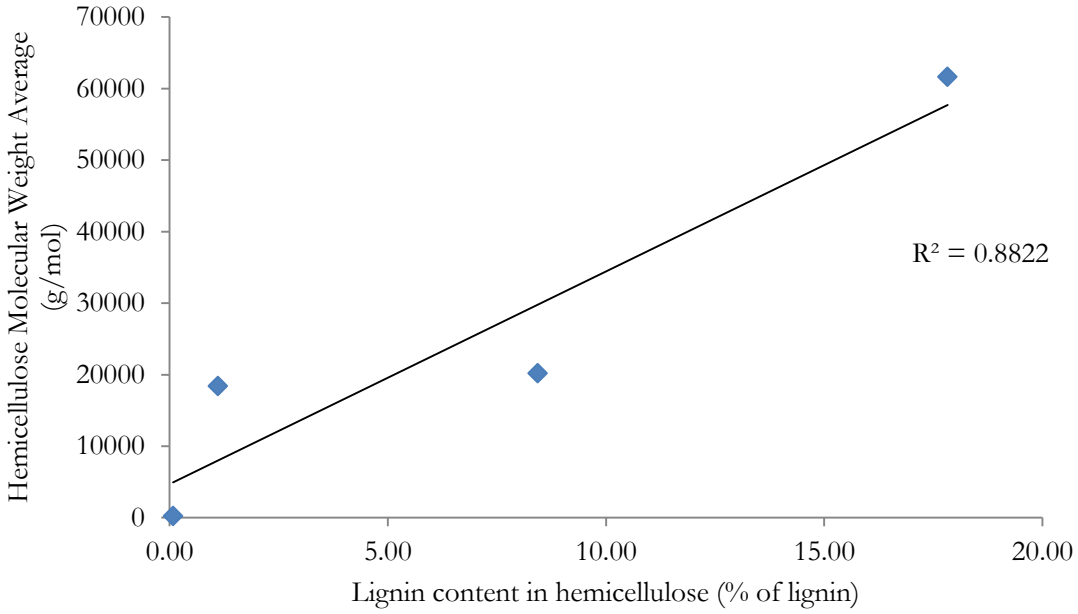


Figure 42: Correlation between *E. grandis* lignin content and *M_W*

Xylitol processes produced the highest *Mw*'s after NaOH extracted hemicelluloses which are also higher than those of Beech wood xylan and Ma et al., (2014s' less than 5000 gmol^{-1} reported M_w of hemicelluloses produced at various hydrothermal treatment conditions (10-240 minutes, 170°C) from bamboo biomass. EG hemicelluloses dissolved below 5.0g of hemicelluloses from both materials, while poor mass balances were also reported. This can be associated with the low M_ws' of hemicelluloses produced from this process at optimum conditions. It is also established that severity of fractionation is directly linked to the molecular weights of hemicelluloses isolated (Ma et al., 2014) as indicated in Figure 42and 43.

Hemicelluloses recovered after fractionation of hemicellulose pre-extracted residues with ethylene glycol recorded the lowest *Mw,* 300gmol^{-1}, almost comparable to commercial D-xylose, 270gmol^{-1}, suggesting that hemicelluloses dissolved at this stage were solely monomers, as confirmed by a DP_w[7] which is less than 25 (Ma et al., 2014). This is probably caused by the double treatment of hemicelluloses remaining in the residual solids after hemicellulose pre-extraction, which is also associated with poor dissolution of hemicellulose, <1.0g as reported in section 4.4.5.

Finally, polydispersity index of hemicelluloses remained nearly concentrated between 1.0-1.50 without a clear pattern between the fractionation processes. However, like Mw it is also influenced by the amount of lignin precipitated with hemicelluloses as shown in Figure 43. As for the materials, SCB had higher PDI's as compared to EC. This implies, hemicellulose molecules from SCB had a broader molecular weight distribution (Peng et al., 2009) as compared to EC hemicelluloses. These values are a little higher than the 0.2-0.8 reported for hemicelluloses extracted from *Populus trichocarpa* using dilute acid at varying times (Cao et al., 2012). Polydispersity is a measure of shape, broad range of size and mass characteristics of hemicelluloses in a given hemicellulose sample (Harmsen et al., 2010). Given this background, and comparing our hemicelluloses to Beechwood xylan, majority of the hemicelluloses have PDI above 1.48 reported for Beechwood xylan, but below 3.36, suggesting a broader spread of hemicellulose molecules in the samples. PDI is particularly high (3.36) for SCB-ethylene glycol derived hemicelluloses, close to PDI of 3.49 reported for hemicelluloses extracted from SCB by treatment with 3% NaOH at 55°C for a period of 3hours and latter precipitated from solution with 60% ethanol(Peng et al., 2009). Others (Sun et al., 2014)reported almost similar results for their hemicelluloses with PDP between 1.11 to 2.17 for their eucalyptus hydrothermal treatment at 100–140◦C, combined with a post treatment with an alkali.In terms of commercial applications, hemicelluloses are highly sought for when their PDI is below 3, as they are considered to be molecularly uniform (Sun et al., 2014).

[7] Degree of polymerization estimated by dividing average molecular weight number by number of xylose units (150)(Cao et al., 2012).

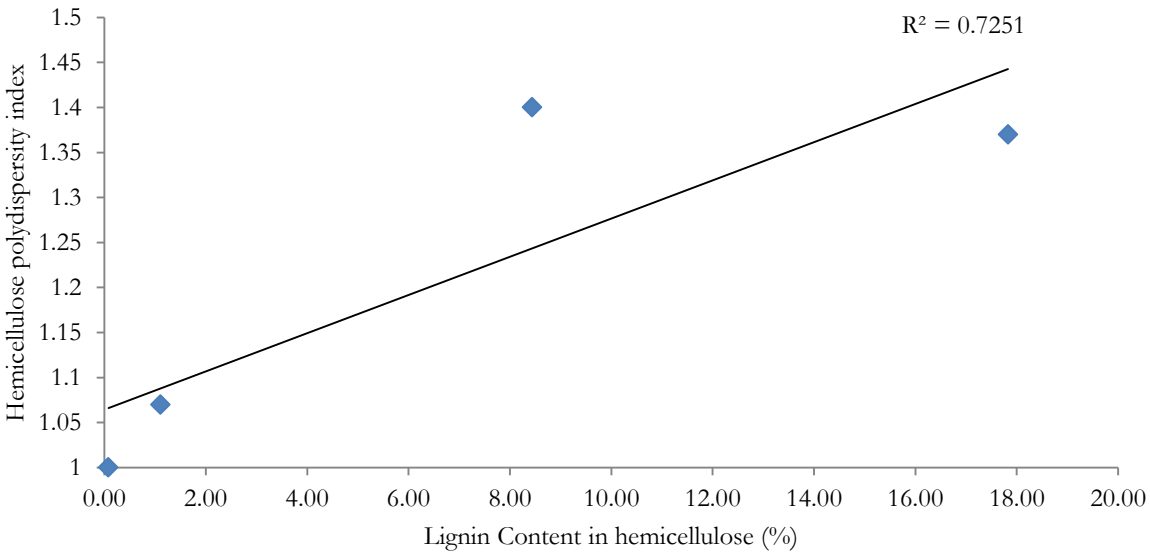


Figure 43: Correlation between *E. grandis'* lignin content and PDI

5.2.3 Quantitative assessment of the quality of lignin rich fraction

a) Lignin functional group determination by FTIR

Lignin FTIR spectras are presented in Figure 44 and 45. Signature lignin bands occurring from 1600 and 1500 cm^{-1}(Leskinen et al., 2013; Ma et al., 2014; Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013), characteristics of aromatic nature of phenolic hydroxyl groups in lignin (Chimphango, 2010; P. Harmsen et al., 2010; Hou et al., 2013; Iqbal et al., 2013; Katahira et al., 2013; J. Li, 2011; Lima et al., 2013; Menon & Rao, 2012; Shahzadi et al., 2014; Singh & Ekhe, 2014; Vena, 2013; B. Yang et al., 2011) and attributed to lignin aromatic skeleton vibrations (Watkins et al., 2014) are observed and well defined in all lignin samples. The presence of bands around 1300 cm^{-1} associated with the lignin monomer, syringyl (Muhammad Safwan et al., 2015; Watkins et al., 2014)and another band at 1200 cm^{-1}associated with another lignin monomer, guaiacyl (Chimphango, 2010; Ma et al., 2014; Watkins et al., 2014) indicates presence of both syringyl and guaiacyl monomers in the lignin samples albeit very low intensities of guaiacyl for ethylene glycol derived lignin samples, agreeing with concentrations of the two monomers in section 5.3.3b.

It is observed with all lignin samples, the absence of bands near 1700 cm^{-1}contrary to what is reported in literature(Cãpraru et al., 2009; Muhammad Safwan et al., 2015; Watkins et al., 2014; Yang et al., 2007). This band near the range 1675-1700cm^{-1} is associated with C=O stretching in conjugated p-substituted aryl ketones (Cãpraru et al., 2009). This suggest fractionation conditions cleaves off the C=O bond on ketones(Cãpraru et al., 2009; Hugo, 2010; Moghaddam et al., 2014; Muhammad Safwan et al., 2015)

associated with lignin phenolic structures (Moghaddam et al., 2014; Pol, Bakker, Zeeland, & Sanchez, n.d.; Zhou et al., 2011).

High intensity and broad bands of apparent hydroxyl groups in phenolic and aliphatic structures is also observed around 3300-3430cm^{-1} (Moghaddam et al., 2014; Singh & Ekhe, 2014; Wang et al., 2012; Yoo, 2012). Because of the physical nature of the lignin samples, which appeared to be very hygroscopic, some of the intensities observed in this region could be contributed by water molecules and also impurities of carbohydrates as argued by high volatiles in lignin from thermogravimetric data presented in section 5.3.3c.

According to Cãpraru et al., 2009, the many peaks displayed in the 1800-900 cm^{-1} region are characteristic of methyl groups, represented mainly by syringyl and guaiacyl units and by other lignin functional groups, which also suggest that our lignin samples are rich with methyl-O-OCH_3, C-O-C stretching and C=C stretching for aromatic ring containing compounds (Yang et al., 2007).

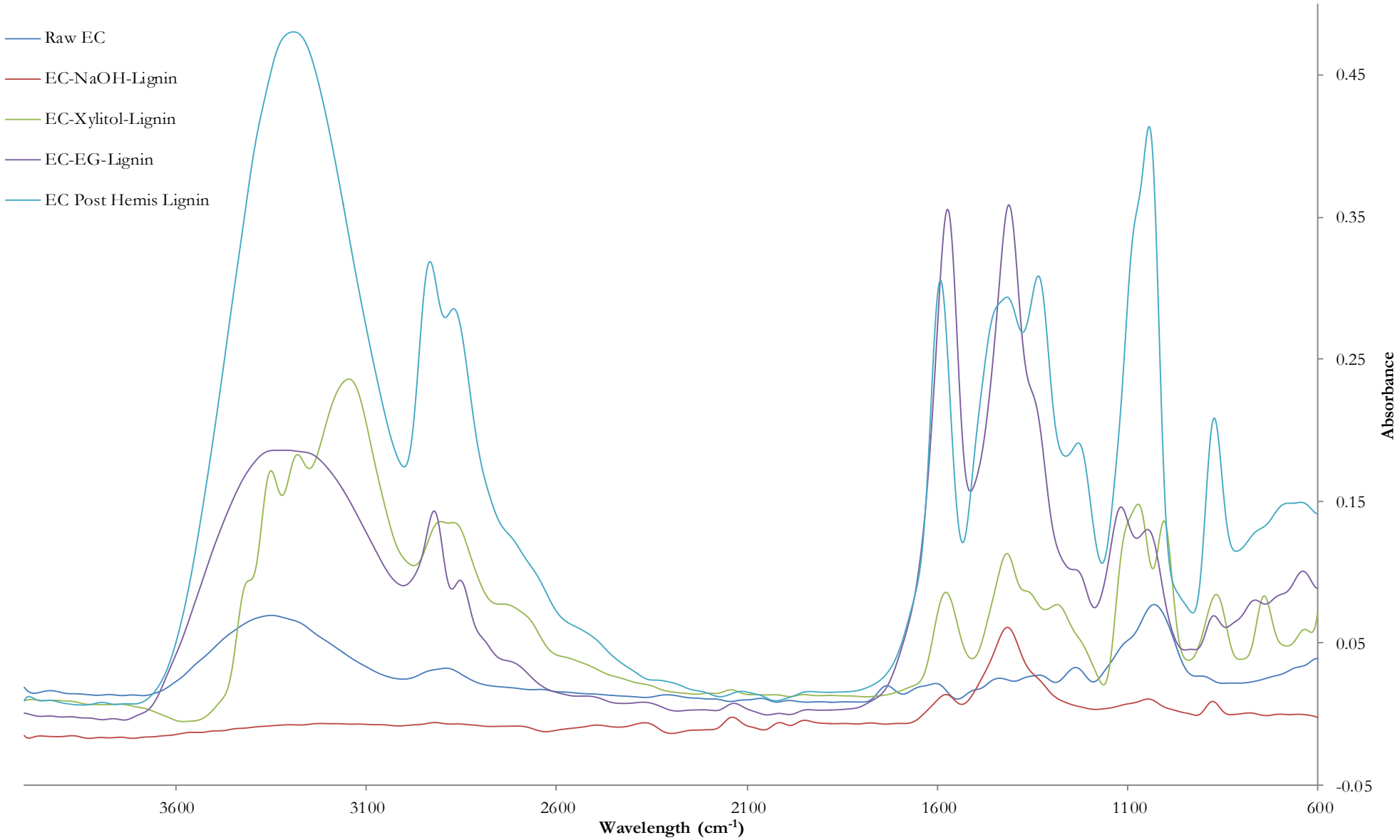


Figure 44: EC Lignin FTIR Spectra

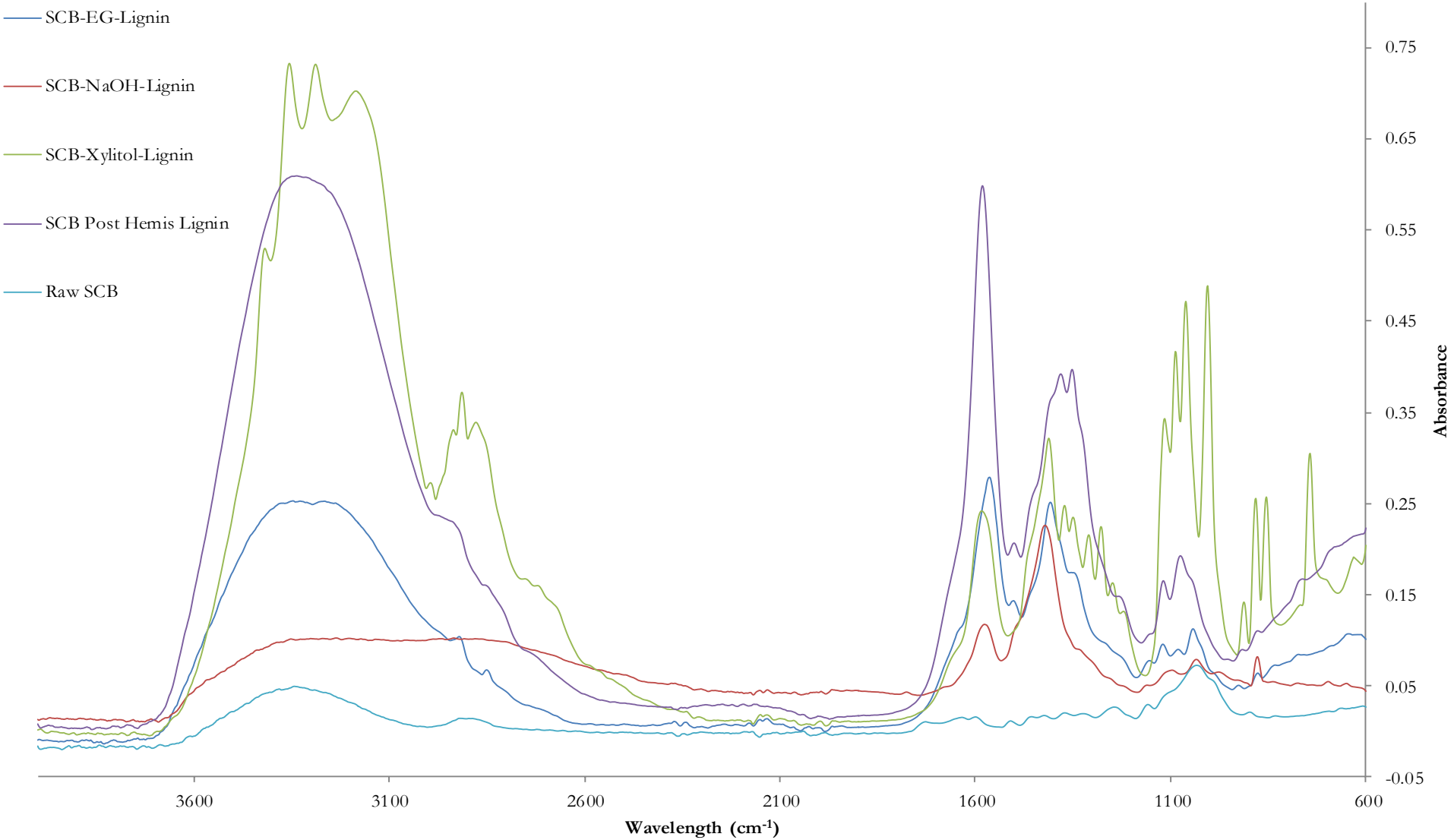


Figure 45: SCB Lignin FTIR Spectra

b) Lignin functional group determination by GC-MS

Our raw sugarcane bagasse reported an S/G ratio of 1.52 (see Table 49), higher than 1.1 reported by van der Hage, Mulder, & Boon, 1993 for their sugarcane bagasse. The S/G ratio obtained for eucalyptus is 3.06, higher than 1.45 to 2.43 reported for *E. grandis* samples from different South African regions (Govender, et al, 2009), but more comparable to ratios of 2.7-3.0 reported for various sample repeats of raw *E. grandis* from Brazil (Lima, et al, 2008). This variation in S/G ratio we suspect can be due to the age of eucalyptus at the time of harvest as reported (Govender, et al., 2009). Methods used to analyse the lignin monomer units are also said to influence the S/G ratio calculation(Brandt et al., 2013; Cao et al., 2012, 2012; Lima, et al., 2008; M. a Lima et al., 2013; Moghaddam et al., 2014; Rodrigues, Meier, Faix, & Pereira, 1999b; Wen et al., 2015; Xu et al., 2012; Yue, Lu, Sun, & Ralph, 2012).

Table 49: S/G ratios of recovered lignins

Lignin source	Guaiacyl (mz 269)	Syringyl (mz 299)	S/G ratio (mz 299/269)
Raw SCB	89874386	136977102	1.52
Raw EC	163014659	499362426	3.06
SCB-NaOH Pre-extraction	22165	59326	2.68
EC-NaOH-Pre-extraction	1318788	4561352	3.46
SCB Xylitol	2040196	4197408	2.06
EC Xylitol	3347074	10782239	3.22
SCB-Ethylene Glycol	2780255	4938725	1.78
EC-Ethylene Glycol	10253568	34099785	3.33
Post Ext SCB	7098515	9419095	1.33
Post Ext EC	18911833	66779763	3.53

Raw *E. grandis* s/g ratio is higher than that of raw SCB, supporting the compositional analysis of the raw materials presented in Chapter Four, section 4.2. This is also supported by others Obst, 1982 and Adler, 1977, who both argues that there is a high concentration of syringil group in hardwoods. Which means the s/g ratio of raw and possibly treated *E. grandis* will be elevated as compared to that of sugarcane

bagasse, grasses which have abundance of the guaiacyl group in their lignin structures (Obst, 1982). The results indeed demonstrated a relative abundance of the syringil monomer in the raw materials before and after treatment as compared to the guaiacyl group. Meanwhile, higher S/G ratio is associated with ease of lignin dissolution, particularly for alkaline environments (Lima et al., 2008), which is in agreement with higher amounts of lignin dissolved from eucalyptus at optimum conditions (see Chapter Four, section 4.4.5), >13.1g/100g of raw material lignin with either of the solvents and as compared to sugarcane bagasse with dissolved lignin capped at below 13.07g/100g of raw material lignin.

