

Deconstruction of Biomass in Ionic Liquids: Reactivity of Cellulose

**Thesis submitted as partial fulfilment of the requirements for
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Declaration of Originality

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This thesis is dedicated to my beloved parents,

Mr Shikh Zahari Shikh Sulaiman and Mrs Rahmah Abdul Ghani

Abstract

The reactivity of cellulose in alkyimidazolium hydrogen sulfate-water ($[\text{C}_n\text{C}_{\text{m}}\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$) mixtures during a biomass deconstruction process at 120 °C was investigated. Two types of sample, *Miscanthus* and a model polymer cellulose, Microcrystalline Sigmacell-Cellulose (MCC), were used. The studied variables included: $[\text{HSO}_4]$ -ionic liquids with different acidities, 1-butylimidazolium hydrogen sulfate, $[\text{HC}_4\text{im}][\text{HSO}_4]$, and 1-butyl-3-methylimidazolium hydrogen sulfate, $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$; acid-to-water ($[\text{C}_n\text{C}_{\text{m}}\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$) ratio, and incubation period. A number of analysis tools and chemical methods were employed to characterise the resultant cellulose products: Scanning Electron Microscopy and Energy Dispersed X-Ray (SEM-EDX), Infrared Spectroscopy, Matrix Assisted Laser Desorption/Ionisation with Time of Flight (MALDI-TOF) Mass Spectroscopy, CHNS elemental analysis, viscosity measurement, compositional analysis and enzymatic saccharification.

Deconstruction of *Miscanthus* in a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ mixture at 120 °C for 22 h successfully separated cellulose, hemicellulose and lignin. A study on the purification of cellulose sample found that inadequate washing allowed the $[\text{HC}_4\text{im}][\text{HSO}_4]$ traces to be physically adsorbed. After an extensive washing, indirect evidence, indicating that $[\text{HSO}_4]^-$ anions had chemically adsorbed, was revealed.

An investigation involving incubation of MCC in $[\text{C}_n\text{C}_{\text{m}}\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ mixtures at 120 °C was conducted, replicating the deconstruction process. MALDI-TOF analysis demonstrated that the “ $[\text{HSO}_4]$ ” anion had chemically adsorbed on the surface of cellulose, forming sulfur-containing oligosaccharides. However, the type of bond responsible for chemisorption could not be identified. The $[\text{HSO}_4]^-$ anion was the active species for chemisorption, regardless of different acidities of ionic liquids.

Incubating MCC in $[\text{C}_n\text{C}_{\text{m}}\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ mixtures at 120 °C also exhibited an interesting interplay between chemisorption and depolymerisation. A positive relationship was predominant in the presence of lower water content. Increasing water content displayed a negative relationship.

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Abbreviations

AICL	Acid insoluble compositional lignin
ASCL	Acid soluble compositional lignin
CRM	Carbohydrate-rich-material
MCC	Microcrystalline Sigmacell-Cellulose
5-HMF	5-Hydroxymethylfurfural
BG	β -Glucosidase enzyme
CBH	Cellobiohydrolase enzyme
EG	Endoglucanase enzyme
CUEN	Bis(ethylenediamine) copper(II) hydroxide solution
DMSO	Dimethyl sulfoxide
ATR-IR	Attenuated total reflectance - infrared spectroscopy
CHNS	Carbon, hydrogen, nitrogen and sulfur
CGA	Combustion gas analysis
GPC	Gel permeation chromatography
EDX	Energy dispersed x-ray
GC	Gas chromatography
LSIMS	liquid secondary ion mass spectrometry
NMR	Nucleus magnetic resonance
MALLS	Multiangle laser light scattering
MALDI-TOF	Matrix assisted laser desorption ionisation – time of flight
RI	Refractive index
SEM	Scanning electron microscope
TCD	Thermal conductivity detector
UV-VIS	Ultraviolet-visible spectrophotometry
XPS	X-ray photoelectron
DP	Degree of polymerisation

H_0	Hammett acidity value
keV	Kilo electrovolt
M_n	Average molecular weight
M_w	Weight average molecular weight
MPa	Mega Pascal
PI	Polydispersity index
r.p.m	Revolutions per minute
ΔT	Observed supercooling temperature
T_g	Glass transition temperature
T_{mp}	Melting point
T_{fp}	Freezing point
v/v%	Volume to volume percentage
wt%	Weight percentage
ϵ	Kinematic viscosity
S	Number of chain scissions
η_{rel}	Relative viscosity
$[\eta]$	Intrinsic viscosity

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1 . Introduction

1.1 General Introduction

To date, the production of energy and fuels has been heavily relying on fossil fuels. However, it is clear that the abundance of these substances is currently dwindling. Additionally, the consumption of fossil fuels has sparked serious concern amongst environmentalists due to the fact that they are mainly responsible for high carbon emissions and pollution.

Biomass is seen as a future feedstock to cope with our heavy dependence on fossil fuels. It is classified as a carbon-neutral substance that can potentially reduce greenhouse effects gasses and atmospheric pollution.^{1,2} More importantly, this feedstock is sustainable. There are two classifications of biomass: first generation and second generation. Agricultural crops are amongst the first generation of biomass, and the current sources employed for deriving biofuels. However, competition with food staples is a commonly-encountered issue regarding this feedstock.² As an alternative, lignocellulose, the second generation of biomass, is now being investigated. This feedstock offer several advantages over the first generation biomass, they include:

1. High yields per unit area of land as compared to starch or vegetable oil materials.³
2. Abundance in nature, less competition with food supply.⁴
3. Capability of grow on less desirable land with less maintenance and inputs required.³

Lignocellulose may benefit the bioethanol production industry, as lignocellulose is capable of generating 0.12-0.30 m³ of bioethanol per ton of dry lignocellulose, slightly higher than the production using agricultural crops.⁴

Converting biomass to end products remains a challenging task. This is because biomass must undergo deconstruction, separating cellulose, hemicellulose and lignin. A number of deconstruction methods are currently being employed to separate the biopolymer. **Figure 1- 1** illustrates a flow chart describing the process of converting biomass to several potential end products.

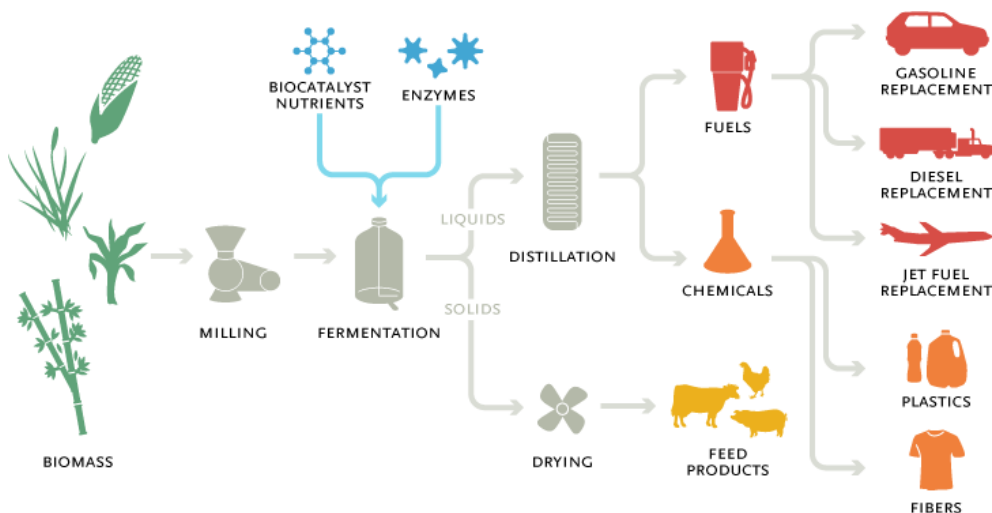


Figure 1- 1. Derivation of potential end products from biomass.