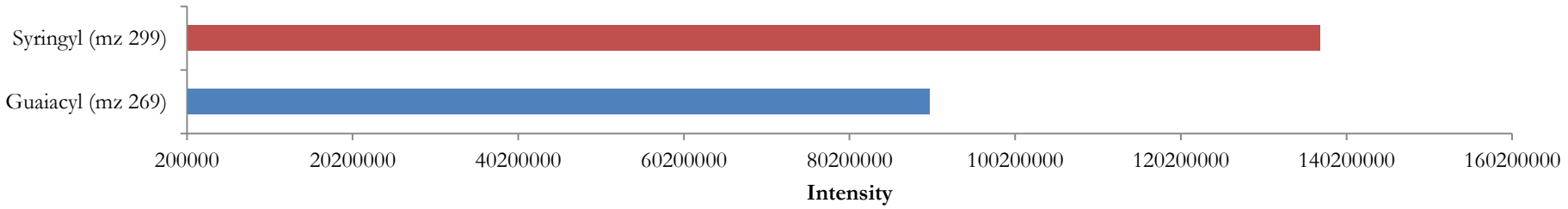


Figure 46: Distribution of syringyl and guaiacyl groups in raw sugarcane bagasse

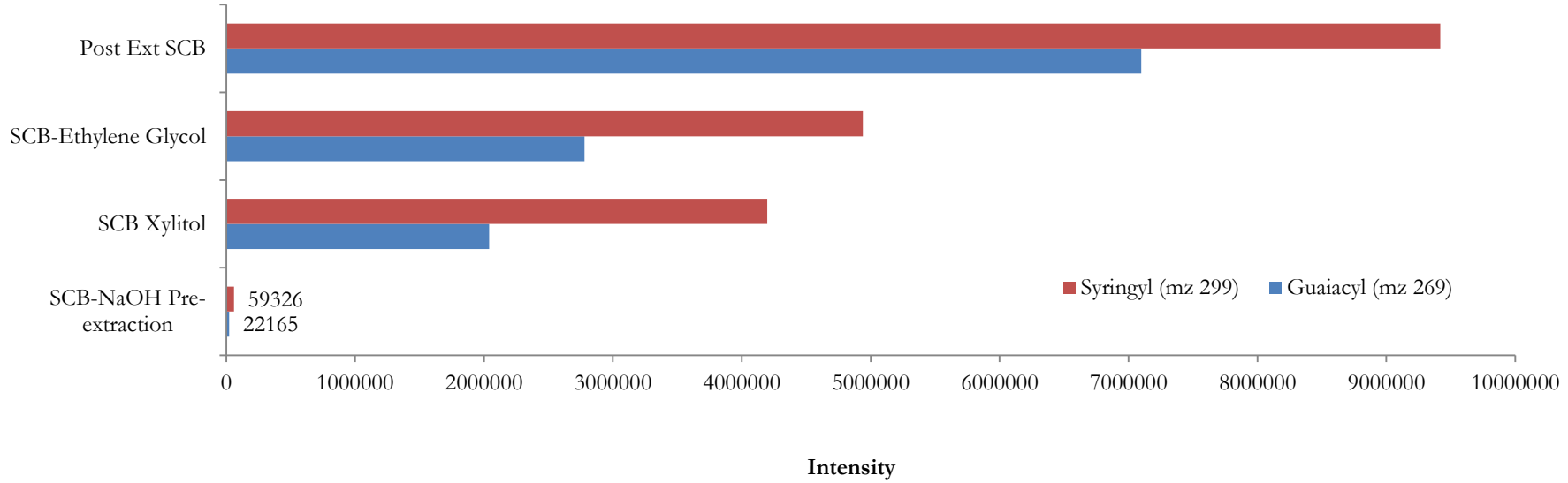


Figure 47: Distribution of syringyl and guaiacyl groups in solid residues after fractionation

There is a major decline in the amount of syringyl and guaiacyl groups after treatment for all processes including hemicellulose pre-extraction as observed in Figure 46 in conjunction with Figure 47. This is a typical behavior as reported by Cao et al, 2012. Syringil group in particular was reduced extensively, considering the initial amount in the raw materials. The syringil group is said to be very susceptible to hydrothermal degradation as compared to the guaiacyl group (Garrote et, al., 1999). Reduction in the syringyl and guaiacyl monomers of the lignin samples was further reiterated with observation of very low intensity bands in FTIR spectras associated with these monomers, as discussed in section 5.3.3a. This also suggests both processes have the ability to cleave lignin from the biomass structure with possibility of degradation.

c) Lignin proximate analysis

Apart from the lignin analysis carried out in section 5.3.3a-b, lignin proximate analysis is very crucial for providing further information on the nature of lignin extracted from the process and also to assist in providing a clear path for possible application as a value added product, which are largely depended on the process used and the biomass type (Muhammad et al., 2015). Proximate analysis results for lignin samples extracted at various conditions as described in section 5.2.6 are presented in Table 50.

As shown in Table 50, lignin extracted alongside the hemicellulose pre-extraction step has very high ash content, between 31.72% and 33.55%as indicated in Table 50. The high ash content in lignin from particularly on hemicelluloses pre-extracted from the two materials we suspect is contributed by the sodium ion (van der Hage, Mulder, & Boon, 1993) concentrated in the 1.5M NaOH solvent used for the hemicellulose pre-extraction. Additionally, traces of sodium ion should be part of lignins of the other samples since it was used as a catalyst throughout, although in minute concentrations and because the NaOH was not recovered separately after the fractionation and hemicellulose pre-extraction process. Nevertheless, our lignins had relatively higher ash content as compared to Muhammad Safwan et al., 2015's findings of lignin ash content between 0.6-1.3%; these lignins were extracted using 1-butyl-3-methylimidazolium chloride ([bmim]Cl), [bmim][CH_3SO_3, EG with either HCl or H_2SO_4 as a catalyst, run at 130°C for 30 minutes.

Ethylene glycol derived lignins have higher ash content as compared to lignin samples from xylitol process. Ethylene glycol has high viscosity as compared to xylitol-water solutions (Jiang, Zu and Ma, 2013) at the working conditions of this study. Viscous solvents carry along dissolved particles in solution (Gírio et al., 2010; Guo et al., 2012; Leskinen et al., 2015; Muhammad Safwan et al., 2015), such as Na ions used as catalyst in this experiment, due to their absorption abilities (Guo et al., 2012), possibly elevating the lignin ash contents. The particle absorption ability of ethylene glycol was demonstrated by

Han et al, 2008. To reduce the ash content of lignin, other researchers (Muhammad Safwan et al., 2015) included a series of hot water washes on their extracted lignin samples between 70°C and 80°C.

Table 50: Proximate analysis of lignin

Lignin Source	Moisture Content (%)	Volatile Matter (%)	Fixed Carbon (%)	Ash Content (%)	Total
EC-NaOH-Pre-extraction	3.20	43.51	20.41	33.55	103.87
SCB-NaOH Pre-extraction	1.19	45.69	20.42	31.72	100.21
SCB-NaOH Pre-extraction Repeat 1	4.90	44.84	16.48	32.80	103.92
EC-Xylitol	1.20	82.46	14.05	2.19	99.91
SCB-Xylitol	1.92	80.26	14.76	3.93	100.86
SCB-Xylitol Repeat 1	1.09	81.74	15.40	1.99	100.22
EC-Ethylene Glycol	15.49	54.36	17.01	13.21	100.07
SCB-Ethylene Glycol	18.47	54.01	15.54	11.62	99.64
EC Post NAOH Extracted	7.58	63.97	13.31	16.55	101.40
SCB Post NAOH Extracted	13.43	59.91	6.51	20.17	100.03
Soda lignin (Felixton Mill, South Africa)	4.26	63.77	28.29	4.27	100.59

All lignins also had a very low fixed carbon content, below 21%, which suggests that those lignins were not pure enough. All lignin samples also have a very low fixed carbon content, below 20%, which suggests impure lignin as compared to Soda lignin extracted from the black liquor supplied by Felixton Mill. This is supported by high volatile matters, which are contributed by the presence of carbohydrates in the phase below 400°C (Watkins et al, 2014). Our lignins, including the Soda lignin were also relatively low in carbon content as compared to lignins from the process of Muhammad et al., 2015 who reported 59.72-60.46% carbon content under the conditions described earlier for their sugarcane bagasse lignins.

5.3 Conclusions

The primary objective of the fractionation processes with regard to the quality of the end product streams were;

- Yield a cellulose rich residue with enzymatic digestibility of 80% or more.
- Dissolve hemicelluloses in polymeric form with minimum molecular weight average of 10 000 gmol-1.
- Defragment the lignin macromolecule into its polymers/oligomers with carbon content of >30% and a syringyl-guaicyl ratio of >1.52 and 3.06 for sugarcane bagasse and E. grandis respectively.
- And finally to understand the effect of set fractionation parameters such as temperature, catalyst and solvent concentration and time of retention on the fragmentation of E. grandis and sugarcane bagasse into the respective components (lignin, hemicellulose and cellulose) providing insights gained from their chemical and structural analysis in terms of the chemistry behind fractionation processes, and how this impacts on yields observed in the previous chapter 4.

Depending on fractionation conditions, for the range of conditions tested, enzymatic hydrolysis efficiency target of 80% for the cellulose rich residue have been achieved. It is recorded that at optimum conditions, digestibility of the cellulose rich residue was below 78% efficiency. This was explained by the amount of lignin and hemicelluloses which were retained in the solid residue as these two hampers accessibility of cellulose by enzymes. However, fractionation of hemicellulose pre-extracted solid residues provided for solid residues with enzymatic digestibility above 80% efficiency which is in line with the objective of the cellulose product outlined in this study. The improved digestibility was explained by the removal of more amorphous components hemicelluloses and lignin first in the pre-extraction step and once again during the fractionation stage of the pre-extracted solid residues (section 4.3 and 4.4.1-4.4.5). Their removal improves solid residue pore size, softens the structure and increase surface area for enzyme access to break-down cellulose (Davison, Parks, Davis, & Donohoe, 2013; Diedericks, 2013; Li, Lu, Zhao, & Qu, 2014). These observations are confirmed with increase in crystallinity of the solid residue (crystallinity of the entire solid material reduced while increasing that of cellulose inside the solid residue) and also with the disappearance of some functional groups associated with bonding of cellulose to hemicelluloses and lignin such as the hydroxyl groups in the FTIR spectras of cellulose (section 5.3.1).

For hemicellulose, the property sought after this work (oligomers and polymer forms of hemicelluloses) was achieved since hemicelluloses isolated in this study are compared to commercial xylan polymer from

Beechwood which was measured to have a molecular weight average of 16882gmol-1. All fractionation processes at optimum conditions provided hemicelluloses with molecular weight average of more than 20 000 gmol-1 (except hemicelluloses from post fractionation of hemicellulose pre-extracted residues) which implies the quality of the hemicelluloses is twice polymeric than the expected (10 000 gmol-1). These quality properties are however do not support the amount of hemicelluloses dissolved from the fractionation processes (<80% target dissolution of hemicelluloses) as discussed in section 4.4.3. Hemicellulose quality is affected by amongst others, the temperature of fractionation i.e. temperatures above 200°C and the amount of lignin impurities.

The lignin stream produced from this study contained high ash content (undesirable), yet with low carbon content below the target carbon content of 30%. All lignins also had a very low fixed carbon content, below 21%, which suggests that those lignins were not pure enough. The low carbon content in the lignin samples is suggesting that the lignin macromolecules were highly degraded, reducing the lignin into monomeric units with lesser C-C covalent bonding which contributes to high fixed carbon content (Elder, 1983). This is in line with the amount of lignin dissolutions experienced in majority of fractionation runs throughout the experiments (section 4.4.4). High delignification is typical for polyols and organic solvents (Lavarack, Griffin, & Rodman, 2002; Zhang, Rackemann, Doherty, & O'Hara, 2013). However, the high dissolution of lignin comes at the advantage of improved enzymatic hydrolysis of the cellulose rich residue as confirmed by S/G ratios >1.73 for lignin samples isolated in this study. Higher syringl ratio is said to be associated with softer lignins and improved enzymatic digestibility (Santos, Gomide, & Hart, 2015).

Chapter Six

6 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

Fractionation studies of lignocellulose materials are complex processes that require thorough understanding of both the feed materials and reaction conditions to be explored. This is especially critical in the case when the fractionation process has a potential for up scaling because the process eventually need to account for costs, sustainability and its effectiveness. For this study, four critical hypotheses were proposed;

- To retain 80% or more cellulose in the solid fraction. The cellulose should also be enzymatically digestible by more than 80% efficiency.
- Dissolve >80% and recover more than 70% of hemicelluloses in the liquid fraction with subsequent extraction with an anti-solvent at optimum fractionation conditions. Recovered hemicelluloses should also be of polymeric form with minimum molecular weight average of 10 000 $gmol^{-1}$.
- Remove more than 70% lignin from the solid fraction, while maintaining high quality of lignin (carbon content of >30%, syringyl-guacyl ratio of >1.52 and 3.06 for sugarcane bagasse and *E. grandis* respectively).
- To determine and understand the effect of set fractionation parameters such as temperature, catalyst and solvent concentration and time of retention on the fragmentation of *E. grandis* and sugarcane bagasse into the respective components (lignin, hemicellulose and cellulose) and use these to optimize best fractionation conditions that maximize components' yields and purity.

This work has extensively looked at the various factors that have the ability to influence the polyol fractionation process, for instance, varying temperature, time and the use of a catalyst as explored in similar polyols fractionation (Li et al., 2013; Moghaddam et al., 2014; Romani et al., 2013; Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013; Zhang, Wong, et al., 2013). Analysis of qualities of materials produced from these combinations also gave further insights into efficiency of these two solvents, ethylene glycol and xylitol as potential fractionation solvents. All in all, some conclusions of note from this work:

a) Cellulose fraction

- Under selected operating conditions, xylitol fractionations of the two materials achieved the 80% target of preserving cellulose in the solid residue, 33.0g/100g and 38.9g/100g of raw material glucose for sugarcane bagasse and eucalyptus respectively, while ethylene glycol fractionations preserved below 70% of initial glucose in the materials, including fractionations of hemicellulose pre-extracted solids achieving only between 54.84-79.02% of initial glucose in raw materials.

- These two solvents, as supported by literature (Ali M. Elshafei, 2011; Deng et al., 2014; Jacobsen & Wyman, 2000; Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013; Zhang, Wong, et al., 2013), induce formation of glycosides that interfere with cellulose (glucose) detection and mass balancing thereof.

- Cellulose is lost in the double treatment process from hemicellulose pre-extraction step to post fractionation of hemicellulose pre-extracted residual solids with EG, i.e 35.39g/100g and 42.44g/100g of raw material glucose remained in the solid fraction after hemicellulose pre-extraction of sugarcane bagasse and eucalyptus respectively, after which further fractionation of the residual solid reduced remaining cellulose in the solid to 28.43g/100g and 26.02g/100g of raw material glucose for sugarcane bagasse and eucalyptus respectively. This represents preservation of 70.65% and 54.84% from the initial raw material glucose for SCB and EC respectively. These are explained by the double treatment of the solids which makes cellulose amenable to dissolution by the two glycols.

- Ethylene glycol fractionations produced a cellulose rich solid highly digestible by selected enzymes, achieving digestibilities of up to 89.0% (run 12, Table 32) and 93.3% (run 12, Table 32) for EC and SCB respectively as compared to highest enzymatic hydrolysis efficiency of 68.85% (run 11, Table 32) reported for both materials when fractionated with xylitol. This is also confirmed by runs at optimized conditions.

- Fractionation of hemicellulose pre-extracted solids with ethylene glycol improved enzymatic hydrolysis efficiency for both materials after optimization of conditions.

- Although highly enzymatic digestible cellulose rich solids were produced at optimum conditions, they comprised undesirably more remaining lignin in their structure i.e. up to 9.14g/100g and 5.91g/100g of initial raw material lignin for SCB and EC respectively after xylitol fractionations, while 5.10g/100g and 4.30g/100gof initial raw material lignin for SCB and EC respectively after ethylene glycol fractionations. Lignin remaining in the cellulose rich solid fraction reduced to below 3.00g/100g of initial raw material lignin for both materials after fractionation of hemicellulose pre-extracted solid residue with 60% ethylene glycol.

- Improvement in crystallinity of cellulose rich solids with increase of more than 15% in some cases indicates the efficiency of the two solvents in removing amorphous materials from eucalyptus and sugarcane bagasse.

b) Hemicellulose fraction

- It is concluded that temperature is the most critical variable for hemicellulose dissolution with maximum to complete dissolution achieved around 180°C while poor recoveries of hemicelluloses as low as 30% of initial raw material hemicellulose is experienced at around 160°C. This is suggested as one of the key factors for high degradation of hemicelluloses.

- The two solvents only managed to dissolve 7.3g/100g of initial hemicellulose (xylose) from raw SCB and EC respectively at optimum conditions, which are all below 70% of hemicellulose in the initial materials respectively. Therefore, the set target for hemicellulose dissolution (70%) was not achieved under these conditions.

- Both processes dissolved xylose lesser than what was dissolved in the NaOH-hemicellulose pre-extraction step. Therefore the hemicellulose pre-extraction step is necessary if the goal of dissolving at least 80% or more hemicelluloses in solution and recovered 70% of the initial hemicellulose. .

- Under the selected operating conditions, ethylene glycol fractionations appeared to have degraded hemicellulose far more than xylitol as observed in the mass balances of the two materials.

- Poor mass balance of xylose was reported possibly due to degradation or formation of glycol-xylosides (Zhang, O'Hara, et al., 2013; Zhang, Wong, et al., 2013).

- Hemicelluloses extracted from the xylitol process have much higher molecular weights as compared to those extracted with ethylene glycol treatments, but lower than those extracted from the NaOH pre-extraction process. This makes this process suitable for producing oligomeric hemicelluloses for the pharmaceutical industries.

c) Lignin fraction

- Fractionation of the two materials with either of the solvents did not achieve the desired target of dissolving more than 70% lignin at optimum conditions even though the fractionations were statistically estimated to dissolve minimum 41% of initial raw material lignin. Just a little above

60% but below 70% lignin was dissolved into the liquid after fractionation of the hemicellulose pre-extracted solid residue with 60% ethylene glycol, 13.07g/100g and 15.72g/100g of initial raw material lignin from SCB and EC respectively.

- A few cases of fractionation runs reported mass balances above 100%, suggesting overestimation of lignin. This was associated with in-situ occurrence of condensation reaction products(Brandt et al., 2013; Deng et al., 2014; Deutschmann & Dekker, 2012; Garrote, Domínguez, et al., 1999; Hage, Mulder, & Boon, 1993; Leskinen et al., 2015; Xuezhi Li et al., 2014; Winkler, 1981) from degradation of either carbohydrates or lignin itself (Cao et al., 2012; P. F. H. Harmsen et al., 2010; Tejado et al., 2007).

- Xylitol fractionations generally solubilised lesser lignin as compared to ethylene glycol fractionations based on the mean estimations (Table 24).

- NaOH as a catalyst improved removal of lignin from xylitol fractionations, especially for sugarcane bagasse. Because this was not the case for eucalyptus fractionations, this observation was associated with the nature of the two materials, a hardwood and softwood.

- Polyols, although widely known to be excellent delignifying agents as reported in literature (Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013), have in this study revealed that they require other variable conditions such as temperature and a catalyst to effectively dissolve lignin. Literature also reported the use of polyols combined with catalysts, mostly acids to fractionate lignocellulose (Moghaddam et al., 2014; Muhammad Safwan et al., 2015).

Due to limited resources and time, other interesting subjects of this project could not be pursued. It would really be interesting, for continuity of this study, that the following items be considered and/or integrated in the processes;

- There was no investigation done on the recovery of xylitol or ethylene glycol from the fractionation process. There are established xylitol recovery processes as previously reported (Faveri, Perego, Converti, & Borghi, 2002). Recovery of the solvents used in the process is a cost effective measure of the process(Menon & Rao, 2012; Moghaddam et al., 2014; Peng et al., 2012; Romani et al., 2013; Zhang, Rackemann, et al., 2013), depending on the easiness of the recovery process and its energy consumption (Romani et al., 2013)This presents an opportunity to experiment if the liquid fraction for possibility of solvent recovery.

- NaOH causes interference in the proximate analysis of lignin as established in section 5.3.3c. There could be a possibility of eliminating it after the process or recovering it for reuse.

- Overestimation of Klason lignin or solvent soluble lignin due to possible interference by polysaccharides (Katahira et al., 2013; Kline, Hayes, Womac, & Libb, 2010; Sathitsuksanoh et al., 2011; Sluiter et al., 2010; J. Sluiter, Nrel, & Sluiter, 2011; Tejado, Peña, Labidi, Echeverria, & Mondragon, 2007) is a major concern and should be investigated to determine the extent of interference and what it mean for the fractionations' interpretations established.

- It was observed that mass balances of hemicelluloses and glucose were quite low and which was concluded to have been a result of either degradation, formation of glycosides or both (Kirk, 1983; Muhammad Safwan et al., 2015; Wyman et al., 2005; Zhang, O'Hara, et al., 2013; Zhang, Rackemann, et al., 2013; Zhang, Wong, et al., 2013). There was no verification done to establish the extend of degradation or formation of the glucosides. No quantitative analysis was done on the supernatants or the solid fractions to determine this conclusion. This could be an opportunity to verify the actual degradation products and how it can be minimized (degradation/glucosides).

- Fractionation of hemicelluloses pre-extracted material with xylitol was not done. It will be interesting to compare it to the complete analysis of ethylene glycol's fractionation of hemicellulose extracted materials.