Ionic liquids are liquids consisting almost or exclusively of ions. Applications of ionic liquids cover a wide range of chemical processes, and the reasons to boost the popularity of these liquids include:

1. They do not evaporate under normal conditions, unlike molecular organic solvents
2. They non-flammable and thermally stable at temperatures higher than conventional organic molecular solvents

Ionic liquids can potentially replace organic solvents known to be toxic and flammable, minimizing chemical waste and pollution. Applications of ionic liquids include metal plating, electropolishing, extracting metals from ores, separation and extraction of chemicals from aqueous and molecular organic solvents.³ Deconstruction of biomass in ionic liquids is currently an area of great interest. It promises higher separation efficiency of biopolymers, increasing people's interest in performing more in depth investigations.^{1,3-8}

1.2 Overview of Thesis

The central objective of this thesis is to examine interactions between cellulose and alkylimidazolium hydrogen sulfate, $[C_nC_mim][HSO_4]$, ionic liquids from the perspective of the deconstruction of biomass process. The thesis contains six chapters, and it is organised as follows:

Chapter 1, *Introduction*, introduces biomass and ionic liquids. It also reviews biomass deconstruction processes, covering current approaches being employed as well as typical assessments to examine the efficiency of separating the biopolymers, cellulose, hemicellulose and lignin.

This chapter also highlights the reactivity of cellulose, the main constituent of biomass, towards a number of chemical reactions. It also describes fundamental understanding and limitations of several analysis tools or methods commonly employed to characterise cellulose products.

Chapter 2, *Methodology*, is divided into three sections. The synthesis of $[C_nC_mim][HSO_4]$ ionic liquids with their chemical identifications is described in the first section. Second section provides detailed procedures on deconstruction of *Miscanthus* and treatment of Microcrystalline Sigmacell-Cellulose (MCC) in the prepared ionic liquids. Third section lists several analyses, and the methodologies used for characterisation of obtained cellulose products.

Chapter 3, *Understanding the Deconstruction Process*, focuses on understanding the entire process of deconstruction of *Miscanthus* in the ionic liquid 1-butylimidazolium hydrogen sulfate, $[HC_4im][HSO_4]$, by providing some explanations of both technical and scientific aspects. It also examines adsorption phenomena between the $[HC_4im][HSO_4]$ and a cellulose-rich-fraction (CRM), which was extracted from *Miscanthus* following the deconstruction process.

Chapter 4, *Understanding the Nature of Adsorption*, concentrates on further examining the adsorption phenomenon using MCC, a model polymer representing cellulose in *Miscanthus*. It also searches for the best analytical tool to identify the formation of sulfur-containing oligosaccharide; an

indicator of the $[C_nC_m\text{im}][\text{HSO}_4]$ chemically adsorbing onto the surface of cellulose.

Chapter 5, *Chemisorption and Depolymerisation*, investigates the relationship between chemisorption and depolymerisation of cellulose in the ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate, $[C_4C_1\text{im}][\text{HSO}_4]$. Several parameters affecting the relationship are also demonstrated in this chapter.

Chapter 6, *Future Work*, proposes potential future work for improvement.

1.3 Structures of Biomass

1.3.1 Carbohydrates – Lignin Complex

Biomass generally contains three polymers: cellulose, hemicellulose and lignin. **Figure 1- 2** illustrates how these polymers are connected.⁹

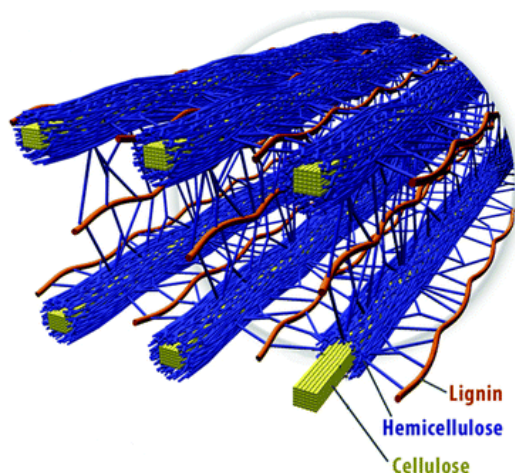


Figure 1- 2. Cellulose-hemicellulose-lignin linkages.⁹

In **Figure 1- 2** it can be seen that cellulose is covered by a lignin-hemicellulose complex lignin-carbohydrate polymeric structure (hemicellulose and arabinose), where lignin is linked to hemicellulose by ether and ester bonds.⁵ In grasses, the lignin-hemicellulose complex is mediated by ferulic acid (as shown in **Figure 1- 3**).⁹ The complex acts as a sheath protecting cellulose from chemical and physical actions.¹⁰

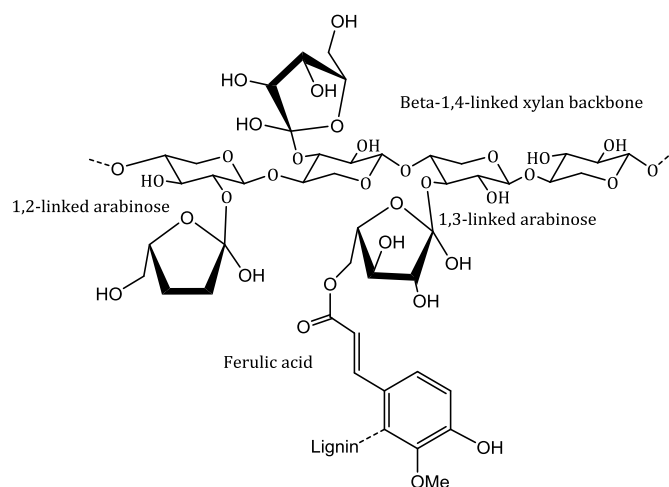


Figure 1- 3. Lignin-carbohydrate complexes mediated by ferulic acid in grasses.⁹

1.3.2 Composition of Biomass

The exact composition of biomass relies on a number of factors: the species, the plant tissue and the growing conditions.⁹ **Table 1- 1** compares the composition of typical biomass species used in bio-refinery applications.

Table 1- 1. Composition of some biomass feedstocks.

Source	Cellulose (wt %)	Hemicellulose (wt %)	Lignin (wt %)	Reference
<i>Miscanthus</i>	41-44	24-25	26-28	3,5,11
Nut shells	25-30	25-30	30-40	12
Corn cobs	45	35	15	12
Wheat straw	30	50	15	12

Miscanthus, a species of perennial grasses, offers several advantages than other biomass species: considerably long life time (10-15 years) and ability to grow to 4 m high.¹¹ *Miscanthus* has a higher cellulose to lignin ratio compared to other species (**Table 1- 1**).

1.3.3 Linkages within Biomass

Cellulose, hemicellulose and lignin are connected either by intra- or interpolymer linkages. **Table 1- 2** provides an overview of related linkages found in biomass.¹²

Table 1- 2. Linkages involved between lignocellulose polymers.¹²

Bonds within different components (intrapolymer linkages)	
Ether bond	Lignin, hemicellulose, cellulose
Carbon to carbon	Lignin
H-bond	Cellulose
Ester bond	Hemicellulose
Bonds connecting different components (interpolymer linkages)	
Ether bond	Cellulose-Lignin
	Hemicellulose-hemicellulose, lignin-lignin
Ester bond	Hemicellulose-Lignin
H- bond	Cellulose-hemicellulose
	Hemicellulose-Lignin
	Cellulose-Lignin

1.3.4 Structure of Biopolymers

Cellulose

Connections involving β -D-glucopyranoside units through β -1-4 glycosidic bonds form the cellulose polymer as shown in **Figure 1- 4**.¹³

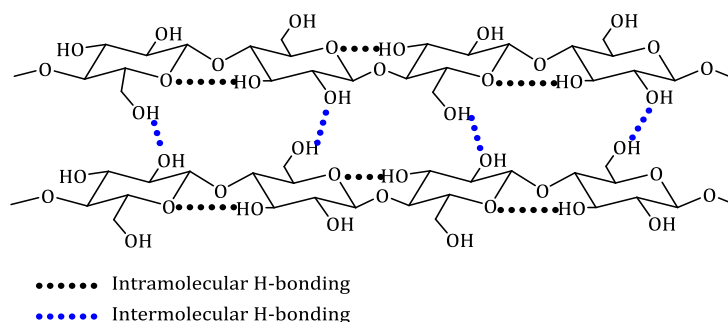


Figure 1- 4. Cellulose polymer.¹³

H-bonding also occurs within the cellulose (**Table 1- 2**, **Figure 1- 4**). It is formed as a result of particular hydroxyl groups, on one glucose unit, interacting with an oxygen on the same or neighbouring units (**Figure 1- 4**).¹⁴ The presence of intra and intermolecular H-bonding holds the cellulose chains firmly, increasing the crystallinity of cellulose. Subsequently, microfibrils are formed (**Figure 1- 5**), comprising amorphous and crystalline regions with higher tensile strength.¹⁴ This explains the low solubility of cellulose in water and many solvents. It has been reported that starch becomes amorphous when heated in water at 60-70 °C, whilst cellulose requires a temperature of 320 °C and a pressure of 25 MPa to do so.¹⁴ The cellulose microfibrils wind around one another to form cables or macrofibrils. **Figure 1- 5** shows the overall structures of cellulose.¹⁶

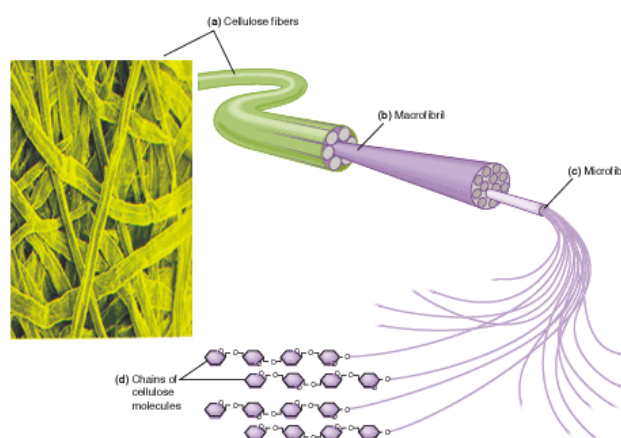


Figure 1- 5. Microfibril, macrofibril and fiber structures in cellulose.¹⁶

Hemicellulose

Hemicellulose consists of various constituents: arabino-xylans, gluco-mannans, and galactans. These constituents are derived from a number of monomers: D-glucose, D-Mannose, D-Galactose, D-Xylose and L-arabinose (chemical structures are shown in **Figure 1- 6**).⁹

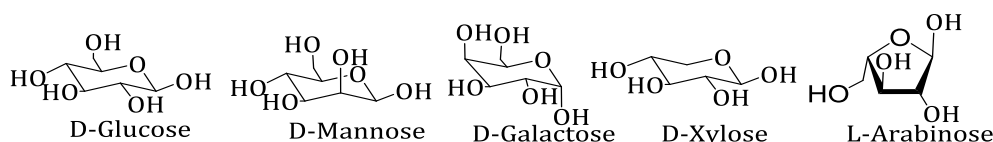


Figure 1- 6. Monomers of hemicellulose.⁹

The hemicellulose's constituents and their monomers are connected together by hydrogen and ether bonds (**Table 1- 2**). In addition, hemicellulose comprises shorter and amorphous branches, making the polymer partially soluble in water.¹⁷

Lignin

Lignin is the most complex polymer in lignocellulose. It is made up of a few units of phenylpropene derivatives, namely p-coumaryl, coniferyl and sinapyl alcohols. **Figure 1- 7** shows the structures of these units.¹²

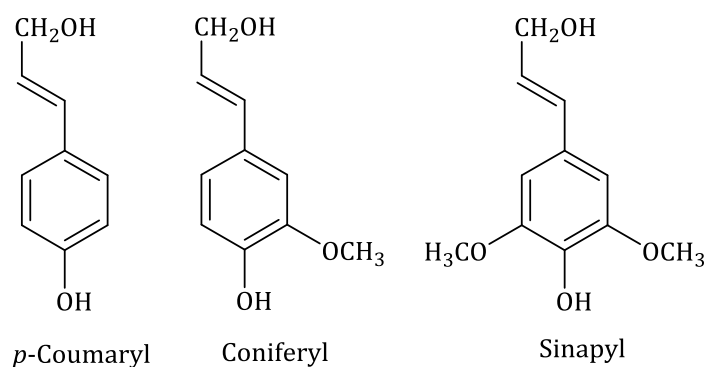


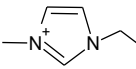
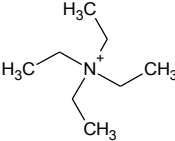
Figure 1- 7. Chemical structures of lignin monomers.¹²

Lignin is a high polydispersity polymer with different branching and bonding; this makes the polymer insoluble in water. It is highly resistant to impact, compression and binding; this enhances the durability of plant cells.¹²

1.4 Ionic Liquids

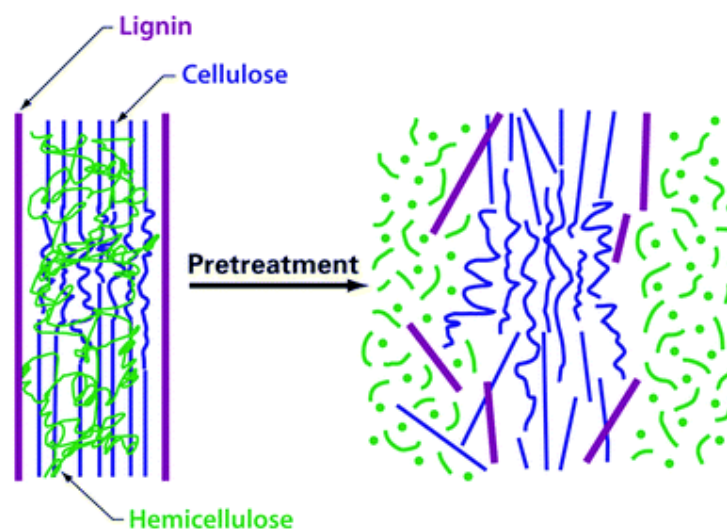
The superior thermal properties of alkyl- and dialkylimidazolium ionic liquids have increased interest in using these compounds for various applications, such as the deconstruction of biomass. Unlike tetraalkylammonium and tetraalkylphosphonium salts, which are commonly high-melting point solids, many alkyl- and dialkyl imidazolium ionic liquids have lower melting and freezing points. A comparison of the melting and freezing points of dialkylimidazolium and a tetraalkylammonium ionic liquids is summarized in **Table 1-3**.¹⁸ The symmetry property of ions influences the thermal properties of ionic liquids.¹⁸⁻²⁰ The melting point decreases as the cation becomes more asymmetrical (**Table 1-3**). In addition, an increase in the size of cation (**Table 1-3**), increases the inter-ion separation, weakening the columbic interaction, eventually suppressing the melting point.¹⁹ There are several other reasons causing alkyl- and dialkylimidazolium ionic liquids to be liquids at room temperature including the delocalized nature of the charge reducing the columbic interactions. The observed supercooling, ΔT for the 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, $[\text{C}_2\text{C}_1\text{im}][\text{NTf}_2]$, is larger than that of the tetraethylammonium bis(trifluoromethylsulfonyl)imide, $[\text{N}_{2222}][\text{NTf}_2]$ ionic liquids (**Table 1-3**). This is due to glass-transforming behavior which is typical for many ionic liquids.^{18,20} The glass transition temperature, T_g , for many imidazolium ionic liquids was reported to be around -80 to -100 °C.¹⁸ Fredlake *et al.* did observe T_g for imidazolium ionic liquids studied around -50 to -98 °C.²⁰ A comparison study on T_g of ionic liquids containing different cations showed that imidazolium salts had T_g values of around -35 to -80 °C while quaternary ammonium salts exhibited the values of T_g around 87 to 200 °C. This makes the quaternary ammonium salts to be solids at room temperature.²²

Table 1-3. Melting and freezing points of dialkylimidazolium and tetraalkylammonium ionic liquids.