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8 APPENDICES

8.1 Appendix A: Experiment designs

Table 51: Central Composite Design-Ethylene Glycol for Raw Substrates

Standard Run	$2^{(4)}$ central composite, nc=16 ns=8 n0=2 Runs=26 + 2 Center points			
	Temperature (°C)	Time (Hours)	Catalyst Concentration (wt. % NaOH)	Solvent Concentration (wt. %)
1	140	2	1.0	50
2	140	2	1.0	70
3	140	2	2.0	50
4	140	2	2.0	70
5	140	4	1.0	50
6	140	4	1.0	70
7	140	4	2.0	50
8	140	4	2.0	70
9	180	2	1.0	50
10	180	2	1.0	70
11	180	2	2.0	50
12	180	2	2.0	70
13	180	4	1.0	50
14	180	4	1.0	70
15	180	4	2.0	50
16	180	4	2.0	70
17	120	3	1.5	60
18	200	3	1.5	60
19	160	1	1.5	60
20	160	5	1.5	60
21	160	3	0.5	60
22	160	3	2.5	60
23	160	3	1.5	40
24	160	3	1.5	80
25 (C)	160	3	1.5	60
26 (C)	160	3	1.5	60
27 (C)	160	3	1.5	60
28 (C)	160	3	1.5	60

Table 52: Central Composite Design-Xylitol-Water Solutions for Raw Substrates

Standard Run	$2^{(4)}$ central composite, nc=16 ns=8 n0=2 Runs=26 + 2 center points			
	Temperature (°C)	Time (Hours)	Catalyst Concentration (wt. % NaOH)	Solvent Concentration (wt. %)
1	140	2	1.0	20
2	140	2	1.0	30
3	140	2	2.0	20
4	140	2	2.0	30
5	140	4	1.0	20
6	140	4	1.0	30
7	140	4	2.0	20
8	140	4	2.0	30
9	180	2	1.0	20
10	180	2	1.0	30
11	180	2	2.0	20
12	180	2	2.0	30
13	180	4	1.0	20
14	180	4	1.0	30
15	180	4	2.0	20
16	180	4	2.0	30
17	120	3	1.5	25
18	200	3	1.5	25
19	160	1	1.5	25
20	160	5	1.5	25
21	160	3	0.5	25
22	160	3	2.5	25
23	160	3	1.5	15
24	160	3	1.5	35
25 (C)	160	3	1.5	25
26 (C)	160	3	1.5	25
27 (C)	160	3	1.5	25
28 (C)	160	3	1.5	25

Table 53: Ethylene Glycol Fractionation Central Composite design for Hemicellulose Pre-extracted Substrates

Standard Run	2 (3) central composite, nc=8 ns=6 n0=2 Runs=16 + 2 center points		
	Temperature (Degree Celcius)	**Time (Hours)**	**Catalyst (wt. % NaOH)**
1	140.0	2.0	1.0
2	140.0	2.0	2.0
3	140.0	4.0	1.0
4	140.0	4.0	2.0
5	180.0	2.0	1.0
6	180.0	2.0	2.0
7	180.0	4.0	1.0
8	180.0	4.0	2.0
9	126.4	3.0	1.5
10	193.6	3.0	1.5
11	160.0	1.3	1.5
12	160.0	4.7	1.5
13	160.0	3.0	0.7
14	160.0	3.0	2.3
15 (C)	160.0	3.0	1.5
16 (C)	160.0	3.0	1.5
17 (C)	160.0	3.0	1.5
18 (C)	160.0	3.0	1.5

8.2 Appendix B: Compositional analysis results of CCD runs

Table 54: Summary of predicted optimum fractionation conditions from model fit, 90% CI and alpha value at 0.1 versus runs actual fractionation runs

					Statistical estimated desirable composition													
					Components dissolved in the Liquid Fraction (g/100g)			Components recovered in the Liquid Fraction (g/100g)			Estimated degraded Components (g/100g)			Solid Fraction			EH[e]	Desir-ability
Fractionation	Temp (°C)	Time (Hours)	Catalyst Conc. (wt. %)	Solvent Conc. (%)	[a]Glc	[b]Xyl	[c]SSL	[a]Glc	[b]Xyl	[c]SSL	[a]Glc	[b]Xyl	[c]SSL	Glc (g/100g)	Xyl (g/100g)	[d]AIL (g/100g)		
Raw sugarcane bagasse														40.24	23.35	22.96	12.76	-
Raw *E. grandis*														47.45	20.90	25.52	21.08	-
Xylitol Sugarcane bagasse	160	4	2	20	6.37	13.12	9.60	1.02	6.39	9.41	5.35	6.73	0.19	33.87	10.23	13.36	30.17	0.65
Xylitol *E. grandis*	180	4	1	15	7.03	12.72	21.74	0.70	5.60	17.43	6.33	7.12	4.31	40.42	8.18	3.78	56.16	0.71
EG. SCB	200	5	1.5	40	1.64	18.46	18.53	1.47	5.53	12.73	0.17	12.93	5.80	38.60	4.89	4.43	64.20	0.81
EG. *E. grandis*	180	4	2.5	80	24.65	14.60	20.04	0.45	1.50	17.80	24.20	13.10	2.24	22.80	6.30	5.48	81.10	0.61
					Actual composition													
Raw sugarcane bagasse														40.24	23.35	22.96	12.76	-
Raw *E. grandis*														47.45	20.90	25.52	21.08	-
Xylitol Sugarcane bagasse	160	4	2	20	6.24	12.85	13.82	1.00	7.30	7.30	5.24	5.55	6.52	33.00	10.50	9.14	28.40	-
Xylitol *E. grandis*	180	4	1	15	7.45	11.08	19.61	1.10	6.70	15.10	6.35	4.38	4.51	38.90	9.82	5.91	57.50	-
EG. SCB	200	5	1.5	40	8.44	16.97	17.86	1.50	4.40	10.50	6.94	12.57	7.36	31.80	6.38	5.10	59.76	-
EG. *E. grandis*	180	4	2.5	80	18.25	13.87	21.22	0.60	1.10	14.20	17.65	12.77	7.02	29.20	8.03	4.30	76.77	-

a-Glucose (g/100g), b-Xylose (g/100g), c- Solvent soluble lignin, d- Acid insoluble lignin e-Enzymatic hydrolysis efficiency

Table 55: Composition of liquid fraction and solid residue from *E. grandis* using xylitol fractionation

Fractionation Conditions						**Recovered Components in Liquid Fraction**			**Residual solid Composition**				**[a]Mass Balance**		
Run	Solvent Conc. (%)	Catalyst Conc.	Temp	Time	Solid Recovery (%)	Glc (g/100g)	Xyl (g/100g)	SSL (g/100g)	Glc (g/100g)	Xyl (g/100g)	AIL (g/100g)	EH Efficiency (%)	Glc (%)	Xylose (%)	[c]Total Lignin (%)
1	20.0	1.0	140.0	2.0	79.6	1.0	2.5	9.5	38.1	12.2	16.9	23.9	82.5	70.1	103.4
2	30.0	1.0	140.0	2.0	81.6	1.1	1.7	12.2	40.4	13.0	15.3	21.7	87.4	70.6	107.8
3	20.0	2.0	140.0	2.0	80.0	1.0	2.1	6.5	37.2	11.8	15.5	25.3	80.6	66.4	86.2
4	30.0	2.0	140.0	2.0	78.3	1.2	1.5	12.7	39.8	12.3	11.7	29.5	86.5	66.2	95.5
5	20.0	1.0	140.0	4.0	83.0	0.9	1.9	8.9	37.9	12.6	18.3	23.8	81.8	69.3	106.7
6	30.0	1.0	140.0	4.0	81.7	0.9	1.9	5.2	38.4	12.2	20.7	25.1	82.8	67.5	101.5
7	20.0	2.0	140.0	4.0	79.7	1.1	2.8	6.6	32.8	10.2	16.0	44.8	71.5	62.0	88.8
8	30.0	2.0	140.0	4.0	76.0	1.4	3.0	8.0	42.8	8.1	16.3	35.9	93.0	53.1	95.1
9	20.0	1.0	180.0	2.0	66.8	0.9	4.7	11.1	30.6	10.7	13.1	53.4	66.5	74.0	94.8
10	30.0	1.0	180.0	2.0	66.7	1.1	3.0	7.1	37.1	7.2	15.3	59.3	80.4	49.2	87.5
11	20.0	2.0	180.0	2.0	57.7	1.3	5.2	10.3	38.4	8.7	9.6	68.8	83.5	66.5	77.8
12	30.0	2.0	180.0	2.0	59.0	1.4	5.8	12.8	42.9	9.3	6.0	61.2	93.3	71.9	73.8
13	20.0	1.0	180.0	4.0	64.8	1.0	4.7	11.7	42.2	7.0	8.8	51.1	91.1	56.0	80.2
14	30.0	1.0	180.0	4.0	47.0	0.9	3.8	11.3	29.7	5.3	10.9	45.2	64.6	43.3	87.2
15	20.0	2.0	180.0	4.0	52.4	0.2	3.6	15.9	40.7	8.4	5.0	52.9	86.3	57.2	82.2
16	30.0	2.0	180.0	4.0	54.3	1.1	3.5	14.5	41.3	9.2	6.6	55.2	89.3	60.5	82.6
17	25.0	1.5	120.0	3.0	82.1	1.2	1.8	5.6	42.3	15.1	20.1	15.0	91.6	80.9	100.4
18	25.0	1.5	200.0	3.0	63.9	1.0	2.3	12.9	38.1	5.5	12.1	62.2	82.4	37.4	97.9
19	25.0	1.5	160.0	1.0	81.8	1.1	3.2	8.8	38.6	13.9	16.3	27.7	83.7	81.9	98.3
20	25.0	1.5	160.0	5.0	63.1	0.9	4.5	10.2	32.8	9.5	14.5	59.6	71.0	67.0	96.8
21	25.0	0.5	160.0	3.0	57.6	0.8	6.4	7.8	26.9	6.9	15.2	51.0	58.4	63.4	90.3
22	25.0	2.5	160.0	3.0	62.0	1.4	4.3	14.2	33.7	8.6	11.1	59.6	73.8	61.5	99.2
23	15.0	1.5	160.0	3.0	70.6	1.2	5.5	12.1	35.5	9.8	14.3	38.4	77.3	73.1	103.5
24	35.0	1.5	160.0	3.0	67.4	1.2	4.2	10.2	32.8	10.2	11.0	58.0	71.7	69.0	83.0
25	25.0	1.5	160.0	3.0	68.8	1.0	6.5	8.7	36.0	10.0	16.2	40.3	78.0	78.5	97.7
26	25.0	1.5	160.0	3.0	76.2	1.2	4.5	7.8	38.1	11.5	17.1	34.7	82.8	76.6	97.8
27	25.0	1.5	160.0	3.0	70.1	1.3	4.4	9.2	33.4	8.0	16.5	32.0	73.3	59.5	100.5
28	25.0	1.5	160.0	3.0	72.2	1.3	5.6	10.4	34.1	8.1	18.0	34.9	74.6	65.4	111.3

Table 56: Composition of liquid fraction and solid residue from *E. grandis* using ethylene glycol fractionation

Fractionation Conditions						Recovered Components in Liquid Fraction			Residual solid Composition				[a]Mass Balance		
Run	Solvent Conc. (%)	Catalyst Conc.	Temp	Time	Solid Recovery (%)	Glc (g/100g)	Xyl (g/100g)	SSL (g/100g)	Glc (g/100g)	Xyl (g/100g)	AIL (g/100g)	EH Efficiency (%)	Glc (%)	Xylose (%)	[c]Total Lignin (%)
1	50	1.0	140.0	2.00	77.2	0.7	0.4	5.7	36.3	11.5	16.6	14.0	77.9	56.9	87.5
2	70	1.0	140.0	2.00	68.7	0.3	0.4	7.7	28.1	8.1	16.1	20.5	59.8	40.6	93.3
3	50	2.0	140.0	2.00	61.7	0.4	0.2	5.7	30.6	9.4	11.9	35.2	65.3	45.7	69.3
4	70	2.0	140.0	2.00	69.4	0.1	0.1	10.3	25.2	7.4	16.9	31.6	53.4	36.0	106.5
5	50	1.0	140.0	4.00	55.4	0.1	0.0	8.0	25.6	8.1	11.3	28.7	54.2	39.1	75.6
6	70	1.0	140.0	4.00	62.9	0.1	0.1	8.4	26.7	7.7	11.1	16.6	56.6	37.3	76.4
7	50	2.0	140.0	4.00	66.0	0.5	0.5	6.6	26.3	7.6	13.2	46.0	56.4	38.7	77.3
8	70	2.0	140.0	4.00	63.4	0.1	0.4	12.1	31.8	6.3	11.6	33.5	67.3	32.1	92.9
9	50	1.0	180.0	2.00	53.5	0.5	0.4	13.1	26.6	6.5	10.9	41.9	57.2	33.2	94.1
10	70	1.0	180.0	2.00	64.6	0.2	0.1	12.0	33.8	8.1	14.0	33.1	71.6	39.5	101.9
11	50	2.0	180.0	2.00	52.5	0.3	0.1	14.9	23.9	6.5	6.9	82.6	51.1	31.5	85.6
12	70	2.0	180.0	2.00	57.7	0.2	0.0	13.7	31.5	6.9	9.3	58.8	66.8	33.2	90.0
13	50	1.0	180.0	4.00	55.3	2.1	2.1	14.5	24.6	7.3	9.8	89.6	56.3	45.1	94.9
14	70	1.0	180.0	4.00	58.0	0.8	2.2	15.8	30.4	10.7	7.0	74.3	65.7	62.0	89.5
15	50	2.0	180.0	4.00	46.0	0.8	2.0	19.3	23.7	8.6	4.1	76.3	51.5	51.0	92.0
16	70	2.0	180.0	4.00	43.2	0.8	1.8	15.1	17.0	6.0	4.3	79.2	37.4	37.8	75.8
17	60	1.5	120.0	3.00	75.4	0.7	0.6	6.0	33.6	12.9	14.2	16.9	72.5	64.6	79.2
18	60	1.5	200.0	3.00	37.0	0.7	2.1	12.8	18.6	5.6	3.4	54.3	40.7	36.9	63.4
19	60	1.5	160.0	1.00	69.9	0.9	1.2	9.5	27.3	11.8	13.0	33.9	59.3	62.1	88.2
20	60	1.5	160.0	5.00	56.1	0.3	0.8	14.2	21.6	6.4	8.4	89.1	46.1	34.3	88.5
21	60	0.5	160.0	3.00	67.9	0.4	1.0	8.4	31.5	10.3	15.6	26.0	67.3	53.8	93.9
22	60	2.5	160.0	3.00	68.6	1.2	3.4	12.4	32.0	9.2	13.5	48.1	70.0	60.2	101.6
23	40	1.5	160.0	3.00	68.2	0.6	3.7	8.6	26.1	12.1	15.6	29.8	56.2	75.5	94.7
24	80	1.5	160.0	3.00	69.5	0.2	0.4	10.8	29.2	12.6	15.1	28.7	62.1	62.0	101.5
25	60	1.5	160.0	3.00	64.9	0.9	2.4	12.0	27.5	12.3	14.0	61.2	59.9	70.5	101.9
26	60	1.5	160.0	3.00	61.0	0.5	1.0	11.0	26.8	10.7	10.4	51.8	57.4	56.4	83.6
27	60	1.5	160.0	3.00	57.7	0.7	1.0	11.0	21.3	9.1	8.1	56.5	46.4	48.4	75.1
28	60	1.5	160.0	3.00	61.6	0.5	1.3	13.8	25.1	11.1	10.3	52.7	53.0	54.8	94.1

Table 57: Composition of liquid fraction and solid residue from sugarcane bagasse using xylitol fractionation

Fractionation Conditions						Recovered Components in Liquid Fraction		Residual solid Composition					Mass Balance		
Run	Solvent Conc. (%)	Catalyst Conc.	Temp	Time	Solid Recovery (%)	Glc (g/100g)	Xyl (g/100g)	SSL (g/100g)	Glc (g/100g)	Xyl (g/100g)	AIL (g/100g)	EH Efficiency (%)	Glc (%)	Xylose (%)	[c]Total Lignin (%)
1.0	20.0	1.0	140.0	2.0	77.4	1.3	4.4	5.8	33.6	16.3	15.5	17.2	86.8	88.7	92.8
2.0	30.0	1.0	140.0	2.0	82.4	1.1	3.3	4.7	35.2	17.5	17.6	20.2	90.2	89.1	97.0
3.0	20.0	2.0	140.0	2.0	76.3	0.9	4.5	7.4	35.8	14.3	13.9	17.5	91.0	80.4	92.8
4.0	30.0	2.0	140.0	2.0	73.9	0.5	2.4	3.8	31.9	16.2	14.7	10.1	80.5	79.9	80.6
5.0	20.0	1.0	140.0	4.0	73.4	1.1	5.7	6.3	28.3	10.9	8.7	21.9	73.2	70.8	65.7
6.0	30.0	1.0	140.0	4.0	71.2	1.2	5.5	6.4	33.0	12.5	13.7	13.8	85.0	76.8	87.7
7.0	20.0	2.0	140.0	4.0	58.5	0.8	6.3	10.2	38.1	15.9	13.9	14.5	96.6	94.9	104.9
8.0	30.0	2.0	140.0	4.0	73.9	1.1	5.4	8.8	33.3	15.3	12.4	12.1	85.4	88.8	92.2
9.0	20.0	1.0	180.0	2.0	64.4	1.0	3.0	5.6	32.5	6.8	17.5	27.4	83.3	41.7	100.8
10.0	30.0	1.0	180.0	2.0	63.6	1.0	3.1	6.6	31.8	5.2	17.3	33.1	81.6	35.4	104.1
11.0	20.0	2.0	180.0	2.0	54.8	0.3	3.3	7.2	31.5	6.7	10.6	34.2	79.0	42.8	77.5
12.0	30.0	2.0	180.0	2.0	59.8	0.8	2.6	7.1	33.0	6.3	13.6	27.6	83.9	38.2	90.3
13.0	20.0	1.0	180.0	4.0	62.0	0.1	1.9	3.9	33.3	5.3	17.7	37.2	83.1	30.7	94.0
14.0	30.0	1.0	180.0	4.0	63.8	0.5	1.8	4.7	33.7	4.8	19.6	43.3	85.0	28.3	105.5
15.0	20.0	2.0	180.0	4.0	56.1	0.5	2.0	7.7	29.4	4.1	15.0	42.8	74.4	26.3	98.6
16.0	30.0	2.0	180.0	4.0	59.9	0.9	3.8	9.7	33.1	5.8	16.7	38.6	84.4	41.0	114.9
17.0	25.0	1.5	120.0	3.0	84.3	0.9	2.6	4.7	35.2	16.7	21.7	10.7	89.6	82.5	115.1
18.0	25.0	1.5	200.0	3.0	55.0	0.3	0.8	1.7	27.8	2.6	15.7	22.8	69.8	14.9	75.6
19.0	25.0	1.5	160.0	1.0	83.5	0.8	5.7	5.6	36.9	16.2	18.2	10.5	93.6	93.8	103.9
20.0	25.0	1.5	160.0	5.0	63.5	0.8	3.1	5.8	33.2	6.9	12.2	26.3	84.5	42.8	78.3
21.0	25.0	0.5	160.0	3.0	79.5	1.4	10.0	2.9	36.9	12.6	20.9	12.8	95.3	96.7	103.8
22.0	25.0	2.5	160.0	3.0	73.4	1.4	7.9	9.4	36.7	13.7	14.4	21.8	94.6	92.6	103.5
23.0	15.0	1.5	160.0	3.0	69.9	1.4	7.2	6.0	34.7	11.9	14.4	21.0	89.7	82.1	88.6
24.0	35.0	1.5	160.0	3.0	67.2	1.3	7.9	5.5	33.0	9.2	14.9	17.0	85.4	73.1	88.8
25.0	25.0	1.5	160.0	3.0	64.0	1.4	9.0	5.4	31.8	9.8	15.5	28.3	82.7	80.6	91.2
26.0	25.0	1.5	160.0	3.0	62.8	1.5	8.5	10.7	30.4	10.0	11.4	29.0	79.2	79.2	96.5
27.0	25.0	1.5	160.0	3.0	67.7	1.4	9.4	7.1	30.3	10.2	16.4	24.0	78.8	83.8	102.6
28.0	25.0	1.5	160.0	3.0	64.2	1.2	7.8	5.4	30.3	9.5	16.0	27.7	78.2	74.0	93.1

Table 58: Composition of liquid fraction and solid residue from sugarcane bagasse using ethylene glycol fractionation