Chemical structure		
Nomenclature	$[C_2C_{1im}][NTf_2]$	$[N_{2222}][NTf_2]$
$T_{mp}(^{\circ}C)$	-15	104
$T_{fp}(^{\circ}C)$	-50	98
ΔT	35	6

1.5 Deconstruction of Biomass

A deconstruction process is conducted to isolate cellulose, hemicellulose and lignin. It is performed prior to subsequent downstream processes, such as enzymatic saccharification and fermentation. **Figure 1- 8** suggests the action of the deconstruction process in isolating polymers in biomass.⁹ To perform this, a number of approaches can be employed, and they include acid hydrolysis (dilute and concentrated acids)²³, Organosolv^{24,25} and ionic liquids.^{3-5,7,26-32}

**Figure 1- 8.** Goal of deconstruction process.⁹

Acid hydrolysis uses either a dilute or a concentrated acid; sulfuric acid (H_2SO_4) is the most preferred selection.²³ However, this approach is preferable only for biomass with low lignin content; this is because lignin is difficult to

remove.³³ The use of H_2SO_4 , on the other hand, arise some concerns, particularly on safety implications.³³

The Organosolv treatment is an alternative to the acid-hydrolysis.^{24,25} A variety of organic solvents are commonly employed, such as alcohols, esters, ketones, glycols, organic acids, phenols, and ethers. To reduce the requirement of high temperatures, a catalyst either an inorganic compound (magnesium chloride, MgCl_2)²⁵ or an acid (H_2SO_4)²⁴ is usually added. Despite being facilitated by a catalyst, the technique still demands high temperatures to achieve an effective separation of polymers.^{24,34}

Deconstruction in Ionic Liquids

Remarkable intrinsic features of ionic liquids promise considerable improvement in a wide range of industrial applications. These features include relatively low viscosity, low vapor pressure, and high thermal as well as chemical stability.³⁴⁻³⁷ Interestingly, chemical properties of ionic liquids can also be tuned to suit various interests, especially in bio-refinery processes.

Deconstruction of biomass in ionic liquids can be accomplished *via* two approaches, the 'Dissolution Process' and the 'Ionosolv Process'. The dissolution process aims at solubilising the entire biomass composite. Alkylimidazolium ionic liquids with strong H-bonding anions, such as chloride, Cl^- ^{1,11}, and acetate $[\text{CH}_3\text{CO}_2]^-$ ^{2,4,7,11,31,38,39}, are most widely studied in this approach.

As an example, Padmanabhan *et al.* treated *Miscanthus* in 1-ethyl-3-methylimidazolium chloride, $[\text{C}_2\text{C}_1\text{im}]\text{Cl}$ at 130 °C for 8 to 10 h.¹¹ However, only 4 wt% of *Miscanthus* particles were dissolved at the end of the process. *Miscanthus* is a species of perennial grasses⁹; therefore, any deconstruction attempts must hydrolyse ester bonds, which connect the carbohydrate-fraction and lignin^{9,11} (as shown in **Figure 1- 3**). To hydrolyse the ester bonds, addition of water is needed. However, the $[\text{C}_2\text{C}_1\text{im}]\text{Cl}$ does not tolerate water, during biomass dissolution, discouraging solubilisation of *Miscanthus*. This is because water can H-bond to the hydroxyl groups of cellulose, or solvate Cl^- anions; this eventually retards Cl^- anions to H-bonding to hydroxyl groups of cellulose.⁸ Meanwhile, poor solubilisation of *Miscanthus* in 1-ethyl-3-methylimidazolium acetate, $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{CO}_2]$, was observed

when the mixture was incubated at 130 °C for 8 to 10 h.¹¹ This could possibly be due to decomposition of $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{CO}_2]$ under applied incubation conditions. According to a thermal decomposition study of Clough *et al.*, $[\text{C}_2\text{C}_1\text{im}][\text{CH}_3\text{CO}_2]$ will decompose when heated at 120 °C or above for lengthened periods.⁴⁰ As regards the technical aspect, the polymer recovery step in the dissolution process is complicated. Two-stages of precipitation step are required. The first step involves adding a mixture of acetone and water with a ratio of 1:1 to treated biomass samples; water precipitates cellulose while acetone dissolves lignin.⁷ Using the right ratio of acetone to water is crucial to avoid cellulose and lignin precipitation at the same time, reducing the separation performance. The second step then deals with precipitation of lignin by evaporating acetone.

The second deconstruction approach, the Ionosolv process, targets (partial) lignin and hemicellulose solubilisation, leaving the cellulose fraction mostly intact. Solubilisation of lignin is the key success of this approach. Pu *et al.* found that ionic liquids with methyl sulfate, $[\text{CH}_3\text{OSO}_3]^-$, anions exhibited the greatest lignin solubility compared to other anions.⁴¹ Brandt *et al.* later tested this concept on real biomass experiments.³ They examined the impact of different anions of 1-butyl-3-methylimidazolium, $[\text{C}_4\text{C}_1\text{im}]^+$, ionic liquids on the removal of lignin from *Miscanthus* at 120 °C for 22 h. In the presence of 20 wt% of water, the effectiveness of the studied anions towards the removal of lignin could be ranked as follows: $[\text{HSO}_4]^- > [\text{CH}_3\text{SO}_3]^- > [\text{CH}_3\text{CO}_2]^- > \text{Cl}^- > [\text{OTf}]^-$.³ It was also found that $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ dissolved up to ca. 90% of the lignin. Unlike the dissolution process, the recovery step in the Ionosolv approach is much simpler. Only EtOH is needed to separate lignin from the intact cellulose fraction.³

1.6 Assessments for Deconstruction of Biomass

The performance of deconstruction trials in separating cellulose, hemicellulose and lignin, is generally assessed through compositional analysis and enzymatic saccharification assays.

1.6.1 Compositional Analysis

Compositional analysis measures the composition of carbohydrates (cellulose and hemicellulose) and lignin in either raw biomass or deconstructed biomass

samples. The analysis was published in 2008 by a group of scientists from National Renewable Energy Laboratory, US.⁴² Ever since, the analysis has been used as an established measure for the effectiveness of deconstruction trials in separating polymers.^{3,9,26-29}

1.6.2 Enzymatic Saccharification Assay

Enzymatic saccharification assays are another assessment to measure the success of deconstruction trials. Unlike diluted acid treatments, the assay guarantees enhancement in the quality of end-products, monomeric sugars, and more importantly zero production of unwanted degradation products, 5-(Hydroxymethyl) furfural, 5-HMF, and furfural. Three enzymes, endoglucanase (EG), exoglucanase/cellobiohydrolase (CBH) and cellobiase/ β -glucosidase (BG), are utilised, and they work synergistically in breaking down cellulose to its glucose monomer (as shown in **Figure 1- 9**).⁴³ Further details on the composition, specificity action of enzymes over carbohydrate substrates and hydrolysis mechanisms are published elsewhere.^{44,45}

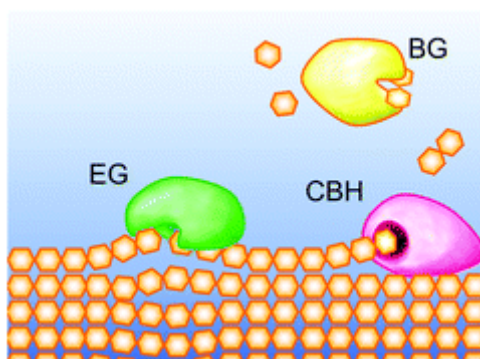


Figure 1- 9. Roles of cellulase and cellobiase in digesting cellulose to glucose.⁴³

However, the assay also has some drawbacks. It is known that there are two major factors affecting the performance of the assay; they are cellulose crystallinity and composition of deconstructed cellulose samples.⁴⁴