Fractionation Conditions						Recovered components in Liquid Fraction			Residual solid composition				[a]Mass Balance		
Run	Solvent Conc. (%)	Catalyst Conc.	Temp	Time	Solid Recovery (%)	Glc (g/100g)	Xyl (g/100g)	SSL (g/100g)	Glc (g/100g)	Xyl (g/100g)	AIL (g/100g)	EH Efficiency (%)	Glc (%)	Xylose (%)	[c]Total Lignin (%)
1	80.0	1.0	140.0	2.0	79.0	1.5	1.8	7.4	35.3	18.4	14.4	18.9	91.4	86.8	94.8
2	90.0	1.0	140.0	2.0	81.2	1.6	1.9	7.6	11.0	6.2	16.2	74.9	31.2	34.8	103.8
3	80.0	2.0	140.0	2.0	65.4	1.6	2.6	9.7	32.1	16.5	11.6	29.8	83.7	81.7	92.5
4	90.0	2.0	140.0	2.0	76.1	1.5	2.2	9.6	22.7	11.6	14.6	37.0	60.3	59.0	105.3
5	80.0	1.0	140.0	4.0	79.9	1.4	2.1	6.4	9.2	4.7	17.4	86.1	26.3	29.2	103.6
6	90.0	1.0	140.0	4.0	79.7	1.4	1.8	7.0	33.4	18.3	17.7	27.0	86.6	86.1	107.5
7	80.0	2.0	140.0	4.0	69.1	1.6	3.2	11.0	29.5	0.8	8.9	38.9	77.3	17.1	86.6
8	90.0	2.0	140.0	4.0	72.1	1.5	2.7	9.5	29.4	0.9	12.2	39.2	76.6	15.2	94.4
9	80.0	1.0	180.0	2.0	83.1	1.5	5.6	8.2	33.7	3.8	13.8	40.5	87.5	39.9	95.7
10	90.0	1.0	180.0	2.0	66.9	1.4	4.1	7.0	30.5	13.0	13.2	49.0	79.2	73.3	87.9
11	80.0	2.0	180.0	2.0	54.7	2.4	6.0	10.2	31.4	11.9	6.2	47.9	84.0	76.6	71.4
12	90.0	2.0	180.0	2.0	62.8	1.3	3.1	11.3	24.0	1.0	9.5	64.4	62.7	17.7	90.3
13	80.0	1.0	180.0	4.0	49.7	1.0	2.4	7.0	26.8	7.7	8.8	76.8	69.2	43.1	68.7
14	90.0	1.0	180.0	4.0	82.9	1.0	2.6	8.1	46.6	21.4	14.4	35.3	118.2	102.8	97.9
15	80.0	2.0	180.0	4.0	68.4	1.3	3.0	11.8	36.4	12.7	11.3	58.1	93.7	67.4	100.6
16	90.0	2.0	180.0	4.0	55.8	1.3	3.4	9.6	33.3	13.3	6.5	61.4	85.8	71.2	70.1
17	85.0	1.5	120.0	3.0	77.3	1.7	2.5	6.7	37.9	19.1	14.3	24.6	98.3	92.3	91.4
18	85.0	1.5	200.0	3.0	57.4	1.1	1.8	7.4	29.8	4.0	12.9	68.7	76.8	24.8	88.2
19	25.0	1.5	160.0	1.0	79.9	1.6	3.3	7.3	36.3	17.4	18.1	30.2	94.2	88.7	110.9
20	25.0	1.5	160.0	5.0	69.7	1.3	4.8	9.7	30.0	12.8	12.0	36.4	77.8	75.4	94.7
21	25.0	0.5	160.0	3.0	75.6	1.5	4.4	4.6	28.8	1.5	18.0	23.3	75.4	25.5	98.5
22	25.0	2.5	160.0	3.0	69.1	1.5	4.5	11.8	24.6	3.4	13.3	50.8	64.8	33.8	109.2
23	15.0	1.5	160.0	3.0	72.8	2.2	7.2	4.7	32.1	0.1	16.9	40.5	85.4	31.4	93.9
24	35.0	1.5	160.0	3.0	76.9	1.4	2.9	5.1	13.9	0.1	17.7	65.3	37.9	12.9	99.3
25	25.0	1.5	160.0	3.0	68.2	1.7	4.2	11.3	34.3	14.7	8.2	44.6	89.3	80.9	85.1
26	25.0	1.5	160.0	3.0	71.2	1.9	4.3	9.4	40.3	17.3	14.9	36.5	104.9	92.5	105.9
27	25.0	1.5	160.0	3.0	73.2	1.6	4.2	8.9	37.8	16.1	12.5	34.9	98.1	86.8	93.1
28	25.0	1.5	160.0	3.0	87.0	1.4	4.0	11.0	38.8	19.5	12.5	38.1	100.0	100.5	102.1

[a] Fractionation mass balance was calculated for each component in the substrates based on the value of the components in the raw materials.*Xyl-Xylose; Glc-Glucose; SSL-Solvent Soluble Lignin (lignin dissolved by either Xylitol or Ethylene Glycol), AIL-Acid insoluble lignin.

Table 59: Hemicellulose extracted *E. grandis* Ethylene glycol fractionation

Fractionation Conditions						Recovered components in Liquid Fraction			Residual solid composition				[a]Mass Balance		
Run	Solvent Conc. (%)	Catalyst Conc.	Temp	Time	Solid Recovery (%)	Glc (g/100g)	Xyl (g/100g)	SSL (g/100g)	Glc (g/100g)	Xyl (g/100g)	AIL (g/100g)	EH Efficiency (%)	Glc (%)	Xylose (%)	[c]Total Lignin (%)
1	60.00	1.00	140.00	2.00	88.9	0.2	0.4	4.1	20.7	5.4	18.4	54.1	44.1	27.7	88.2
2	60.00	2.00	140.00	2.00	83.9	0.6	0.4	8.9	24.7	6.4	13.2	60.6	53.2	32.4	86.7
3	60.00	1.00	140.00	4.00	84.9	0.6	0.4	6.9	34.6	7.0	15.0	48.1	74.1	35.4	85.9
4	60.00	2.00	140.00	4.00	75.3	0.7	0.9	14.9	24.9	6.4	7.7	85.5	54.1	34.7	88.6
5	60.00	1.00	180.00	2.00	70.4	0.9	1.2	14.1	28.7	7.1	6.0	78.4	62.3	39.8	78.4
6	60.00	2.00	180.00	2.00	44.7	0.8	1.5	20.7	17.8	4.3	0.5	89.8	39.3	27.8	83.2
7	60.00	1.00	180.00	4.00	63.4	0.6	1.3	19.0	29.2	6.9	4.1	79.6	62.9	39.6	90.6
8	60.00	2.00	180.00	4.00	55.6	0.6	1.5	18.9	20.0	5.4	0.6	93.2	43.5	33.0	76.5
9	60.00	1.50	126.40	3.00	87.1	0.6	0.3	8.1	22.2	5.5	17.5	52.9	48.2	27.8	100.0
10	60.00	1.50	193.60	3.00	54.5	0.6	1.9	19.3	22.9	5.3	0.8	72.6	49.5	34.5	78.8
11	60.00	1.50	160.00	1.30	74.5	0.4	0.8	11.0	17.9	4.7	12.1	91.4	38.6	26.1	90.6
12	60.00	1.50	160.00	4.70	65.9	0.5	1.0	19.6	23.7	6.0	3.2	76.5	51.0	33.5	89.0
13	60.00	0.70	160.00	3.00	78.3	0.4	0.5	9.5	27.2	6.7	12.6	76.0	58.1	34.4	86.7
14	60.00	2.30	160.00	3.00	64.8	0.2	0.4	18.2	23.8	6.0	2.8	85.5	50.6	30.4	82.4
15	60.00	1.50	160.00	3.00	69.6	0.4	1.2	17.9	25.6	6.3	5.8	90.2	55.0	35.9	93.0
16	60.00	1.50	160.00	3.00	68.0	0.4	1.2	18.6	25.5	6.2	5.1	93.4	54.7	35.5	92.6
17	60.00	1.50	160.00	3.00	70.8	0.6	1.6	17.6	24.2	5.7	6.0	94.5	52.2	34.7	92.2
18	60.00	1.50	160.00	3.00	69.8	0.5	0.9	19.8	27.1	6.1	4.2	93.0	58.1	33.5	93.9

Table 60: Hemicellulose extracted SCB Ethylene glycol fractionation

Fractionation Conditions						Recovered components in Liquid Fraction			Residual solid composition				[a]Mass Balance		
Run	Solvent Conc. (%)	Catalyst Conc.	Temp	Time	Solid Recovery (%)	Glc (g/100g)	Xyl (g/100g)	SSL (g/100g)	Glc (g/100g)	Xyl (g/100g)	AIL (g/100g)	EH Efficiency (%)	Glc (%)	Xylose (%)	[c]Total Lignin (%)
1	60	1	140	2	60.9	0.2	1.1	13.4	28.1	14.3	3.9	82.2	70.1	65.9	75.2
2	60	2	140	2	56.6	0.6	1.1	10.0	26.8	12.6	6.8	87.9	68.1	58.9	73.0
3	60	1	140	4	56.0	0.5	1.0	12.9	26.8	13.8	3.2	81.5	67.9	63.4	70.4
4	60	2	140	4	56.4	0.6	1.1	13.1	26.8	12.7	2.6	76.1	68.0	58.9	68.2
5	60	1	180	2	60.0	0.7	1.6	17.3	28.0	15.0	3.7	76.6	71.3	70.8	91.3
6	60	2	180	2	69.7	1.2	2.4	13.7	34.8	15.8	3.5	75.7	89.4	77.9	74.7
7	60	1	180	4	74.5	1.4	2.2	13.1	35.2	16.9	4.3	73.0	91.1	81.8	76.0
8	60	2	180	4	68.6	2.1	2.8	5.6	34.7	16.4	2.2	33.1	91.4	82.0	34.2
9	60	1.5	126.4	3	77.9	1.5	1.4	2.0	36.1	18.7	15.8	72.8	93.6	86.0	77.4
10	60	1.5	193.6	3	68.7	1.4	2.9	11.1	34.8	14.6	4.3	57.1	90.0	74.7	67.3
11	60	1.5	160	1.3	55.3	0.6	1.6	13.1	25.8	12.8	2.8	76.3	65.6	61.7	69.5
12	60	1.5	160	4.7	74.0	1.2	1.6	14.4	36.0	17.7	2.8	62.6	92.3	82.4	74.8
13	60	0.7	160	3	76.8	0.8	1.4	11.7	32.7	16.0	6.4	40.1	83.3	74.5	78.9
14	60	2.3	160	3	70.4	0.4	2.3	16.4	31.4	15.2	1.6	68.6	79.1	74.8	78.4
15	60	1.5	160	3	74.9	0.2	0.9	14.5	31.1	15.3	3.2	68.1	77.9	69.2	77.1
16	60	1.5	160	3	75.4	0.3	0.9	13.9	31.7	15.2	3.1	74.7	79.4	69.0	74.0
17	60	1.5	160	3	74.7	0.7	1.9	13.9	30.8	14.8	3.7	63.2	78.2	71.4	77.0
18	60	1.5	160	3	74.7	0.9	1.5	13.8	30.5	14.7	3.5	70.9	78.0	69.4	75.3

8.3 Appendix C: Component mass balances

8.3.1 Cellulose Mass Balances

Table 61: Cellulose Mass Balance- Raw EC Xylitol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Recovered cellulose in Liquid Fraction (g/100g)	Cellulose in Residual Solid (g/100g)	Total Cellulose (g/100)	% Yield in Solid fraction	Total Recovery (%)
1	20	1	140	2	0.98	38.15	39.13	80.40	82.47
2	30	1	140	2	1.09	40.36	41.45	85.05	87.35
3	20	2	140	2	0.98	37.24	38.23	78.49	80.56
4	30	2	140	2	1.24	39.79	41.03	83.86	86.47
5	20	1	140	4	0.89	37.91	38.81	79.90	81.78
6	30	1	140	4	0.92	38.36	39.28	80.84	82.79
7	20	2	140	4	1.15	32.77	33.92	69.07	71.49
8	30	2	140	4	1.36	42.76	44.12	90.12	92.99
9	20	1	180	2	0.93	30.64	31.57	64.58	66.54
10	30	1	180	2	1.09	37.08	38.17	78.15	80.44
11	20	2	180	2	1.27	38.37	39.64	80.87	83.55
12	30	2	180	2	1.37	42.91	44.28	90.43	93.33
13	20	1	180	4	1.01	42.21	43.22	88.97	91.09
14	30	1	180	4	0.94	29.69	30.63	62.58	64.56
15	20	2	180	4	0.20	40.73	40.93	85.84	86.26
16	30	2	180	4	1.11	41.28	42.39	86.99	89.33
17	25	1.5	120	3	1.23	42.25	43.48	89.04	91.64
18	25	1.5	200	3	1.04	38.05	39.10	80.19	82.39
19	25	1.5	160	1	1.15	38.57	39.72	81.29	83.70
20	25	1.5	160	5	0.90	32.78	33.68	69.08	70.98
21	25	0.5	160	3	0.84	26.86	27.70	56.61	58.38
22	25	2.5	160	3	1.35	33.69	35.04	71.00	73.85
23	15	1.5	160	3	1.17	35.51	36.68	74.84	77.30
24	35	1.5	160	3	1.22	32.79	34.01	69.10	71.68
25	25	1.5	160	3	0.98	36.02	37.00	75.92	77.99
26	25	1.5	160	3	1.23	38.06	39.29	80.22	82.81
27	25	1.5	160	3	1.32	33.44	34.76	70.47	73.26
28	25	1.5	160	3	1.30	34.10	35.40	71.87	74.60

Table 62: Cellulose Mass Balance- Raw EC Ethylene glycol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Recovered cellulose in Liquid Fraction (g/100g)	Cellulose in Residual Solid (g/100g)	Total Cellulose (g/100)	% Yield in Solid fraction	Total Recovery (%)
1	50.0	1	140	2	0.72	36.25	36.97	76.40	77.91
2	70.0	1	140	2	0.31	28.07	28.38	59.16	59.81
3	50.0	2	140	2	0.38	30.59	30.97	64.46	65.26
4	70.0	2	140	2	0.12	25.23	25.35	53.16	53.42
5	50.0	1	140	4	0.12	25.58	25.70	53.90	54.15
6	70.0	1	140	4	0.12	26.75	26.86	56.37	56.61
7	50.0	2	140	4	0.47	26.29	26.76	55.40	56.40
8	70.0	2	140	4	0.07	31.84	31.92	67.11	67.26
9	50.0	1	180	2	0.52	26.63	27.15	56.13	57.22
10	70.0	1	180	2	0.17	33.79	33.96	71.22	71.57
11	50.0	2	180	2	0.35	23.88	24.23	50.33	51.06
12	70.0	2	180	2	0.17	31.50	31.67	66.39	66.75
13	50.0	1	180	4	2.08	24.64	26.72	51.93	56.31
14	70.0	1	180	4	0.79	30.38	31.16	64.02	65.68
15	50.0	2	180	4	0.78	23.66	24.44	49.87	51.51
16	70.0	2	180	4	0.78	16.97	17.75	35.76	37.40
17	60.0	1.5	120	3	0.75	33.64	34.39	70.90	72.48
18	60.0	1.5	200	3	0.72	18.61	19.33	39.21	40.74
19	60.0	1.5	160	1	0.90	27.26	28.15	57.44	59.33
20	60.0	1.5	160	5	0.30	21.55	21.85	45.42	46.06
21	60.0	0.5	160	3	0.42	31.53	31.95	66.45	67.33
22	60.0	2.5	160	3	1.20	32.01	33.21	67.46	69.99
23	40.0	1.5	160	3	0.59	26.07	26.66	54.95	56.19
24	80.0	1.5	160	3	0.22	29.25	29.47	61.64	62.10
25	60.0	1.5	160	3	0.88	27.55	28.43	58.05	59.91
26	60.0	1.5	160	3	0.45	26.80	27.26	56.49	57.44
27	60.0	1.5	160	3	0.69	21.33	22.03	44.96	46.42
28	25	1.5	160	3	0.50	25.11	25.61	52.92	53.97

Table 63: Cellulose Mass Balance- Raw SCB Xylitol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Recovered cellulose in Liquid Fraction (g/100g)	Cellulose in Residual Solid (g/100g)	Total Cellulose (g/100)	% Yield in Solid fraction	Total Recovery (%)
1	20	1	140	2	1.35	33.59	34.94	83.48	86.83
2	30	1	140	2	1.12	35.19	36.31	87.46	90.23
3	20	2	140	2	0.85	35.77	36.62	88.89	91.01
4	30	2	140	2	0.55	31.85	32.40	79.16	80.52
5	20	1	140	4	1.11	28.33	29.44	70.40	73.15
6	30	1	140	4	1.17	33.03	34.20	82.07	84.99
7	20	2	140	4	0.77	38.11	38.88	94.71	96.61
8	30	2	140	4	1.10	33.28	34.38	82.71	85.44
9	20	1	180	2	1.03	32.48	33.51	80.72	83.28
10	30	1	180	2	1.01	31.83	32.84	79.11	81.62
11	20	2	180	2	0.32	31.46	31.79	78.19	79.00
12	30	2	180	2	0.77	32.99	33.75	81.98	83.88
13	20	1	180	4	0.12	33.32	33.44	82.81	83.10
14	30	1	180	4	0.54	33.67	34.21	83.67	85.02
15	20	2	180	4	0.53	29.42	29.95	73.11	74.44
16	30	2	180	4	0.89	33.06	33.96	82.17	84.39
17	25	1.5	120	3	0.90	35.17	36.07	87.39	89.64
18	25	1.5	200	3	0.32	27.76	28.08	68.98	69.78
19	25	1.5	160	1	0.77	36.89	37.66	91.68	93.58
20	25	1.5	160	5	0.77	33.23	34.01	82.58	84.51
21	25	0.5	160	3	1.40	36.95	38.34	91.82	95.29
22	25	2.5	160	3	1.39	36.68	38.06	91.14	94.59
23	15	1.5	160	3	1.40	34.71	36.11	86.25	89.73
24	35	1.5	160	3	1.30	33.05	34.35	82.12	85.35
25	25	1.5	160	3	1.45	31.82	33.27	79.09	82.69
26	25	1.5	160	3	1.46	30.42	31.89	75.61	79.24
27	25	1.5	160	3	1.43	30.29	31.72	75.27	78.83
28	25	1.5	160	3	1.18	30.28	31.46	75.24	78.17

Table 64: Cellulose Mass Balance- Raw SCB Ethylene glycol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Recovered cellulose in Liquid Fraction (g/100g)	Cellulose in Residual Solid (g/100g)	Total Cellulose (g/100)	% Yield in Solid fraction	Total Recovery (%)
1	50	1	140	2	1.52	35.25	36.77	87.61	91.38
2	70	1	140	2	1.56	11.00	12.56	27.33	31.20
3	50	2	140	2	1.59	32.11	33.69	79.79	83.73
4	70	2	140	2	1.51	22.75	24.26	56.54	60.29
5	50	1	140	4	1.39	9.20	10.60	22.87	26.33
6	70	1	140	4	1.40	33.43	34.83	83.08	86.56
7	50	2	140	4	1.56	29.55	31.11	73.43	77.31
8	70	2	140	4	1.47	29.37	30.84	72.99	76.65
9	50	1	180	2	1.52	33.69	35.21	83.72	87.51
10	70	1	180	2	1.41	30.46	31.87	75.69	79.20
11	50	2	180	2	2.43	31.37	33.80	77.95	83.99
12	70	2	180	2	1.27	23.98	25.25	59.59	62.75
13	50	1	180	4	1.02	26.84	27.86	66.70	69.22
14	70	1	180	4	0.96	46.61	47.57	115.83	118.22
15	50	2	180	4	1.32	36.37	37.69	90.37	93.66
16	70	2	180	4	1.27	33.26	34.52	82.65	85.79
17	60	1.5	120	3	1.67	37.87	39.55	94.12	98.28
18	60	1.5	200	3	1.08	29.83	30.91	74.13	76.81
19	60	1.5	160	1	1.55	36.35	37.90	90.33	94.19
20	60	1.5	160	5	1.28	30.01	31.29	74.57	77.76
21	60	0.5	160	3	1.51	28.84	30.35	71.66	75.41
22	60	2.5	160	3	1.48	24.58	26.06	61.08	64.76
23	40	1.5	160	3	2.25	32.13	34.37	79.84	85.42
24	80	1.5	160	3	1.36	13.89	15.25	34.53	37.89
25	60	1.5	160	3	1.65	34.28	35.94	85.20	89.31
26	60	1.5	160	3	1.94	40.28	42.22	100.11	104.92
27	60	1.5	160	3	1.64	37.84	39.48	94.04	98.11
28	60	1.5	160	3	1.42	38.81	40.23	96.44	99.97

8.3.2 Hemicellulose Mass Balances

Table**65:** Hemicellulose Mass Balance- Raw EC Xylitol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Xylose recovered in Liquid Fraction (g/100g)	Xylose recovered in Residual Solid (g/100g)	Total Hemicellulose (g/100)	% Yield in Liquid fraction	Total Recovery (%)
1	20	1	140	2	2.49	12.17	14.66	11.89	70.12
2	30	1	140	2	1.73	13.03	14.75	8.27	70.59
3	20	2	140	2	2.11	11.77	13.89	10.11	66.44
4	30	2	140	2	1.53	12.30	13.83	7.30	66.16
5	20	1	140	4	1.89	12.59	14.48	9.02	69.28
6	30	1	140	4	1.87	12.24	14.11	8.93	67.50
7	20	2	140	4	2.79	10.17	12.96	13.35	61.99
8	30	2	140	4	3.05	8.06	11.10	14.57	53.12
9	20	1	180	2	4.75	10.71	15.46	22.71	73.98
10	30	1	180	2	3.05	7.23	10.28	14.59	49.17
11	20	2	180	2	5.19	8.70	13.89	24.82	66.46
12	30	2	180	2	5.77	9.26	15.03	27.61	71.94
13	20	1	180	4	4.68	7.03	11.71	22.38	56.03
14	30	1	180	4	3.79	5.26	9.05	18.14	43.32
15	20	2	180	4	3.59	8.37	11.95	17.16	57.20
16	30	2	180	4	3.48	9.17	12.65	16.66	60.52
17	25	1.5	120	3	1.80	15.12	16.91	8.60	80.93
18	25	1.5	200	3	2.31	5.51	7.82	11.05	37.43
19	25	1.5	160	1	3.19	13.93	17.13	15.29	81.95
20	25	1.5	160	5	4.53	9.49	14.01	21.66	67.04
21	25	0.5	160	3	6.37	6.88	13.25	30.50	63.41
22	25	2.5	160	3	4.28	8.59	12.86	20.46	61.53
23	15	1.5	160	3	5.50	9.78	15.28	26.33	73.11
24	35	1.5	160	3	4.18	10.24	14.41	19.98	68.96
25	25	1.5	160	3	6.45	9.96	16.41	30.86	78.50
26	25	1.5	160	3	4.53	11.47	16.01	21.69	76.58
27	25	1.5	160	3	4.41	8.03	12.44	21.12	59.54
28	25	1.5	160	3	5.62	8.06	13.68	26.87	65.44

Table**66:** Hemicellulose Mass Balance- Raw EC Ethylene glycol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Xylose recovered in Liquid Fraction (g/100g)	Xylose recovered in Residual Solid (g/100g)	Total Hemicellulose (g/100)	% Yield in Liquid fraction	Total Recovery (%)
1	50	1	140	2	0.40	11.49	11.90	1.93	56.93
2	70	1	140	2	0.40	8.09	8.49	1.93	40.64
3	50	2	140	2	0.16	9.39	9.55	0.76	45.68
4	70	2	140	2	0.09	7.43	7.52	0.43	35.97
5	50	1	140	4	0.03	8.14	8.17	0.16	39.09
6	70	1	140	4	0.06	7.74	7.80	0.28	37.32
7	50	2	140	4	0.54	7.55	8.09	2.57	38.71
8	70	2	140	4	0.40	6.31	6.71	1.93	32.11
9	50	1	180	2	0.43	6.52	6.95	2.06	33.25
10	70	1	180	2	0.11	8.14	8.25	0.52	39.47
11	50	2	180	2	0.12	6.46	6.59	0.60	31.51
12	70	2	180	2	0.00	6.94	6.94	0.00	33.20
13	50	1	180	4	2.09	7.34	9.43	10.00	45.12
14	70	1	180	4	2.21	10.75	12.95	10.56	61.98
15	50	2	180	4	2.01	8.64	10.65	9.64	50.97
16	70	2	180	4	1.85	6.04	7.89	8.85	37.76
17	60	1.5	120	3	0.64	12.85	13.50	3.07	64.58
18	60	1.5	200	3	2.10	5.62	7.72	10.03	36.92
19	60	1.5	160	1	1.19	11.78	12.97	5.69	62.08
20	60	1.5	160	5	0.78	6.38	7.16	3.71	34.26
21	60	0.5	160	3	0.97	10.26	11.24	4.66	53.76
22	60	2.5	160	3	3.41	9.17	12.58	16.29	60.19
23	40	1.5	160	3	3.68	12.10	15.78	17.60	75.49
24	80	1.5	160	3	0.37	12.58	12.96	1.79	61.99
25	60	1.5	160	3	2.41	12.33	14.74	11.54	70.54
26	60	1.5	160	3	1.05	10.73	11.78	5.02	56.37
27	60	1.5	160	3	0.98	9.14	10.12	4.70	48.41
28	60	1.5	160	3	0.33	11.13	11.45	1.56	54.80

Table**67:** Hemicellulose Mass Balance- Raw SCB Xylitol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Xylose recovered in Liquid Fraction (g/100g)	Xylose recovered in Residual Solid (g/100g)	Total Hemicellulose (g/100)	% Yield in Liquid fraction	Total Recovery (%)
1	20	1	140	2	4.38	16.32	20.70	17.18	81.13
2	30	1	140	2	3.30	17.49	20.80	12.95	81.50
3	20	2	140	2	4.49	14.28	18.77	17.58	73.54
4	30	2	140	2	2.43	16.24	18.67	9.52	73.14
5	20	1	140	4	5.65	10.88	16.54	22.15	64.80
6	30	1	140	4	5.46	12.46	17.93	21.40	70.24
7	20	2	140	4	6.28	15.87	22.15	24.60	86.80
8	30	2	140	4	5.44	15.31	20.74	21.30	81.29
9	20	1	180	2	2.97	6.76	9.73	11.62	38.11
10	30	1	180	2	3.08	5.18	8.26	12.06	32.36
11	20	2	180	2	3.34	6.66	10.00	13.10	39.18
12	30	2	180	2	2.61	6.30	8.91	10.22	34.91
13	20	1	180	4	1.87	5.31	7.18	7.32	28.12
14	30	1	180	4	1.81	4.79	6.61	7.11	25.89
15	20	2	180	4	2.01	4.12	6.13	7.87	24.03
16	30	2	180	4	3.80	5.77	9.58	14.91	37.53
17	25	1.5	120	3	2.56	16.70	19.26	10.03	75.46
18	25	1.5	200	3	0.83	2.64	3.47	3.25	13.61
19	25	1.5	160	1	5.66	16.24	21.90	22.17	85.81
20	25	1.5	160	5	3.06	6.94	10.00	12.00	39.19
21	25	0.5	160	3	9.99	12.58	22.57	39.15	88.45
22	25	2.5	160	3	7.88	13.73	21.61	30.89	84.69
23	15	1.5	160	3	7.24	11.93	19.16	28.36	75.09
24	35	1.5	160	3	7.91	9.16	17.08	31.01	66.91
25	25	1.5	160	3	8.97	9.84	18.81	35.16	73.72
26	25	1.5	160	3	8.47	10.03	18.50	33.19	72.49
27	25	1.5	160	3	9.37	10.20	19.57	36.70	76.68
28	25	1.5	160	3	7.82	9.46	17.27	30.63	67.68

Table**68:** Hemicellulose Mass Balance- Raw SCB Ethylene glycol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Xylose recovered in Liquid Fraction (g/100g)	Xylose recovered in Residual Solid (g/100g)	Total Hemicellulose (g/100)	% Yield in Liquid fraction	Total Recovery (%)
1	50	1	140	2	1.84	18.43	20.27	7.19	79.41
2	70	1	140	2	1.89	6.22	8.12	7.43	31.81
3	50	2	140	2	2.59	16.48	19.07	10.15	74.73
4	70	2	140	2	2.15	11.63	13.78	8.44	54.01
5	50	1	140	4	2.13	4.69	6.81	8.33	26.70
6	70	1	140	4	1.76	18.34	20.10	6.88	78.74
7	50	2	140	4	3.23	0.77	3.99	12.65	15.65
8	70	2	140	4	2.67	0.88	3.55	10.45	13.91
9	50	1	180	2	5.55	3.75	9.31	21.76	36.47
10	70	1	180	2	4.10	13.00	17.11	16.08	67.03
11	50	2	180	2	6.02	11.87	17.89	23.57	70.08
12	70	2	180	2	3.14	1.00	4.14	12.30	16.22
13	50	1	180	4	2.36	7.69	10.05	9.26	39.40
14	70	1	180	4	2.58	21.42	24.00	10.13	94.06
15	50	2	180	4	3.01	12.72	15.73	11.80	61.65
16	70	2	180	4	3.35	13.28	16.63	13.14	65.18
17	60	1.5	120	3	2.45	19.10	21.55	9.60	84.43
18	60	1.5	200	3	1.75	4.03	5.78	6.87	22.66
19	60	1.5	160	1	3.33	17.37	20.70	13.06	81.13
20	60	1.5	160	5	4.80	12.80	17.60	18.79	68.96
21	60	0.5	160	3	4.42	1.54	5.96	17.31	23.35
22	60	2.5	160	3	4.46	3.42	7.89	17.49	30.90
23	40	1.5	160	3	7.19	0.15	7.33	28.16	28.74
24	80	1.5	160	3	2.92	0.08	3.00	11.45	11.76
25	60	1.5	160	3	4.19	14.70	18.90	16.43	74.05
26	60	1.5	160	3	4.33	17.28	21.61	16.97	84.67
27	60	1.5	160	3	4.19	16.07	20.26	16.42	79.38
28	60	1.5	160	3	3.98	19.49	23.48	15.61	91.99

8.3.3 Lignin Mass Balances

Table**69:** Lignin Mass Balance- Raw EC Xylitol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Lignin recovered in Liquid Fraction (g/100g)	Lignin recovered in Residual Solid (g/100g)	Total lignin (g/100)	% Yield in liquid fraction	Total Recovery (%)
1	20	1	140	2	9.51	16.88	26.39	37.25	103.40
2	30	1	140	2	12.19	15.32	27.51	47.76	107.79
3	20	2	140	2	6.49	15.51	22.00	25.42	86.19
4	30	2	140	2	12.65	11.72	24.37	49.58	95.51
5	20	1	140	4	8.93	18.30	27.23	34.99	106.72
6	30	1	140	4	5.19	20.71	25.90	20.33	101.49
7	20	2	140	4	6.61	16.05	22.66	25.90	88.78
8	30	2	140	4	8.00	16.27	24.28	31.35	95.13
9	20	1	180	2	11.13	13.07	24.20	43.61	94.84
10	30	1	180	2	7.07	15.26	22.33	27.71	87.50
11	20	2	180	2	10.28	9.58	19.86	40.28	77.81
12	30	2	180	2	12.82	6.03	18.84	50.23	73.84
13	20	1	180	4	11.66	8.81	20.47	45.70	80.22
14	30	1	180	4	11.30	10.94	22.25	44.28	87.17
15	20	2	180	4	15.94	5.04	20.97	62.45	82.19
16	30	2	180	4	14.50	6.58	21.08	56.80	82.60
17	25	1.5	120	3	5.56	20.07	25.62	21.77	100.40
18	25	1.5	200	3	12.89	12.09	24.97	50.49	97.85
19	25	1.5	160	1	8.82	16.26	25.09	34.57	98.30
20	25	1.5	160	5	10.22	14.48	24.70	40.03	96.78
21	25	0.5	160	3	7.83	15.23	23.06	30.69	90.35
22	25	2.5	160	3	14.23	11.09	25.32	55.77	99.22
23	15	1.5	160	3	12.09	14.33	26.42	47.38	103.54
24	35	1.5	160	3	10.21	10.98	21.19	40.00	83.02
25	25	1.5	160	3	8.71	16.22	24.92	34.12	97.67
26	25	1.5	160	3	7.83	17.14	24.97	30.69	97.84
27	25	1.5	160	3	9.17	16.48	25.65	35.95	100.52
28	25	1.5	160	3	10.35	18.05	28.40	40.57	111.28

Table**70:** Lignin Mass Balance- Raw EC Ethylene glycol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Lignin recovered in Liquid Fraction (g/100g)	Lignin recovered in Residual Solid (g/100g)	Total lignin (g/100)	% Yield in liquid fraction	Total Recovery (%)
1	50	1	140	2	5.73	16.60	22.34	22.47	87.52
2	70	1	140	2	7.68	16.12	23.80	30.11	93.28
3	50	2	140	2	5.75	11.93	17.68	22.53	69.28
4	70	2	140	2	10.29	16.89	27.18	40.31	106.50
5	50	1	140	4	7.96	11.34	19.30	31.20	75.63
6	70	1	140	4	8.37	11.14	19.51	32.79	76.44
7	50	2	140	4	6.57	13.15	19.72	25.74	77.28
8	70	2	140	4	12.13	11.58	23.70	47.52	92.88
9	50	1	180	2	13.12	10.89	24.02	51.43	94.11
10	70	1	180	2	11.98	14.01	25.99	46.95	101.86
11	50	2	180	2	14.93	6.92	21.85	58.50	85.61
12	70	2	180	2	13.67	9.29	22.96	53.57	89.96
13	50	1	180	4	14.46	9.77	24.22	56.65	94.92
14	70	1	180	4	15.81	7.04	22.85	61.94	89.54
15	50	2	180	4	19.34	4.15	23.49	75.79	92.04
16	70	2	180	4	15.09	4.27	19.36	59.13	75.85
17	60	1.5	120	3	6.00	14.21	20.21	23.51	79.21
18	60	1.5	200	3	12.77	3.41	16.19	50.04	63.42
19	60	1.5	160	1	9.52	12.98	22.50	37.30	88.17
20	60	1.5	160	5	14.22	8.37	22.59	55.72	88.51
21	60	0.5	160	3	8.41	15.55	23.97	32.97	93.92
22	60	2.5	160	3	12.45	13.47	25.92	48.77	101.56
23	40	1.5	160	3	8.56	15.62	24.18	33.55	94.74
24	80	1.5	160	3	10.81	15.10	25.90	42.35	101.50
25	60	1.5	160	3	11.97	14.04	26.01	46.89	101.92
26	60	1.5	160	3	10.95	10.38	21.33	42.91	83.59
27	60	1.5	160	3	11.00	8.15	19.15	43.12	75.06
28	60	1.5	160	3	13.76	10.27	24.03	53.92	94.14

Table**71:** Lignin Mass Balance- Raw SCB Xylitol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Lignin recovered in Liquid Fraction (g/100g)	Lignin recovered in Residual Solid (g/100g)	Total lignin (g/100)	% Yield in liquid fraction	Total Recovery (%)
1	20	1	140	2	5.83	15.48	21.31	25.37	92.80
2	30	1	140	2	4.68	17.60	22.28	20.37	97.03
3	20	2	140	2	7.43	13.87	21.30	32.37	92.76
4	30	2	140	2	3.77	14.75	18.52	16.41	80.65
5	20	1	140	4	6.34	8.74	15.08	27.62	65.68
6	30	1	140	4	6.39	13.74	20.13	27.83	87.65
7	20	2	140	4	10.16	13.93	24.09	44.24	104.92
8	30	2	140	4	8.80	12.37	21.16	38.32	92.17
9	20	1	180	2	5.63	17.52	23.14	24.50	100.79
10	30	1	180	2	6.63	17.27	23.90	28.87	104.08
11	20	2	180	2	7.17	10.61	17.78	31.25	77.46
12	30	2	180	2	7.08	13.65	20.73	30.85	90.30
13	20	1	180	4	3.86	17.73	21.58	16.80	94.00
14	30	1	180	4	4.66	19.56	24.21	20.28	105.46
15	20	2	180	4	7.68	14.97	22.65	33.43	98.65
16	30	2	180	4	9.65	16.74	26.39	42.03	114.94
17	25	1.5	120	3	4.69	21.73	26.42	20.44	115.08
18	25	1.5	200	3	1.69	15.67	17.35	7.34	75.58
19	25	1.5	160	1	5.62	18.25	23.86	24.47	103.93
20	25	1.5	160	5	5.82	12.15	17.97	25.34	78.27
21	25	0.5	160	3	2.89	20.95	23.83	12.57	103.80
22	25	2.5	160	3	9.41	14.35	23.76	40.98	103.48
23	15	1.5	160	3	5.97	14.37	20.33	25.99	88.55
24	35	1.5	160	3	5.49	14.89	20.38	23.92	88.76
25	25	1.5	160	3	5.44	15.50	20.94	23.69	91.20
26	25	1.5	160	3	10.75	11.42	22.16	46.80	96.53
27	25	1.5	160	3	7.11	16.45	23.56	30.99	102.63
28	25	1.5	160	3	5.43	15.95	21.38	23.65	93.13

Table**72:** Lignin Mass Balance- Raw SCB Ethylene glycol Fractionation

Run	Solvent Conc. (%, w/v)	Catalyst Conc. (%, w/v)	Temp (°C)	Time (Hours)	Lignin recovered in Liquid Fraction (g/100g)	Lignin recovered in Residual Solid (g/100g)	Total lignin (g/100)	% Yield in liquid fraction	Total Recovery (%)
1	50	1	140	2	7.38	14.40	21.77	32.13	94.83
2	70	1	140	2	7.59	16.25	23.84	33.06	103.84
3	50	2	140	2	9.66	11.58	21.24	42.08	92.52
4	70	2	140	2	9.55	14.63	24.18	41.60	105.32
5	50	1	140	4	6.35	17.43	23.79	27.68	103.60
6	70	1	140	4	6.98	17.69	24.67	30.41	107.47
7	50	2	140	4	11.01	8.87	19.89	47.97	86.62
8	70	2	140	4	9.50	12.18	21.68	41.36	94.41
9	50	1	180	2	8.15	13.83	21.98	35.51	95.75
10	70	1	180	2	7.00	13.18	20.18	30.47	87.87
11	50	2	180	2	10.20	6.20	16.40	44.42	71.44
12	70	2	180	2	11.27	9.46	20.73	49.09	90.29
13	50	1	180	4	6.98	8.78	15.76	30.41	68.66
14	70	1	180	4	8.14	14.35	22.49	35.44	97.94
15	50	2	180	4	11.81	11.29	23.10	51.42	100.61
16	70	2	180	4	9.59	6.50	16.09	41.78	70.08
17	60	1.5	120	3	6.72	14.26	20.98	29.27	91.36
18	60	1.5	200	3	7.38	12.86	20.24	32.15	88.17
19	60	1.5	160	1	7.33	18.14	25.47	31.95	110.94
20	60	1.5	160	5	9.75	11.99	21.74	42.46	94.70
21	60	0.5	160	3	4.60	18.00	22.61	20.06	98.45
22	60	2.5	160	3	11.80	13.28	25.07	51.39	109.21
23	40	1.5	160	3	4.67	16.89	21.55	20.33	93.88
24	80	1.5	160	3	5.13	17.67	22.81	22.35	99.33
25	60	1.5	160	3	11.32	8.22	19.54	49.32	85.11
26	60	1.5	160	3	9.42	14.89	24.32	41.05	105.92
27	60	1.5	160	3	8.86	12.52	21.38	38.58	93.10
28	60	1.5	160	3	10.97	12.47	23.45	47.79	102.12

8.4 Appendix D: ANOVA AND Regression Analysis

8.4.1 ANOVA Single factor Analysis of solid yields

Table 73: Single Factor ANOVA analysis of solid yields between Xylitol fractionations

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
EC-xylitol	28	1944.453492	69.44476757	106.29951
SCB-xylitol	28	1906.315784	68.08270659	74.631587

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	25.97294168	1	25.97294168	0.2871031	0.594283155	4.01954096
Within Groups	4885.139602	54	90.46554818			
Total	4911.112543	55				

Table 74: Single Factor ANOVA analysis of solid yields between EG fractionations

SUMMARY				
Groups	*Count*	*Sum*	*Average*	*Variance*
SCB-EG	28	2004.962986	71.60582094	86.714138
EC-EG	28	1713.107525	61.18241161	86.569214

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1521.064469	1	1521.064469	17.555806	0.000103934	4.01954096
Within Groups	4678.650488	54	86.6416757			
Total	6199.714957	55				

Table 75: Single Factor ANOVA analysis of solid yields between EC fractionations

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
EC-xylitol	28	1944.453492	69.44476757	106.29951		
EC-EG	28	1713.107525	61.18241161	86.569214		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	955.7313652	1	955.7313652	9.9106931	0.00267558	4.01954096
Within Groups	5207.455518	54	96.43436145			
Total	6163.186884	55				

Table 76: Single Factor ANOVA analysis of solid yields between SCB fractionations

SUMMARY						
Groups	*Count*	*Sum*	*Average*	*Variance*		
SCB-xylitol	28	1906.315784	68.08270659	74.631587		
SCB-EG	28	2004.962986	71.60582094	86.714138		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	173.7726861	1	173.7726861	2.1540414	0.147995908	4.01954096
Within Groups	4356.334571	54	80.67286243			
Total	4530.107257	55				

Table 77: Descriptive statistical analysis of solid yield data

	Fractionation's Solid Yield (%)					
	EC-xylitol	*EC-EG*	*SCB-xylitol*	*SCB-EG*	Hemicellulose Extracted EC-EG	Hemicellulose Extracted SCB-EG
Mean	69.44	61.18	68.08	71.61	70.58	68.08
Standard Error	1.95	1.76	1.63	1.76	2.78	1.93
Median	69.49	62.31	65.80	72.45	70.12	70.05
Standard Deviation	10.31	9.30	8.64	9.31	11.78	8.19
Sample Variance	106.30	86.57	74.63	86.71	138.76	67.03
Minimum	46.95	36.96	54.80	49.72	44.69	55.26
Maximum	82.95	77.17	84.28	86.98	88.88	77.87
Count	28	28	28	28	18	18
Confidence Level (95.0%)	4.00	3.61	3.35	3.61	5.86	4.07