Cellulose comprises amorphous and crystalline regions. During the course of the enzymatic saccharification assay, the amorphous regions hydrolyse more rapidly than the crystalline regions. The maximum reaction rate for hydrolysing amorphous cellulose was reported to be 40 times faster than for crystalline cellulose.⁴⁴ Thus, prolonging the period of reaction is needed to

ensure a complete hydrolysis is achieved. Brandt *et al.* performed enzymatic saccharification on deconstructed-cellulose-rich samples following deconstruction trials, at 50 °C for 72 h.³ In addition, the laboratory analytical procedure (LAP) “Enzymatic saccharification of lignocellulosic biomass” (NREL/TP-510-42629) recommends performing the assay at 50 °C for a period of 70 to 168 h.⁴⁶

The second factor, the composition of deconstructed-cellulose-rich samples, is associated with the presence of unseparated lignin and hemicellulose. Lignin is found to be the main inhibitor for the assay. This is because the cellulase and the cellobiase enzymes are also prone to unproductively bind to lignin substrates, impeding the access of the enzymes to cellulose substrates,⁴⁴ eventually reducing the glucose released.⁴⁴ For hemicellulose, factors such as high degree of branching and heterogeneity in substituent groups, allow the enzymes to depolymerise the hemicellulose readily.⁴⁴

It is known that approximately 10-15% (v/v) of ionic liquid residues are typically retained in the deconstructed-cellulose-rich samples following deconstruction trials.⁴⁷ However, such a quantity is enough to impair the enzymes, especially cellulase. Engel *et al.* investigated the cellulase enzyme to ionic liquid over hydrolysis of α -cellulose. Buffered aqueous media, containing cellulase and α -cellulose, were contaminated with various concentrations of the ionic liquid 1,3-dimethylimidazolium dimethyl phosphate, [C₁C₁im][DMP]. The activity of cellulase markedly dropped to zero as the concentration of ionic liquid was increased to more than 30% (v/v).⁴⁷ Other factors, such as type of ionic liquid, pH, viscosity and ionic strength, were also found to have a negative impact on the enzymatic saccharification assay.^{45,47}

1.7 Chemical Reactivity of Cellulose

1.7.1 Recalcitrance towards Dissolution

It is known that most conventional solvents, either polar or non-polar, cannot dissolve cellulose. For non-polar solvents, this occurrence can easily be understood. Each glucose unit contains three free hydroxyl groups: OH-C2, OH-C3 and OH-C6 (**Figure 1- 4, Section 1.3.4**); thus, cellulose forms a

network of H-bonds and is quite polar, rendering it insoluble in any solvent incapable of disrupting these H-bonds.

Despite its good ability to form H-bonds, cellulose is generally insoluble in polar solvents, including water. The general view of the insolubility of cellulose in water is that water molecules are incapable of disrupting strong inter- and intramolecular H-bonding within cellulose. However, this view neglects the fact that cellulose also inherently holds amphiphilic (hydrophilic and lipophilic), and more importantly hydrophobic characters.^{48,49} As emphasised by 'Lindman Hypothesis', these characters have a significant influence on solubility and insolubility behaviours of cellulose in solvents.^{48,49}

Hydrophilic character in cellulose is acquired from OH-C2, OH-C3 and OH-C6 located on the equatorial directions of glucopyranose rings (**Figure 1- 4, Section 1.3.4**).⁴⁹ Hydrophobic character is gained from: (1) hydrogen atoms of C-H groups located on the axial directions of glucopyranose rings,⁴⁹ and; (2) glucopyranose rings that contain only one polar oxygen unit per ring.⁴⁸ The combination of both characters forms strong inter-sheet interactions *via* H-bonding (between OH-C6 and OH-C2) and *van der Waals* attraction (between C-H groups). Interestingly, the hydrophobic aspects are more dominant than hydrophilic ones. This is because although the energy of H-bonding (12-30 kJmole⁻¹) is greater than that of *van der Waals* (0.4-4.0 kJmole⁻¹), there are a lot of C-H groups in cellulose.⁴⁸

A study of Nishiyama *et al.* could be the best example to understand the role of amphiphilic and hydrophobic characters when dissolving cellulose in water.⁵⁰ A computer model of a small cellulose I_β crystal, which contains four-cellulose-chains, in water molecules was simulated, and the outcome is shown in **Figure 1- 10**.

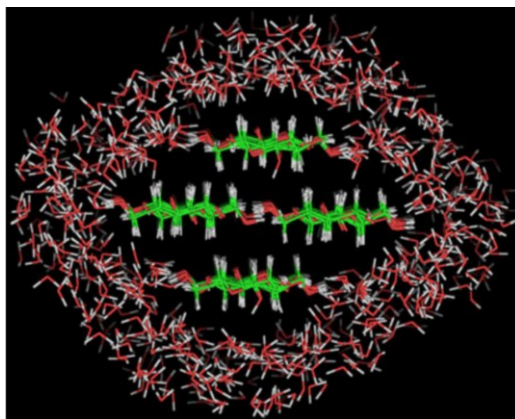


Figure 1- 10. Solvation of a four-cellulose-chain crystal by water molecules.⁵⁰

The simulation gave two interesting observations: (1) only hydroxyl groups at the two ends of each cellulose chain had close contact with water molecules; and (2) substantial gaps were observed between water molecules and C-H groups on the top and bottom of the crystal (**Figure 1- 10**).⁵⁰ From **Figure 1- 10**, it can also be seen that water molecules are not found between the cellulose inter-sheets. This likely explains the role of hydrophobic interactions preventing water molecules from forming H-bonds with the hydroxyl groups on the cellulose chains. Thus, the simulation works of Nishiyama *et al.*⁵⁰ further support the *Lindman Hypothesis*^{48,49} that hydrophobic interactions are the key interactions rendering cellulose crystals recalcitrant towards dissolution in water or any conventional solvents.

1.7.2 Depolymerisation of Cellulose

Cellulose can be depolymerised *via* acid-catalysed hydrolysis of β -1,4-glycosidic bonds, yielding a number of cellulose chains with varying degree of polymerisations (DP). The depolymerisation consists of two mechanisms, A1 which is predominant, and possibly A2 (as shown in **Figure 1- 11**).⁵¹

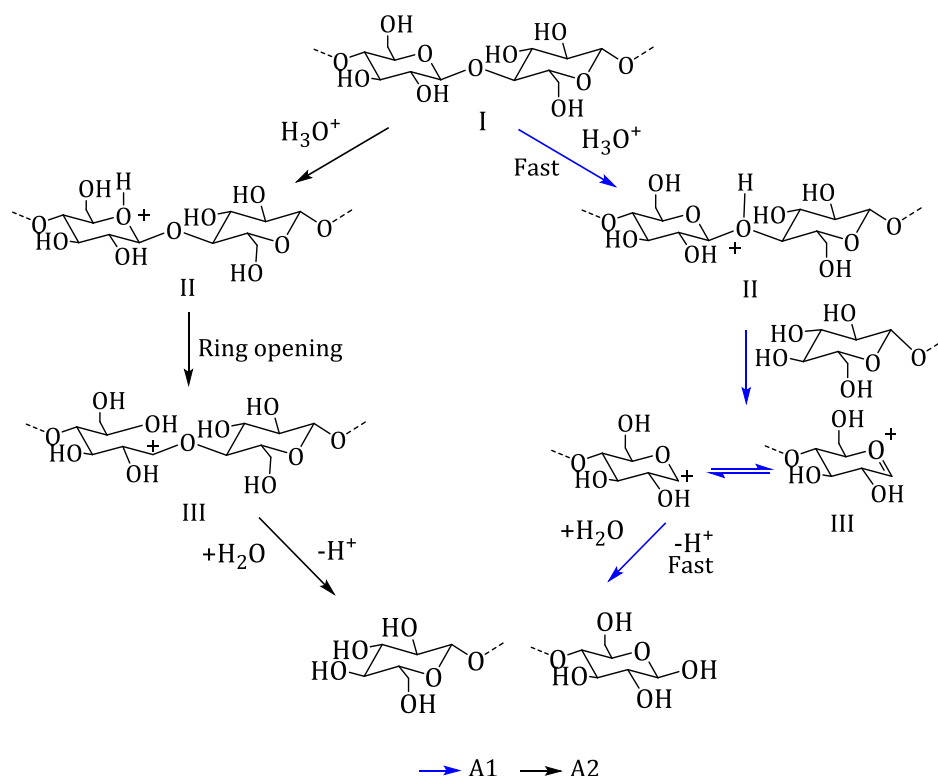


Figure 1- 11. Depolymerisation of cellulose *via* acid-catalysed hydrolysis.⁵¹

In the A-1 mechanism, the reaction proceeds *via* three steps: (1) formation of conjugate acid *via* rapid protonation of glycosidic oxygen atom (II), (2) a slow transfer of the positive charge to C1, forming a cyclic carbocation (III), and (3) rapid addition of water to the carbocation.⁵¹

The depolymerisation may also occur through the A-2 mechanism. This involves protonation of the oxygen on a pyranose ring (II), forming a non-cyclic carbocation (III).⁵¹ The carbocations are very reactive electrophiles; they can react even with a weak nucleophile, like water, and the reaction is fast. During the hydrolysis, the cellulose chains continue to degrade, eventually forming glucose monomers.