8.4.2 Selected ANOVA Multiple factor Analysis

Table 78: Summary of ANOVA on glucose remaining in solid residue for EC-Ethylene glycol Fractionation

Factor	ANOVA; Var.:Glucose SF; R-sqr=.82594; Adj:.5231 (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in SCB-Xylitol) 4 factors, 1 Blocks, 28 Runs; MS Residual=5.225705 DV: Glucose SF				
	SS	df	MS	F	P
(1)Temperature(L)	27.5204	1	27.52042	5.266355	0.039030
Temperature(Q)	0.0115	1	0.01148	0.002198	0.963322
(2)Time (L)	4.5937	1	4.59375	0.879068	0.365550
Time (Q)	17.9834	1	17.98336	3.441327	0.086398
(3)Catalyst Conc.(L)	0.7704	1	0.77042	0.147428	0.707212
Catalyst Conc.(Q)	40.7552	1	40.75523	7.798992	0.015242
(4)Solvent Conc.(L)	0.0337	1	0.03375	0.006458	0.937171
Solvent Conc.(Q)	7.6784	1	7.67836	1.469344	0.247020
1L by 2L	1.2656	1	1.26563	0.242192	0.630833
1L by 3L	11.0556	1	11.05562	2.115624	0.169519
1L by 4L	3.3306	1	3.33063	0.637354	0.439012
2L by 3L	2.6406	1	2.64062	0.505315	0.489732
2L by 4L	1.8906	1	1.89062	0.361793	0.557856
3L by 4L	5.6406	1	5.64063	1.079400	0.317770
Error	67.9342	13	5.22571		
Total SS	181.6125	27			

Table 79: Summary of recovred Xylose ANOVA Analysis-EC-Ethylene glycol Fractionation

Factor	ANOVA; Var.:Xylose LF; R-sqr=.62114; Adj:.4544 (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in EC Glycol) 4 factors, 1 Blocks, 28 Runs; MS Residual=1.055962 DV: Xylose LF				
	SS	df	MS	F	P
(1)Temperature(L)	3.84000	1	3.840000	3.636496	0.078867
Temperature(Q)	0.78844	1	0.788438	0.746654	0.403200
(2)Time (L)	1.81500	1	1.815000	1.718813	0.212532
Time (Q)	1.73344	1	1.733437	1.641573	0.222493
(3)Catalyst Conc.(L)	0.73500	1	0.735000	0.696048	0.419182
Catalyst Conc.(Q)	0.02344	1	0.023437	0.022195	0.883855
(4)Solvent Conc.(L)	2.16000	1	2.160000	2.045529	0.176247
Solvent Conc.(Q)	0.00094	1	0.000937	0.000888	0.976682
1L by 2L	3.61000	1	3.610000	3.418685	0.087327
1L by 3L	0.09000	1	0.090000	0.085230	0.774937
1L by 4L	0.01000	1	0.010000	0.009470	0.923961
2L by 3L	0.09000	1	0.090000	0.085230	0.774937
2L by 4L	0.01000	1	0.010000	0.009470	0.923961
3L by 4L	0.01000	1	0.010000	0.009470	0.923961
Error	13.72750	13	1.055962		
Total SS	28.66679	27			

Table 80: Summary of recovred Xylose ANOVA Analysis-SCB-Ethylene glycol Fractionation

Factor	ANOVA; Var.:Xylose LF; R-sqr=.65029; Adj:.57368 (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in SCB Glycol) 4 factors, 1 Blocks, 28 Runs; MS Residual=1.352308 DV: Xylose LF				
	SS	df	MS	F	P
(1)Temperature(L)	1.30937	1	1.30937	0.968246	0.343078
Temperature(Q)	10.30315	1	10.30315	7.618940	0.016217
(2)Time (L)	1.53597	1	1.53597	1.135813	0.305933
Time (Q)	0.77940	1	0.77940	0.576349	0.461292
(3)Catalyst Conc.(L)	0.42603	1	0.42603	0.315042	0.584149
Catalyst Conc.(Q)	0.15440	1	0.15440	0.114176	0.740833
(4)Solvent Conc.(L)	0.53025	1	0.53025	0.392108	0.542033
Solvent Conc.(Q)	0.11690	1	0.11690	0.086446	0.773387
1L by 2L	4.73063	1	4.73063	3.498187	0.084117
1L by 3L	0.33062	1	0.33062	0.244489	0.629233
1L by 4L	0.45563	1	0.45563	0.336924	0.571535
2L by 3L	0.52563	1	0.52563	0.388687	0.543774
2L by 4L	1.26563	1	1.26563	0.935900	0.350989
3L by 4L	0.22563	1	0.22563	0.166844	0.689580
Error	17.58000	13	1.35231		
Total SS	50.27000	27			

Table 81: Summary of recovered Xylose ANOVA Analysis-SCB-Xylitol Fractionation

Factor	ANOVA; Var.:Xylose LF; R-sqr=.82346; Adj:.63334 (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in SCB-Xylitol) 4 factors, 1 Blocks, 28 Runs; MS Residual=2.566154 DV: Xylose LF				
	SS	df	MS	F	P
(1)Temperature(L)	16.0067	1	16.0067	6.23761	0.026713
Temperature(Q)	102.9204	1	102.9204	40.10688	0.000026
(2)Time (L)	0.0150	1	0.0150	0.00585	0.940221
Time (Q)	46.7604	1	46.7604	18.22198	0.000915
(3)Catalyst Conc.(L)	0.2817	1	0.2817	0.10976	0.745695
Catalyst Conc.(Q)	1.6017	1	1.6017	0.62415	0.443682
(4)Solvent Conc.(L)	0.1350	1	0.1350	0.05261	0.822155
Solvent Conc.(Q)	8.8817	1	8.8817	3.46108	0.085597
1L by 2L	7.2900	1	7.2900	2.84083	0.115735
1L by 3L	0.3025	1	0.3025	0.11788	0.736833
1L by 4L	1.8225	1	1.8225	0.71021	0.414612
2L by 3L	0.8100	1	0.8100	0.31565	0.583792
2L by 4L	1.2100	1	1.2100	0.47152	0.504352
3L by 4L	0.0225	1	0.0225	0.00877	0.926825
Error	33.3600	13	2.5662		
Total SS	188.9668	27			

8.4.1 Predictions and desirability profiling analysis

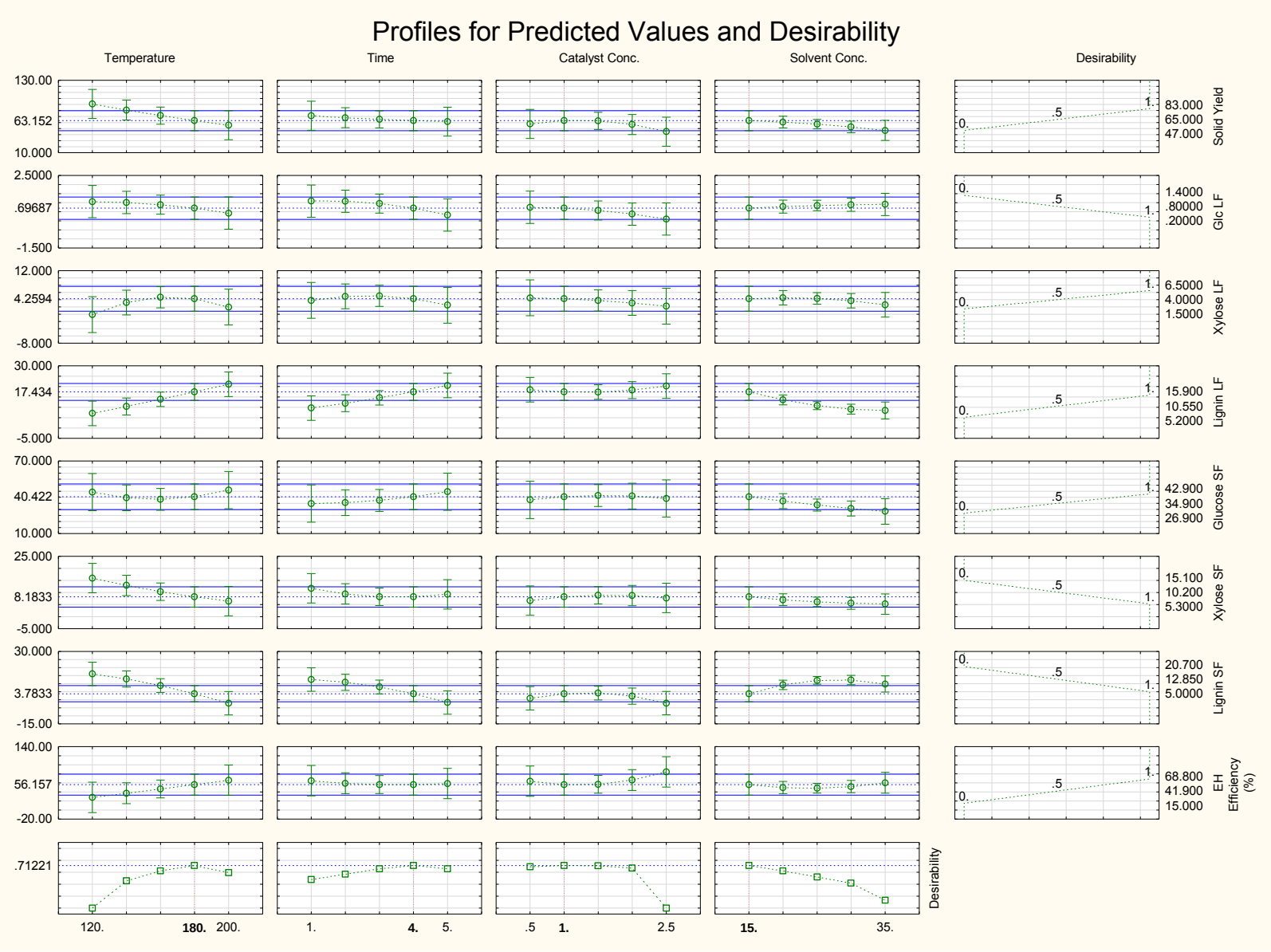

Figure 48: Profiles for predicted values and desirability- Xylitol EC fractionation

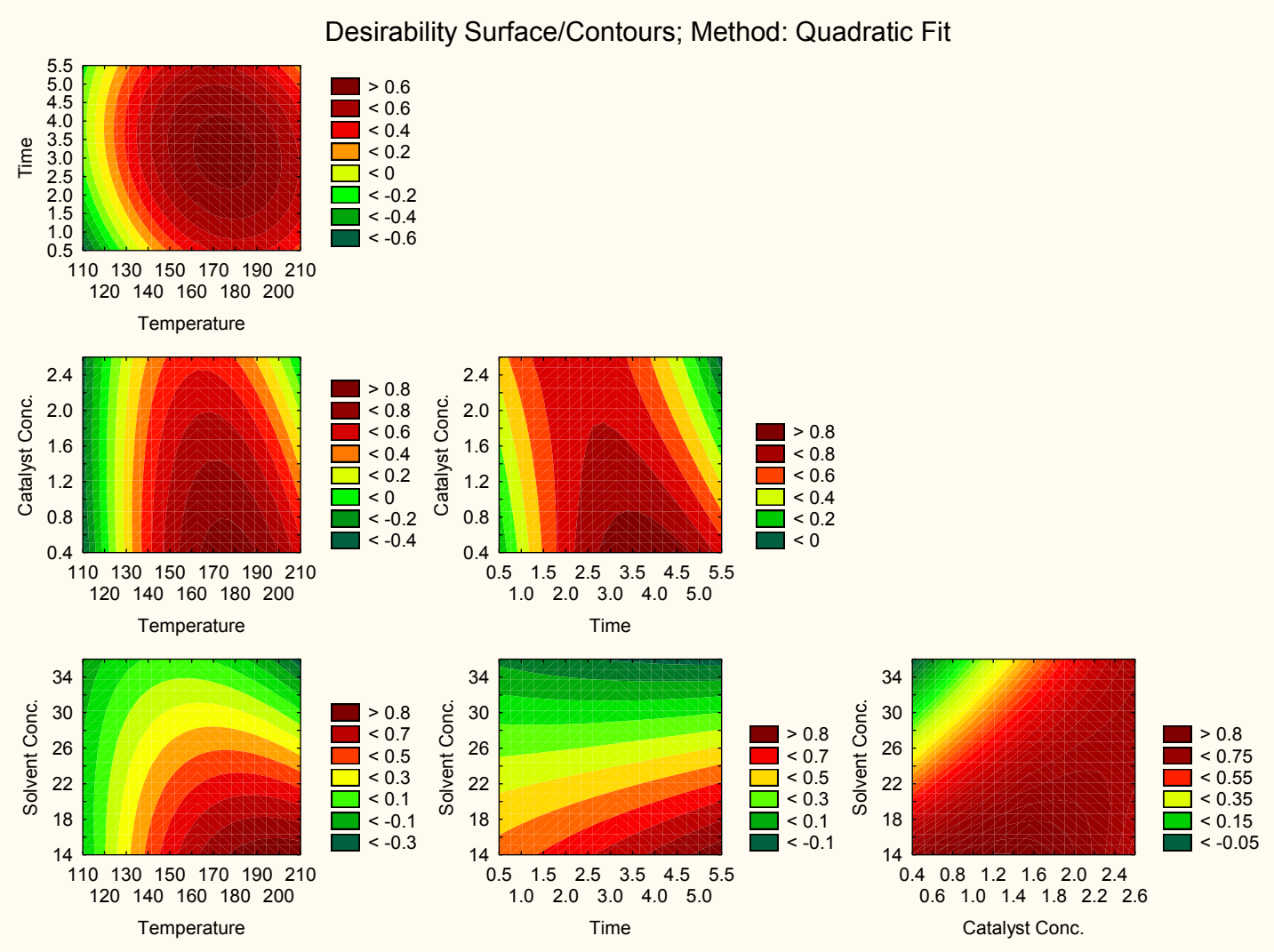

Figure 49: Desirability surface contours- Xylitol EC fractionation

Table 82: Factor levels and predicted responses- Xylitol EC fractionation

Factor	Factor levels and predicted responses (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 in Eucalyptus-Xylitol.stw) Predicted responses at each level of each factor holding all other factors constant at their current setting								
	Factor Level	Predictd Glc LF	Predictd Xylose LF	Predictd Lignin LF	Predictd Glucose SF	Predictd Xylose SF	Predictd Lignin SF	Predictd EH Efficiency (%)	Desirbty Value
Temperature	120.	0.984375	0.246875	10.82188	46.57188	16.88333	11.57083	33.51979	0.000000
Temperature	140.	0.979167	3.533333	13.25417	40.23750	13.03750	8.94583	43.44583	0.496667
Temperature	160.	0.901042	4.955208	15.81354	37.32188	9.62500	5.41250	53.56146	0.687217
Temperature	180.	0.750000	4.512500	18.50000	37.82500	6.64583	0.97083	63.86667	0.779334
Temperature	200.	0.526042	2.205208	21.31354	41.74688	4.10000	-4.37917	74.36146	0.714772
Time	1.	1.009375	3.671875	11.73438	31.77188	10.18333	10.32083	75.85729	0.507917
Time	2.	1.045833	4.916667	13.73750	32.62083	7.87083	8.46250	69.14583	0.614489
Time	3.	0.959375	5.196875	15.99271	34.63854	6.69167	5.34583	65.14896	0.722433
Time	4.	0.750000	4.512500	18.50000	37.82500	6.64583	0.97083	63.86667	0.779334
Time	5.	0.417708	2.863542	21.25938	42.18021	7.73333	-4.66250	65.29896	0.762477
Catalyst Conc.	.5	0.750000	4.512500	18.50000	37.82500	6.64583	0.97083	63.86667	0.779334
Catalyst Conc.	1.	0.696875	4.259375	17.43438	40.42188	8.18333	3.78333	56.15729	0.760813
Catalyst Conc.	1.5	0.570833	3.791667	17.37083	41.48750	8.87917	4.21250	56.98750	0.757446
Catalyst Conc.	2.	0.371875	3.109375	18.30938	41.02188	8.73333	2.25833	66.35729	0.763291
Catalyst Conc.	2.5	0.100000	2.212500	20.25000	39.02500	7.74583	-2.07917	84.26667	0.698390
Solvent Conc.	15.	0.750000	4.512500	18.50000	37.82500	6.64583	0.97083	63.86667	0.779334
Solvent Conc.	20.	0.755208	4.576042	13.76354	32.90104	5.19583	7.09167	57.99896	0.682163
Solvent Conc.	25.	0.737500	4.175000	10.10417	28.37083	4.02917	10.57917	57.12083	0.484653
Solvent Conc.	30.	0.696875	3.309375	7.52188	24.23438	3.14583	11.43333	61.23229	0.000000
Solvent Conc.	35.	0.633333	1.979167	6.01667	20.49167	2.54583	9.65417	70.33333	0.000000

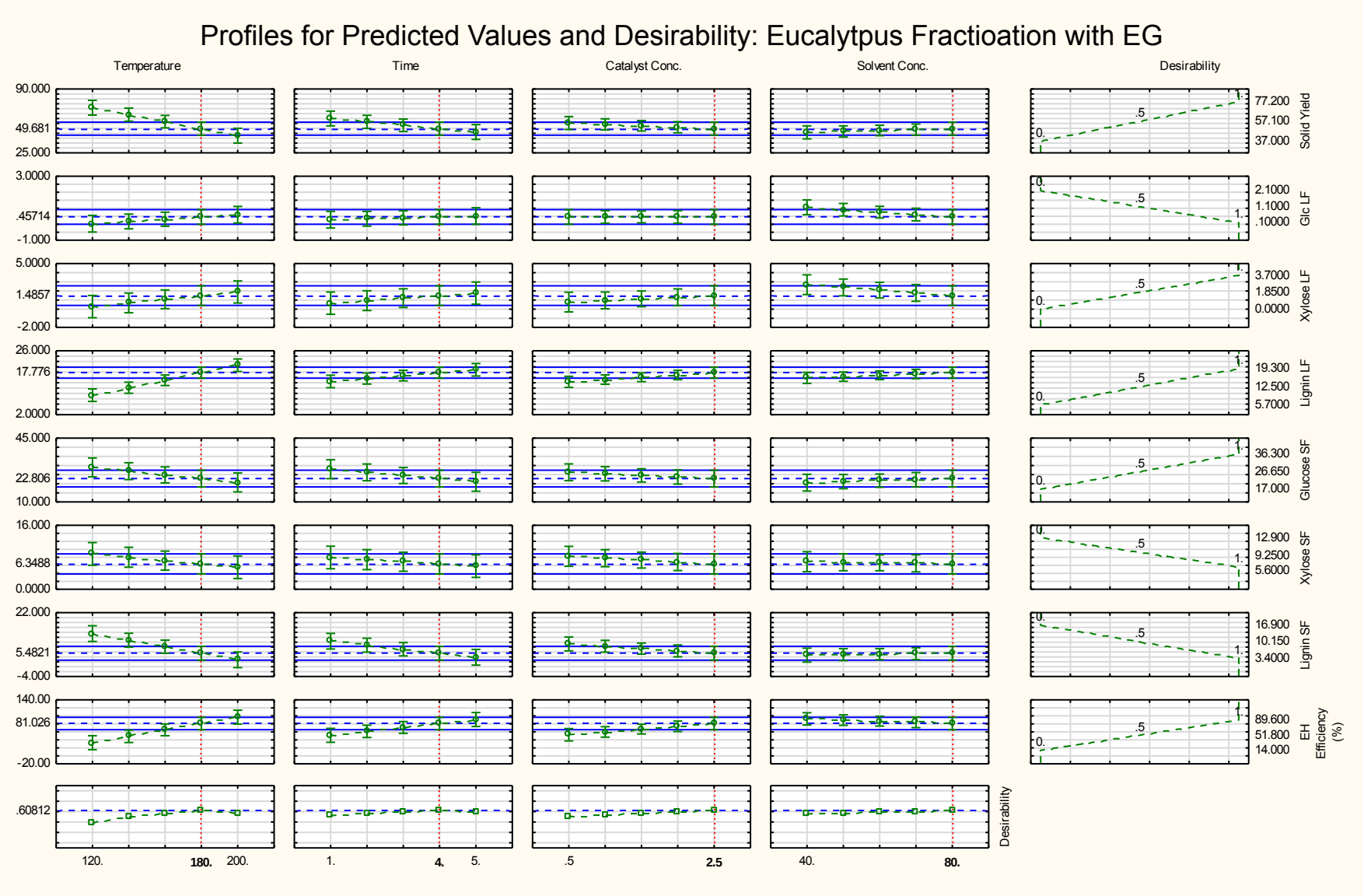

Figure 50: Profiles for predicted values and desirability- Eucalyptus Ethylene glycol fractionation

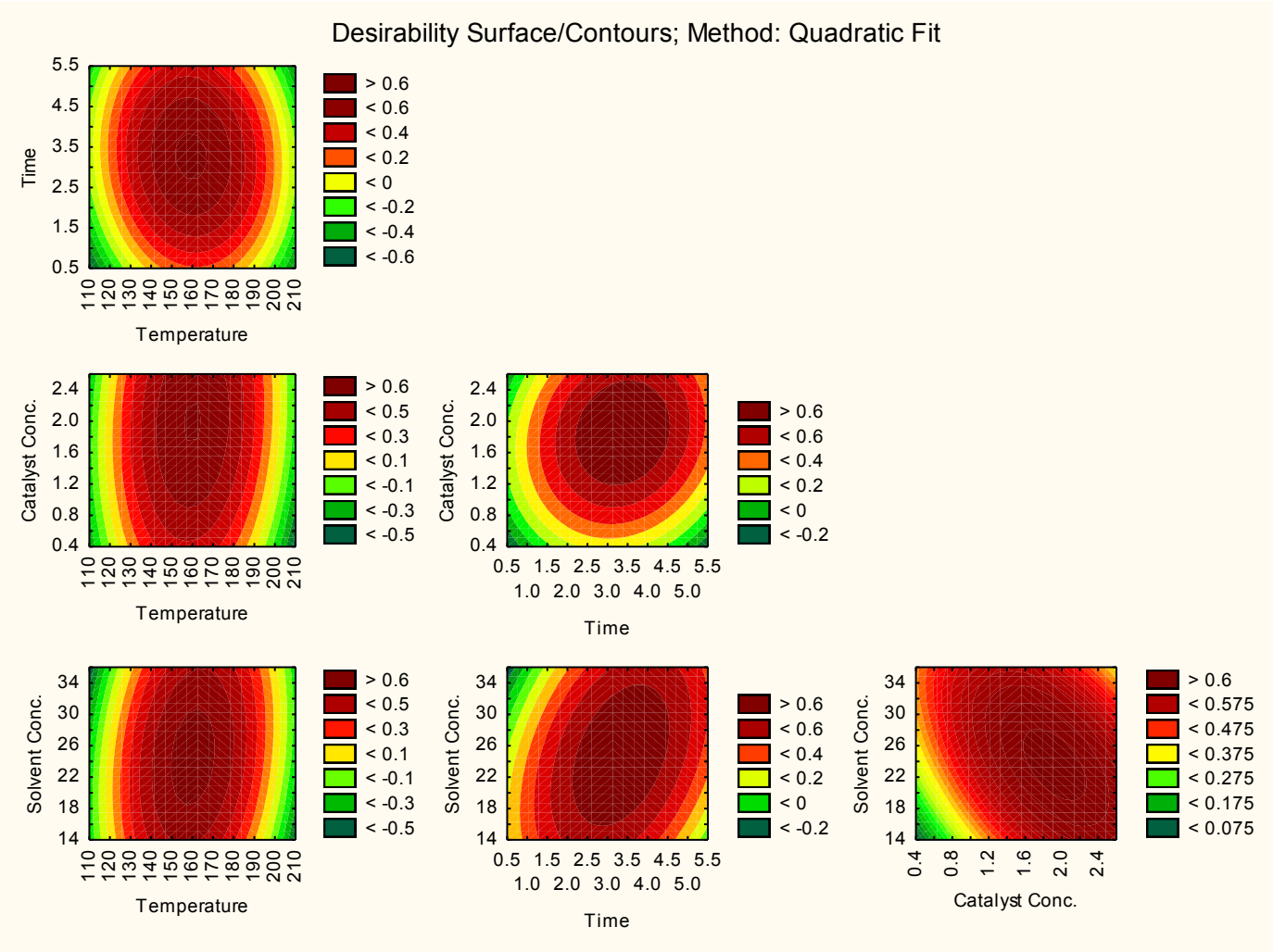

Figure 51: Desirability surface contours- Ethylene glycol EC fractionation

Table 83: Factor levels and predicted responses- Eucalyptus Ethylene glycol fractionation

	Factor levels and predicted responses (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in EC Glycol.stw) Predicted responses at each level of each factor holding all other factors constant at their current setting									
Factor	Factor Level	Predictd Solid Yield	Predictd Glc LF	Predictd Xylose LF	Predictd Lignin LF	Predictd Glucose SF	Predictd Xylose SF	Predictd Lignin SF	Predictd EH Efficiency (%)	Desirability Value
Temperature	120.	71.01845	0.032143	0.285714	9.33869	28.94345	8.861310	13.48214	32.96369	0.373673
Temperature	140.	63.90595	0.173810	0.685714	12.15119	26.89762	8.023810	10.81548	48.98452	0.502243
Temperature	160.	56.79345	0.315476	1.085714	14.96369	24.85179	7.186310	8.14881	65.00536	0.576663
Temperature	180.	49.68095	0.457143	1.485714	17.77619	22.80595	6.348810	5.48214	81.02619	0.608119
Temperature	200.	42.56845	0.598810	1.885714	20.58869	20.76012	5.511310	2.81548	97.04702	0.564585
Time	1.	60.01845	0.282143	0.660714	14.51369	27.96845	7.961310	10.40714	51.41369	0.521990
Time	2.	56.57262	0.340476	0.935714	15.60119	26.24762	7.423810	8.76548	61.28452	0.567006
Time	3.	53.12679	0.398810	1.210714	16.68869	24.52679	6.886310	7.12381	71.15536	0.595754
Time	4.	49.68095	0.457143	1.485714	17.77619	22.80595	6.348810	5.48214	81.02619	0.608119
Time	5.	46.23512	0.515476	1.760714	18.86369	21.08512	5.811310	3.84048	90.89702	0.599428
Catalyst Conc.	.5	55.39762	0.457143	0.785714	14.35952	26.32262	8.265476	9.28214	52.90952	0.509304
Catalyst Conc.	1.	53.96845	0.457143	0.960714	15.21369	25.44345	7.786310	8.33214	59.93869	0.541963
Catalyst Conc.	1.5	52.53929	0.457143	1.135714	16.06786	24.56429	7.307143	7.38214	66.96786	0.569215
Catalyst Conc.	2.	51.11012	0.457143	1.310714	16.92202	23.68512	6.827976	6.43214	73.99702	0.591291
Catalyst Conc.	2.5	49.68095	0.457143	1.485714	17.77619	22.80595	6.348810	5.48214	81.02619	0.608119
Solvent Conc.	40.	45.86429	1.057143	2.685714	15.82619	20.62262	6.898810	4.71548	92.50952	0.552273
Solvent Conc.	50.	46.81845	0.907143	2.385714	16.31369	21.16845	6.761310	4.90714	89.63869	0.574262
Solvent Conc.	60.	47.77262	0.757143	2.085714	16.80119	21.71429	6.623810	5.09881	86.76786	0.589729
Solvent Conc.	70.	48.72679	0.607143	1.785714	17.28869	22.26012	6.486310	5.29048	83.89702	0.601120
Solvent Conc.	80.	49.68095	0.457143	1.485714	17.77619	22.80595	6.348810	5.48214	81.02619	0.608119

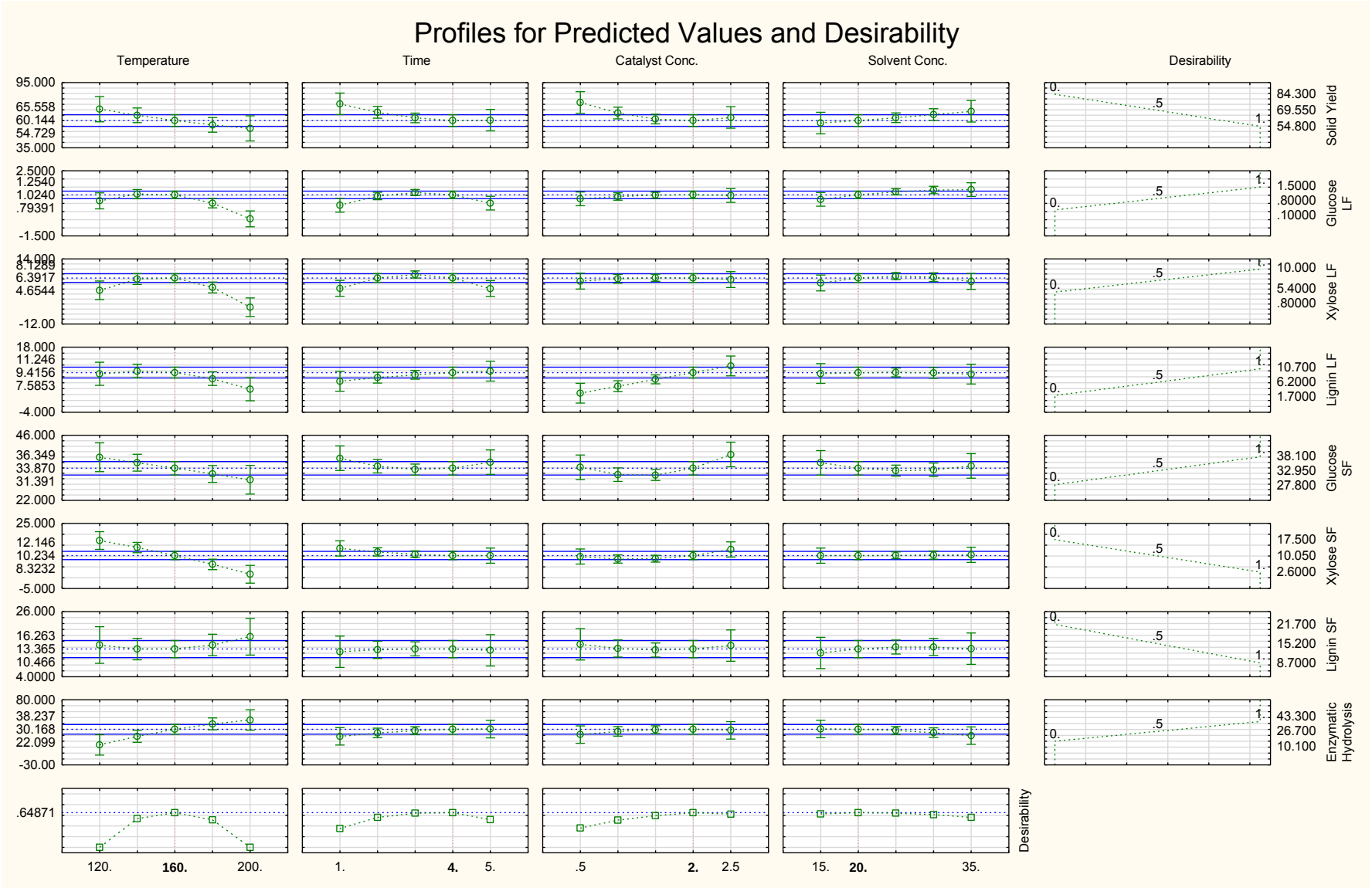

Figure 52: Profile for predicted values and desirability- SCB xylitol fractionation

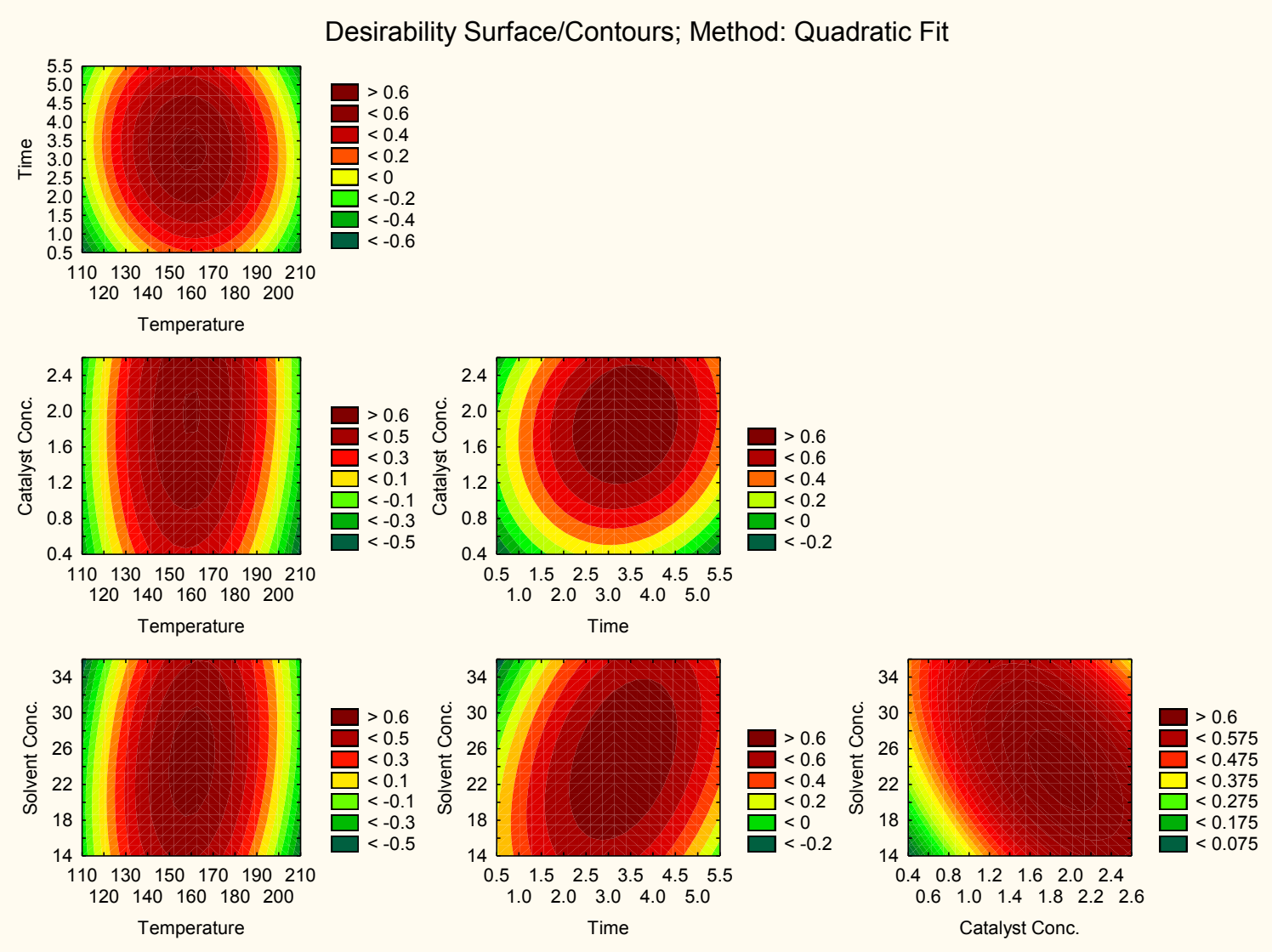

Figure 53: Desirability surface contours- Xylitol SCB fractionation

Table 84: Factor levels and predicted responses- SCB xylitol fractionation

Factor	Factor levels and predicted responses (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in SCB-Xylitol) Predicted responses at each level of each factor holding all other factors constant at their current setting									
	Factor Level	Predictd Solid Yield	Predictd Glucose LF	Predictd Xylose LF	Predictd Lignin LF	Predictd Glucose SF	Predictd Xylose SF	Predictd Lignin SF	Predictd Enzymatic Hydrolysis	Desirbty Value
Temperature	120.	70.56875	0.657292	1.49167	9.02396	37.93646	17.12604	14.74792	3.87604	0.000000
Temperature	140.	64.95833	1.070833	6.01250	9.97083	35.92500	13.90000	13.36250	18.33750	0.539527
Temperature	160.	60.14375	1.023958	6.39167	9.41563	33.86979	10.23438	13.36458	30.16771	0.648706
Temperature	180.	56.12500	0.516667	2.62917	7.35833	31.77083	6.12917	14.75417	39.36667	0.514677
Temperature	200.	52.90208	-0.451042	-5.27500	3.79896	29.62813	1.58438	17.53125	45.93437	0.000000
Time	1.	75.51250	0.383333	2.27917	6.48750	37.59167	13.51250	12.44583	18.22083	0.350621
Time	2.	67.66875	0.957292	6.44167	7.71563	34.61979	11.90938	13.11458	24.00938	0.560395
Time	3.	62.54583	1.170833	7.81250	8.69167	33.37917	10.81667	13.42083	27.99167	0.639152
Time	4.	60.14375	1.023958	6.39167	9.41563	33.86979	10.23438	13.36458	30.16771	0.648706
Time	5.	60.46250	0.516667	2.17917	9.88750	36.09167	10.16250	12.94583	30.53750	0.520530
Catalyst Conc.	.5	76.77500	0.783333	5.15417	2.47500	34.24167	9.76250	14.97083	21.29583	0.360877
Catalyst Conc.	1.	67.03542	0.923958	6.08333	4.81563	31.51146	8.60938	13.57292	26.60937	0.510001
Catalyst Conc.	1.5	61.49167	1.004167	6.49583	7.12917	31.38750	8.76667	13.03750	29.56667	0.590958
Catalyst Conc.	2.	60.14375	1.023958	6.39167	9.41563	33.86979	10.23438	13.36458	30.16771	0.648706
Catalyst Conc.	2.5	62.99167	0.983333	5.77083	11.67500	38.95833	13.01250	14.55417	28.41250	0.622283
Solvent Conc.	15.	57.79583	0.745833	4.40417	9.12500	35.85417	10.15000	12.04583	30.73333	0.626874
Solvent Conc.	20.	60.14375	1.023958	6.39167	9.41563	33.86979	10.23438	13.36458	30.16771	0.648706
Solvent Conc.	25.	62.73750	1.216667	7.16250	9.47917	33.01667	10.32917	14.04583	28.09583	0.637003
Solvent Conc.	30.	65.57708	1.323958	6.71667	9.31563	33.29479	10.43438	14.08958	24.51771	0.610985
Solvent Conc.	35.	68.66250	1.345833	5.05417	8.92500	34.70417	10.55000	13.49583	19.43333	0.560299

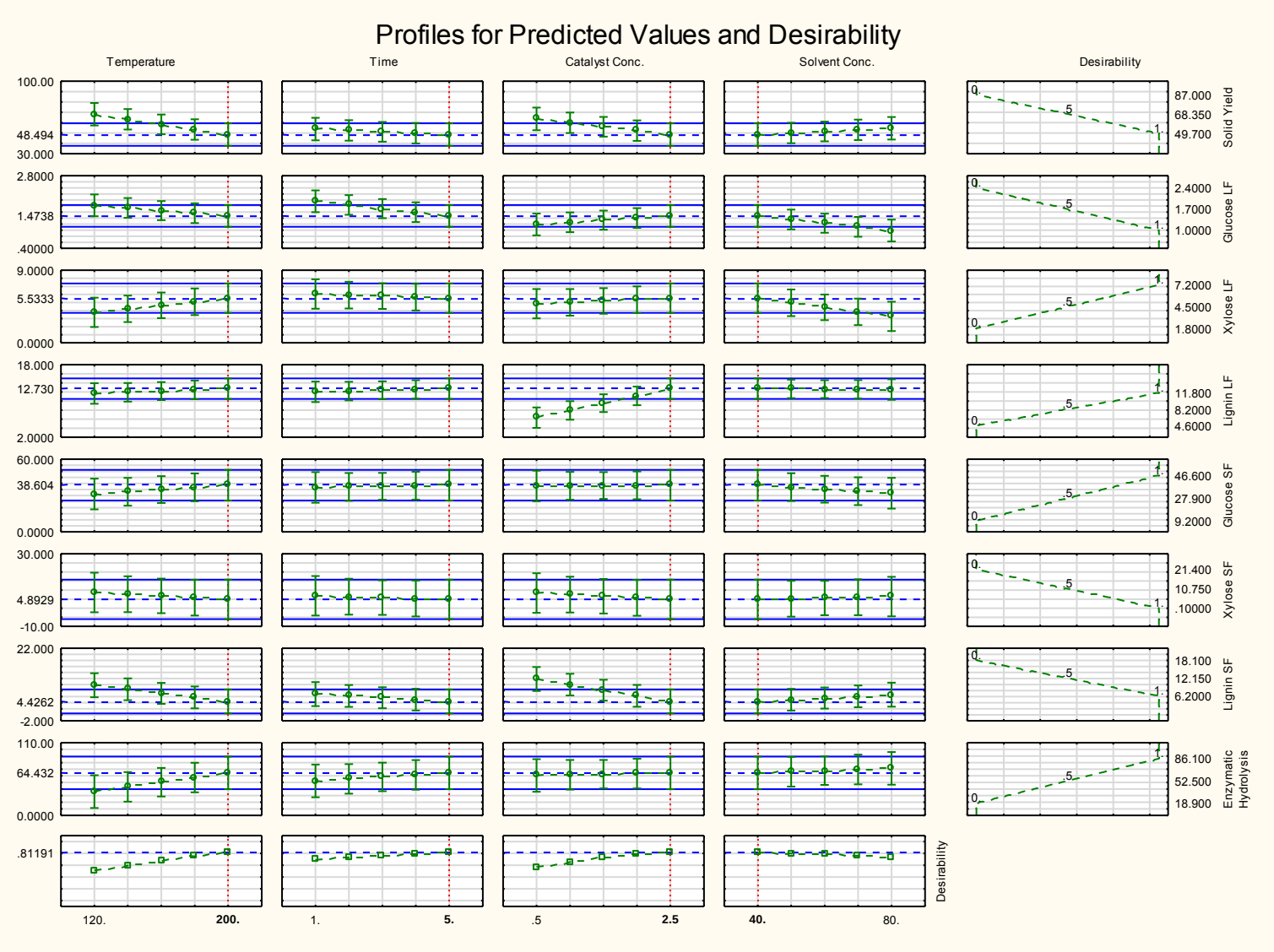

Figure 54: Profile for predicted values and desirability for SCB-EG fractionation