Currently, hydrolysis of cellulose is performed with the use of a strong acid at a high concentration. For example, the standard protocol for determining the composition of carbohydrates in biomass samples (**Section 1.6.1**) uses sulfuric acid (H_2SO_4) at a concentration of 72 wt%, at 30 °C;⁴² however, only soluble oligomers are able to be detected. Thus, an additional step, heating the resultant mixture at a high temperature under pressure, is performed to achieve complete hydrolysis of these oligomers to glucose.⁵²

1.7.3 Reactivity of Cellulose

1.7.3.1 Hydroxyl Groups of Cellulose

Cellulose is a trihydric alcohol consisting of three hydroxyls in each anhydroglucose unit;⁵³ OH-C2 (secondary hydroxyl), OH-C3 (secondary hydroxyl) and OH-C6 (primary alcohol).⁵⁴ All hydroxyls can be functionalised through several chemical reactions, such as esterification and oxidation.⁵⁴

An esterification reaction forms a cellulose-sulfate-ester compound through the formation of a carbon-oxygen-sulfur (-C-O-S-) bond. The reaction, also called 'sulfation', primarily involves OH-C6, which reacts with sulfating agents, such as sulfamic acid (NH₂SO₃H)⁵⁵ and sulfuric acid (H₂SO₄).⁵⁶ On the other hand, Rajalaxmi *et al.* demonstrated that the OH-C3 and the OH-C2 of cellulose underwent an oxidation reaction using sodium periodate (NaIO₄), yielding aldehydes.⁵⁷

1.7.3.2 Reactivity of Reducing End Groups

Cellulose is a unique polymer. Each cellulose chain contains a non-reducing end and a reducing-end group (as shown in **Figure 1- 12**). The non-reducing end refers to a closed ring glucose unit with a free hydroxyl at C4 located at the end of cellulose chain. At another end, there is also a glucose unit containing both cyclic hemiacetal and aldehyde structures. Both structures exist in equilibrium. The existence of aldehyde enables cellulose to acquire reducing properties.¹³

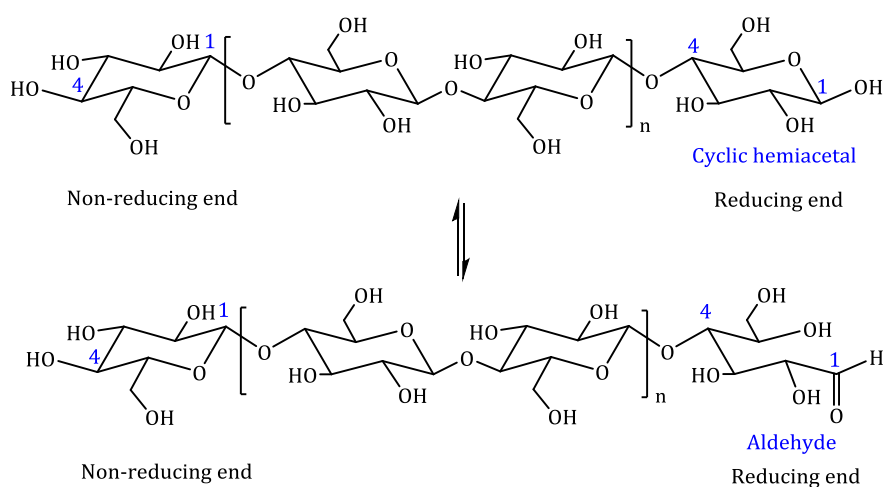


Figure 1- 12. Non-reducing and reducing end groups in equilibrium.¹³

Several chemical derivitisations can take place at the aldehyde on the reducing group (**Figure 1- 12**). Hydrazinolysis reaction of aldehyde followed by re-N-acetylation reaction using Girard's T reagent introduces acetylamino groups at the reducing end (**Figure 1- 13**).⁵⁸

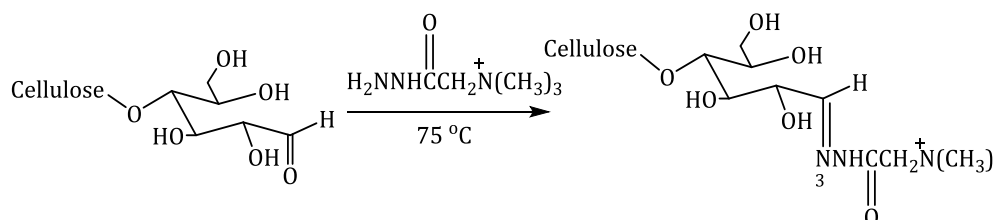


Figure 1- 13. Derivitisation of aldehyde of cellulose with Girard's T reagent.⁵⁸

This chemical derivitisation (**Figure 1- 13**) was used to enable detection of cellulose oligosaccharides in positive ion mode of MALDI-TOF.⁵⁸ In a review article of Pinkert *et al.*, it was reported that 1-ethyl-3-methylimidazolium acetate, $[C_2C_1im][CH_3CO_2]$, formed a covalent bond with the aldehyde group on the reducing end of oligomers (DP 6-10) (**Figure 1- 14**).⁵⁹ The covalent bond involved C2 of the $[C_2C_1im]^+$ cation and C1 of the reducing end.⁵⁹ It has also been demonstrated in the literature that $[C_2C_1im][CH_3CO_2]$ forms a N-heterocyclic carbene that react with aldehydes.⁶⁰

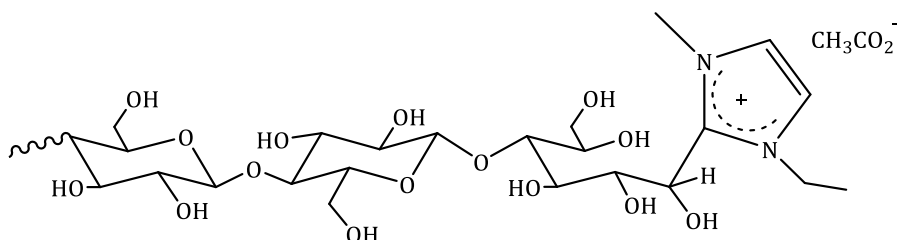


Figure 1- 14. Derivitisation of aldehyde of cellulose with $[C_2C_1im]^+$ cation.⁵⁹

1.8 Characterisation of Cellulose Products

1.8.1 Scanning Electron Microscopy and Energy Dispersed X-Ray

Scanning electron microscopy (SEM) has been used to examine morphological changes of cellulose samples following chemical treatments.^{1,2,56,61} The technique uses a beam of high-energy target electrons, which then bombard the surface of a specimen under vacuum environment. The bombardment process ejects electrons from atoms of the specimen. The ejected electrons are called 'Secondary Electrons' (SE). A secondary electron (SE) detector, a component of the SEM, then captures the SE electrons, which are then processed to give micrographs. The SEM is attached to an Electron Dispersed X-ray (EDX) detector. During the event of the bombardment process, the target electrons eject electrons occupied K-shell in the specimen. This creates vacant holes which are then filled by electrons from L-shell. The transition of the electrons from L-shell to K-shell emits a series of K_{α} characteristic x-rays, captured by the EDX detector. The emitted K_{α} x-rays are then analysed to produce a spectrum. The spectrum reveals a plot of x-ray counts versus energy (in keV). To date, the EDX has been utilised in determining sulfur on treated cellulose.^{61,62}

Limitations

The EDX is a semi-quantitative analysis with several limitations. Among them are:

1. Possess high detection limit: around 1% or higher, preventing detection of a small quantity of trace elements.⁶³
2. Provides a total ratio of very narrow segments of a specimen.
3. Unable to identify the existence of chemical bonds.

1.8.2 CHNS Elemental Analysis

The composition of carbon, hydrogen, nitrogen and sulfur (CHNS) elements in a given sample can be determined by combustion gas analysis (CGA). It has been used to quantify sulfate groups present in treated-cellulose samples.⁵⁶ During the analysis, the sample of interest experiences complete and

instantaneous oxidation by “flash combustion” at a very high temperature. This converts all organic substances into combusted gasses, which are then passed through a GC column attached to a thermal conductivity detector (TCD). Finally, the composition of carbon, hydrogen, nitrogen and sulfur are known.⁶⁴

Limitations

CHNS elemental analysis is known as a quantitative technique with a detection limit of 0.3%. However, like the SEM-EDX technique (**Section 1.8.1**), the analysis cannot firmly verify the characteristic of chemical bonds that existed in the analysed sample.

1.8.3 Infrared Spectroscopy

Infrared Spectroscopy is a great tool for identification of functional groups of chemical substances. It has been used to identify IR vibrations, which represent carbon to oxygen to sulfur (-C-O-S)^{55,56} and carbon to sulfur (-C-S)^{57,65} bonds in cellulose samples treated in organosulfur acids, such as sulfuric acid (H₂SO₄) and sulfamic acid (NH₂SO₃H). This tool overcomes the limitations of the aforementioned techniques, SEM-EDX (**Section 1.8.1**) and CHNS elemental analysis (**Section 1.8.2**), in identifying chemical bonds present in the analysed sample.

Limitations

It is very challenging to obtain structural information of polymers, for example end group and repeating unit, using IR spectroscopy.⁶⁶ Additionally, this may not be the most sensitive tool for determination of carbonyls in cellulose. This is because the average quantity of the aldehyde group in cellulose (**Figure 1- 12**) is extremely low, ranging in the order of μmolg^{-1} .^{67,68} Other conventional techniques, such as nucleus magnetic resonance (NMR) or Raman spectroscopy, are also unable to report such minor quantities.^{67,68}

1.8.4 Matrix Assisted Laser Desorption/Ionisation Mass Spectroscopy

Matrix Assisted Laser Desorption/Ionisation attached to a Time-of-Flight mass analyser (MALDI-TOF) is a powerful and quick tool to characterise large

molecules.⁶⁹ It has also been frequently used to elucidate the structure of a wide range of synthetic polymers^{66,70,71} and biopolymers.^{69,72} Using this tool, detailed structural information can be obtained, including compositions of end group^{70,73} and repeating unit.^{69,70}

Figure 1- 15 illustrates a proposed working principle of MALDI-TOF.⁷⁴ In general, the analysis is divided into two steps. The first steps involves co-crystallisation of a small quantity of an analyte with an excess quantity of an organic matrix prior to the analysis.⁷⁵ Detailed methodologies on preparing analyte/matrix mixtures have been reported by Hanton *et al.*⁷⁶

The second step, ionisation of analyte, is then performed upon completion of the co-crystallisation. When a high energy laser (for example N₂ laser, 337 nm) irradiates the analyte/matrix mixture, the matrix absorbs the energy of the irradiation. Such action induces vaporisation and ionisation of the matrix as well as the analyte molecules³, yielding single charged positive and negative ions. The charged ions are then desorbed and accelerated through an acceleration zone (a flight separation tube) to a detector.⁷⁷

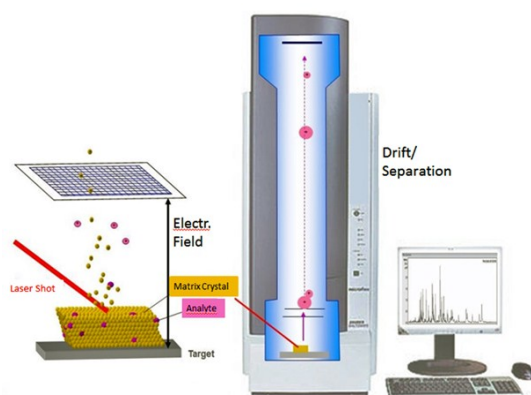


Figure 1- 15. Working principle of MALDI-TOF.⁷⁴

In this analysis, compatibility between a matrix and the analyte of interest is crucial in order to obtain high resolution spectra, which lead to accurate information.^{71,78,79}

Limitation

Mass discrimination⁷⁵ commonly encountered when analysing high molecular weight polymers, giving low signal intensities of produced charged ions.⁷¹ High molecular weight polymers usually have broader polydispersities

(greater total number of chain lengths).⁷¹ Based on a study of Belu *et al.*, the mass discrimination was likely to be associated with two factors.⁷¹

The first factor related to sample preparations,⁷¹ arising from inadequate quantities of matrix molecules to solvate high molecular weight fractions in a given polymer.⁷¹ This causes the high molecular weight fractions to be directly exposed to high energy laser irradiation, absorbing the excessive energy which eventually leads to fragmentation.

The second factor was proposed to be associated with an instrumental limitation.^{71,75} High molecular weight ions give a lower detector response than low molecular weight ions.⁷⁵ In this analysis, the abundance of the produced charged ions captured by the detector is determined by the velocities of the ions travelling through the acceleration zone to reach the detector (**Figure 1- 15**). The velocity decreases as the molecular weight of ions increases.

Thus, determining polymer distribution patterns, such as average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity index (PI), through MALDI-TOF analysis may result in inaccurate readings. This is likely to happen when characterising a massive polymer, particularly cellulose.⁸⁰

1.8.5 Viscosity

Gel permeation chromatography (GPC) is a well-established method to determine the molecular weight distribution of polymers. Unfortunately, analysing biopolymer like cellulose, is not easy since the polymer does not dissolve in common organic solvents.⁸¹ Owing to this, cellulose is subjected to either activation or derivitisation prior to dissolving in typical non-derivating solvents, such as dimethylacetamide/LiCl or 1,3-dimethyl-2-imidazoleidinone/LiCl.⁸¹ A GPC set-up, however, must be equipped with a multiangle laser light scattering (MALLS) detector because none of available GPC standards suits cellulose analysis.⁸¹ One of the disadvantages of this method is that the sample preparation is time consuming.⁸¹

Another existing method to determine molecular weight of cellulose is through viscosity measurements.⁸²⁻⁸⁷ In general, cellulose is dissolved with a metal amine complex, such as copper (II) ethylene diamine hydroxide (CUEN). This process is commonly completed in a relatively short period of time.⁸⁰ During the dissolution process, hydroxyls at C2 and C3 on the glucose unit of cellulose are believed to coordinate to Cu(II) centre, displacing one molecule of ethylenediamine in CUEN.⁸⁸ The resultant mixture then passes through a viscometer under atmospheric pressure, and the efflux time is recorded. The viscosity value is then determined followed by determination of molecular weight using a set of equations as proposed in several articles.⁸²⁻⁸⁷

2 .Methodology

2.1 Synthesis of Ionic Liquids

2.1.1 1-Butylimidazolium Hydrogen Sulfate, [HC₄im][HSO₄]

95 wt% (2.5 moles, 245 g) of sulfuric acid (H₂SO₄) was dissolved in distilled water (200 ml), resulting in 12.5 M sulfuric acid. The acid was added dropwise into 1-butylimidazole ([C₄im]) (2.5 moles, 310 g) over 30 minutes. The addition of the acid was conducted in an ice bath under vigorous stirring. The resultant liquid was passed through a C-18 purification column, whereupon, a purified ionic liquid was obtained. The excess water was removed from the ionic liquid by rotary evaporation and subsequent drying at 50 °C *in vacuo* overnight.