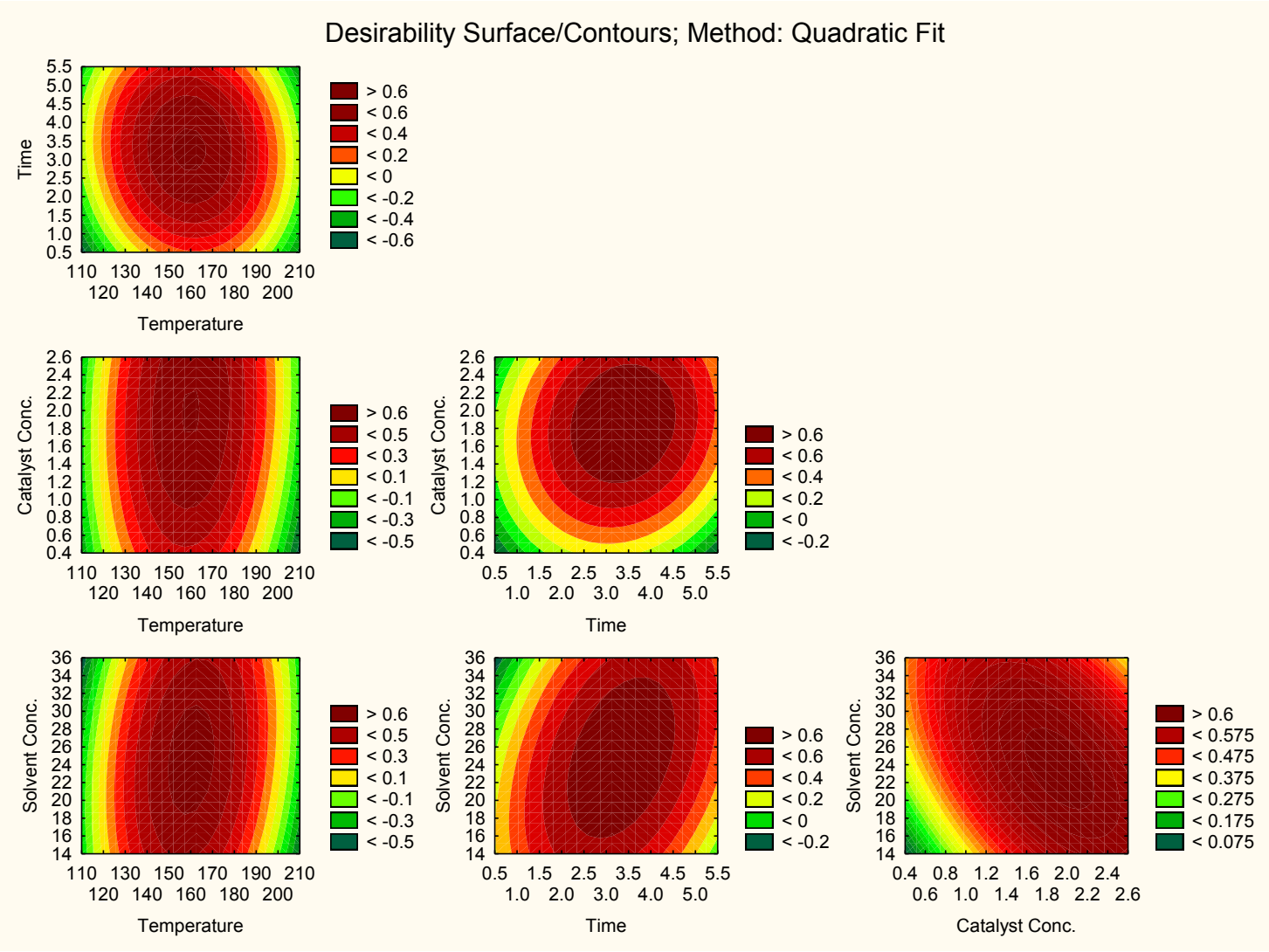

Figure 55: Desirability surface contours- Ethylene glycol EC fractionation

Table 85: Factor levels and predicted responses- SCB Ethylene glycol fractionation

Factor	Factor levels and predicted responses (2**(4) central composite, nc=16 ns=8 n0=2 Runs=26 ([No active dataset]) in SCB Glycol) Predicted responses at each level of each factor holding all other factors constant at their current setting									
	Factor Level	Predicted Solid Yield	Predicted Glucose LF	Predicted Xylose LF	Predicted Lignin LF	Predicted Glucose SF	Predicted Xylose SF	Predicted Lignin SF	Predicted Enzymatic Hydrolysis	Desirability Value
Temperature	120.	68.16071	1.823810	3.783333	11.66310	31.28690	8.692857	9.77619	36.13214	0.512144
Temperature	140.	63.24405	1.736310	4.220833	11.92976	33.11607	7.742857	8.43869	43.20714	0.597744
Temperature	160.	58.32738	1.648810	4.658333	12.19643	34.94524	6.792857	7.10119	50.28214	0.677685
Temperature	180.	53.41071	1.561310	5.095833	12.46310	36.77440	5.842857	5.76369	57.35714	0.751262
Temperature	200.	48.49405	1.473810	5.533333	12.72976	38.60357	4.892857	4.42619	64.43214	0.811906
Time	1.	53.82738	1.957143	6.050000	12.02976	36.72024	6.859524	6.84286	52.29881	0.691517
Time	2.	52.49405	1.836310	5.920833	12.20476	37.19107	6.367857	6.23869	55.33214	0.730546
Time	3.	51.16071	1.715476	5.791667	12.37976	37.66190	5.876190	5.63452	58.36548	0.761538
Time	4.	49.82738	1.594643	5.662500	12.55476	38.13274	5.384524	5.03036	61.39881	0.789436
Time	5.	48.49405	1.473810	5.533333	12.72976	38.60357	4.892857	4.42619	64.43214	0.811906
Catalyst Conc.	.5	63.66071	1.190476	4.850000	6.32976	37.95357	8.392857	11.84286	60.56548	0.570539
Catalyst Conc.	1.	59.86905	1.261310	5.020833	7.92976	38.11607	7.517857	9.98869	61.53214	0.658974
Catalyst Conc.	1.5	56.07738	1.332143	5.191667	9.52976	38.27857	6.642857	8.13452	62.49881	0.728796
Catalyst Conc.	2.	52.28571	1.402976	5.362500	11.12976	38.44107	5.767857	6.28036	63.46548	0.788803
Catalyst Conc.	2.5	48.49405	1.473810	5.533333	12.72976	38.60357	4.892857	4.42619	64.43214	0.811906
Solvent Conc.	40.	48.49405	1.473810	5.533333	12.72976	38.60357	4.892857	4.42619	64.43214	0.811906
Solvent Conc.	50.	50.01071	1.352976	4.970833	12.67976	36.94107	5.276190	4.98869	66.13214	0.802413
Solvent Conc.	60.	51.52738	1.232143	4.408333	12.62976	35.27857	5.659524	5.55119	67.83214	0.784714
Solvent Conc.	70.	53.04405	1.111310	3.845833	12.57976	33.61607	6.042857	6.11369	69.53214	0.761092
Solvent Conc.	80.	54.56071	0.990476	3.283333	12.52976	31.95357	6.426190	6.67619	71.23214	0.725062

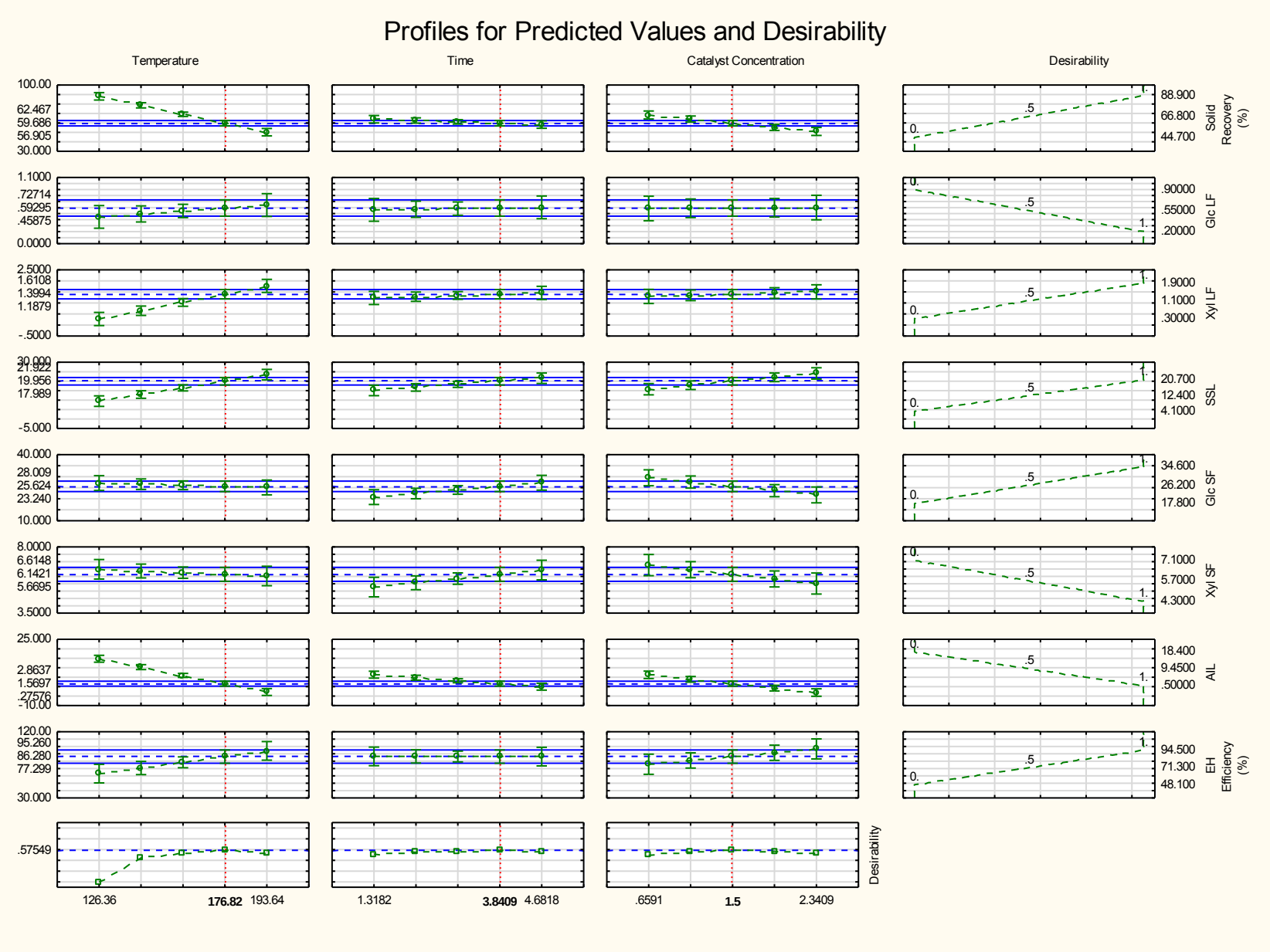

Figure 56: Profile for predicted values and desirability for Post hemicellulose-extracted eucalyptus EG fractionation

Table 86: Factor levels and predicted responses -Post hemicellulose-extracted eucalyptus EG fractionation

Factor	Factor levels and predicted responses (2**(3) central composite, nc=8 ns=6 n0=2 Runs=16 ([No active dataset]) in E Grandis Post hemis Extraction.stw) Predicted responses at each level of each factor holding all other factors constant at their current setting									
	Factor Level	Predicted Solid Recovery (%)	Predicted Glc LF	Predicted Xyl LF	Predicted SSL	Predicted Glc SF	Predicted Xyl SF	Predicted AIL	Predicted EH Efficiency (%)	Desirability Value
Temperature	126.36	88.08239	0.445172	0.274253	9.47532	27.10635	6.481360	14.71917	63.03613	0.000000
Temperature	143.18	78.61696	0.494430	0.649287	12.96875	26.61236	6.368290	10.33602	70.78396	0.445995
Temperature	160.	69.15153	0.543689	1.024321	16.46217	26.11837	6.255219	5.95287	78.53180	0.532530
Temperature	176.82	59.68610	0.592947	1.399356	19.95559	25.62439	6.142149	1.56973	86.27964	0.575491
Temperature	193.64	50.22067	0.642206	1.774390	23.44901	25.13040	6.029078	-2.81342	94.02748	0.537838
Time	1.3182	63.96484	0.561881	1.226392	15.08575	20.71927	5.276491	6.31110	86.56757	0.517091
Time	2.1591	62.53859	0.572237	1.284046	16.70903	22.35431	5.565044	4.73064	86.47159	0.549273
Time	3.	61.11235	0.582592	1.341701	18.33231	23.98935	5.853596	3.15019	86.37562	0.567988
Time	3.8409	59.68610	0.592947	1.399356	19.95559	25.62439	6.142149	1.56973	86.27964	0.575491
Time	4.6818	58.25985	0.603303	1.457010	21.57887	27.25943	6.430701	-0.01073	86.18367	0.565019
Catalyst Concentration	.6591	68.40538	0.585110	1.296920	15.77704	29.50573	6.767394	6.24702	75.82734	0.494073
Catalyst Concentration	1.0796	64.04574	0.589029	1.348138	17.86631	27.56506	6.454772	3.90837	81.05349	0.548019
Catalyst Concentration	1.5	59.68610	0.592947	1.399356	19.95559	25.62439	6.142149	1.56973	86.27964	0.575491
Catalyst Concentration	1.9204	55.32646	0.596866	1.450574	22.04487	23.68372	5.829526	-0.76892	91.50579	0.569914
Catalyst Concentration	2.3409	50.96682	0.600784	1.501791	24.13415	21.74305	5.516903	-3.10757	96.73194	0.528003

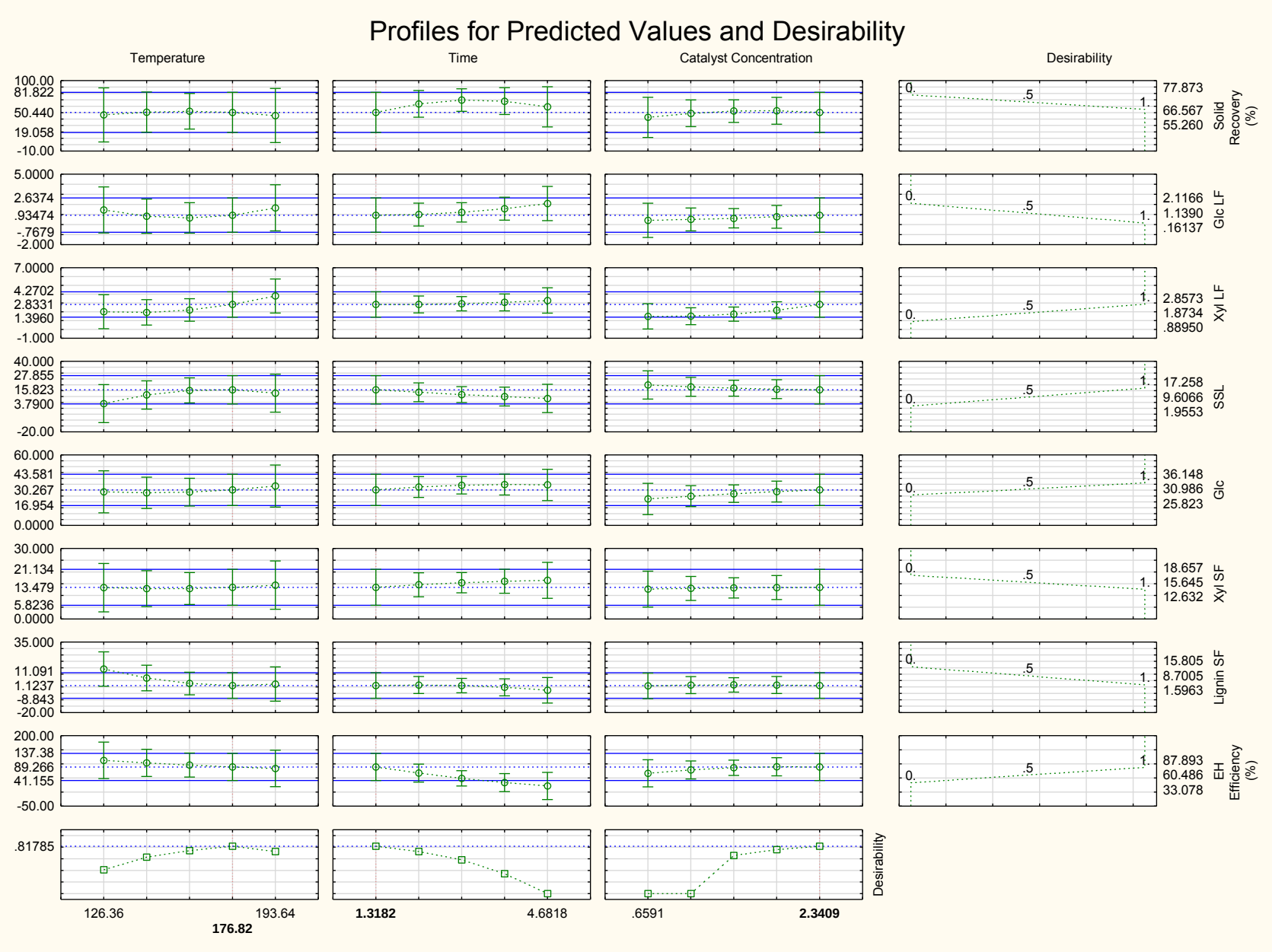

Figure 57: Profile for predicted values and desirability for Post hemicellulose-extracted SCB-EG fractionation

Table 87: Factor levels and predicted responses -Post hemicellulose-extracted SCB-EG fractionation

Factor	Factor levels and predicted responses (2**(3) central composite, nc=8 ns=6 n0=2 Runs=16 ([No active dataset]) in SCB Post hemis Extractions) Predicted responses at each level of each factor holding all other factors constant at their current setting									
	Factor Level	Predicted Solid Recovery (%)	Predicted Glc LF	Predicted Xyl LF	Predicted SSL	Predicted Glc	Predicted Xyl SF	Predicted Lignin SF	Predicted EH Efficiency (%)	Desirability Value
Temperature	126.36	46.44656	1.446513	2.018367	4.03978	28.61013	13.33264	13.99824	112.5467	0.410883
Temperature	143.18	50.86105	0.829536	1.942319	11.38201	27.79060	12.92589	6.97264	103.6525	0.628833
Temperature	160.	52.19228	0.658945	2.213898	15.30962	28.34306	12.97468	2.68114	95.8921	0.743232
Temperature	176.82	50.44026	0.934738	2.833101	15.82260	30.26749	13.47902	1.12375	89.2656	0.817849
Temperature	193.64	45.60499	1.656916	3.799931	12.92097	33.56389	14.43889	2.30046	83.7729	0.726654
Time	1.3182	50.44026	0.934738	2.833101	15.82260	30.26749	13.47902	1.12375	89.2656	0.817849
Time	2.1591	63.73045	0.994920	2.841035	13.69480	32.71177	14.58385	1.54284	67.2860	0.725605
Time	3.	69.59872	1.207403	2.919852	11.75781	34.22910	15.44758	1.07060	48.7057	0.583715
Time	3.8409	68.04506	1.572189	3.069552	10.01164	34.81949	16.07022	-0.29297	33.5245	0.343533
Time	4.6818	59.06948	2.089276	3.290134	8.45627	34.48293	16.45176	-2.54786	21.7424	0.000000
Catalyst Concentration	.6591	42.67509	0.420843	1.487379	19.89513	22.50360	12.73191	0.93039	66.5554	0.000000
Catalyst Concentration	1.0796	49.02728	0.509042	1.509760	18.36817	24.95627	13.02904	1.42833	78.4630	0.000000
Catalyst Concentration	1.5	52.43886	0.624091	1.741507	17.18043	27.06781	13.25260	1.62653	86.2172	0.656272
Catalyst Concentration	1.9204	52.90986	0.765990	2.182621	16.33191	28.83822	13.40259	1.52501	89.8181	0.757687
Catalyst Concentration	2.3409	50.44026	0.934738	2.833101	15.82260	30.26749	13.47902	1.12375	89.2656	0.817849