¹H-NMR (400 MHz, DMSO-d₆) δ_H: 9.14 (1H, s, CH-2), 7.79 (1H, s, CH-4), 7.66 (1H, s, CH-5), 4.20 (2H, t, N-CH₂-), 1.75 (2H, m, N-CH₂-CH₂-), 1.22 (2H, m, N-(CH₂)₂-CH₂-), 0.853 (3H, t, N-(CH₂)₃-CH₃) ppm. ¹³C-NMR (100 MHz, DMSO-d₆) δ_C: 135 (C-2), 122 (C-4), 120 (C-5), 48.3 (N-CH₂-), 31.6 (N-CH₂-CH₂-), 18.9 (N-(CH₂)₂-CH₂-), 13.4 (N-(CH₂)₃-CH₃) ppm. *m/z* (LSIMS⁺): 125 (100%, [HC₄im]⁺); *m/z* (LSIMS⁻): 97 (100%, [HSO₄]⁻).

2.1.2 1-Butyl-3-methylimidazolium Hydrogen Sulfate, [C₄C₁im][HSO₄]

The synthesis of 1-butyl-3-methylimidazolium hydrogen sulfate, [C₄C₁im][HSO₄] involved two steps.

(1) 1-Butyl-3-Methylimidazolium Methyl Sulfate, [C₄C₁im][CH₃OSO₃]

Prior to the synthesis, precursors were pre-dried under a nitrogen atmosphere and later distilled under vacuum: C₄im with potassium hydroxide (KOH) and dimethyl sulfate ((CH₃)₂SO₄) was dried with calcium oxide (CaO). Under a nitrogen atmosphere, the C₄im (0.79 mole, 99.2 g) was dissolved in toluene (1.8 mole, 200 ml) under vigorous stirring at room temperature. (CH₃)₂SO₄ (0.79 mole, 100.9 g) was then added dropwise into the [C₄im]. This resulted in a mixture of two layers upon completion of the reaction. The upper layer, containing toluene and possibly unreacted [C₄im] and (CH₃)₂SO₄, was removed. The

bottom layer, containing the ionic liquid 1-butyl-3-methylimidazolium methyl sulfate, $[\text{C}_4\text{C}_1\text{im}][\text{CH}_3\text{OSO}_3]$, was washed with toluene several times. The excess toluene was removed from the ionic liquid by rotary evaporation. The ionic liquid was then dried at 50 °C in *vacuo* overnight.

^1H -NMR (400 MHz, DMSO- d_6) δ_{H} : 9.10 (1H, s, CH-2), 7.77 (1H, s, CH-4), 7.70 (1H, s, CH-5), 4.16 (2H, t, N-CH $_2$ -), 3.84 (3H, s, N-CH $_3$), 3.37 (3H, t, S-O-CH $_3$), 1.76 (2H, m, N-CH $_2$ -CH $_2$ -), 1.24 (2H, m, N-(CH $_2$) $_2$ -CH $_2$ -), 0.90 (3H, t, N-(CH $_2$) $_3$ -CH $_3$) ppm. ^{13}C -NMR (100 MHz, DMSO- d_6) δ_{C} : 136 (C-2), 123 (C-4), 122 (C-5), 52.7 (N-CH $_2$ -), 48.4 (N-CH $_3$), 35.7 (S-OCH $_3$), 31.5 (N-CH $_2$ -CH $_2$ -), 18.7 (N-(CH $_2$) $_2$ -CH $_2$ -), 13.2 (N-(CH $_2$) $_3$ -CH $_3$) ppm. m/z (LSIMS+): 139 (100%, $[\text{C}_4\text{C}_1\text{im}]^+$); m/z (LSIMS-): 111 (100%, $[\text{HSO}_4]^-$).

(2) 1-Butyl-3-methylimidazolium Hydrogen Sulfate, $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$

$[\text{C}_4\text{C}_1\text{im}][\text{CH}_3\text{OSO}_3]$ was initially mixed with distilled water. A few drops of H_2SO_4 catalyst was then added into the solution. The resultant mixture was heated to 170 °C. Upon reaching this temperature, distilled water was constantly added dropwise into the mixture. The addition of water continued throughout the course of the reaction of 3 h. After cooling to room temperature, the mixture was flushed through a C-18 purification column. The excess water was removed from the ionic liquid by rotary evaporation. The ionic liquid was dried at 50 °C in *vacuo* overnight.

^1H -NMR (400 MHz, DMSO- d_6) δ_{H} : 9.14 (1H, s, CH-2), 7.76 (1H, s, CH-4), 7.71 (1H, s, CH-5), 4.16 (2H, t, N-CH $_2$ -), 3.85 (3H, s, N-CH $_3$), 1.70 (2H, m, N-CH $_2$ -CH $_2$ -), 1.24 (2H, m, N-(CH $_2$) $_2$ -CH $_2$ -), 0.89 (3H, t, N-(CH $_2$) $_3$ -CH $_3$) ppm. ^{13}C -NMR (100 MHz, DMSO- d_6) δ_{C} : 136 (C-2), 123 (C-4), 122 (C-5), 48.4 (N-CH $_2$ -), 35.7 (N-CH $_3$), 31.3 (N-CH $_2$ -CH $_2$ -), 18.7 (N-(CH $_2$) $_2$ -CH $_2$ -), 13.3 (N-(CH $_2$) $_3$ -CH $_3$) ppm. m/z (LSIMS+): 139, (100%, $[\text{HC}_4\text{im}]^+$); m/z (LSIMS-): 97 (100%, $[\text{HSO}_4]^-$).

2.1.3 Characterisation of Ionic Liquids

The ionic liquids were characterised by the following analytical instruments: Nuclear Magnetic Resonance (NMR) and Liquid Secondary Ion Mass Spectroscopy (LSIMS).

2.2 Deconstruction of Biomass

The overall process of deconstruction of *Miscanthus* in ionic liquids has been described in **Chapter 3** (Figure 3- 4, Section 3.2.2).

2.2.1 Processing Raw Biomass Feedstocks

Miscanthus whole stems were obtained from the Silwood Park Campus, Imperial College London. **Figure 2- 1** provides a flowchart of the processing step. *Miscanthus* sticks (**Figure 2- 1** (a)) were sliced; the slices (**Figure 2- 1** (b)) were then air-dried at room temperature. Using a mechanical grinder and a mechanical sieve, the air-dried slices were ground to an average particle size of 0.18-0.85 mm (-20 + 80 of US mesh scale) (**Figure 2- 1** (c)). The moisture content of the ground *Miscanthus* particles (method described in **Section 2.2.2**) was determined to be 5.6 wt%.

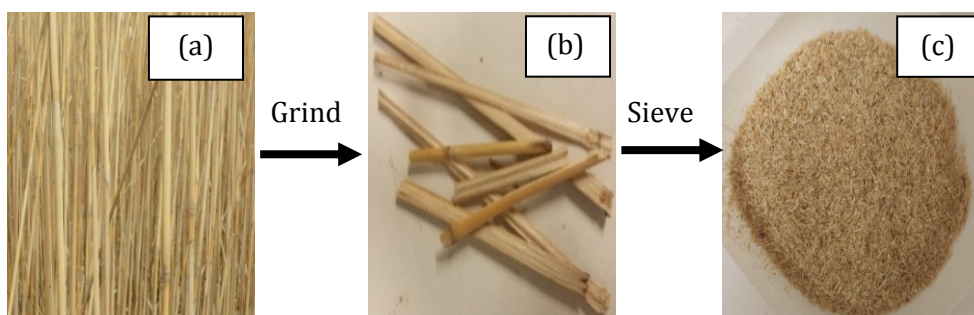


Figure 2- 1. Reduction of size of biomass feedstock.

2.2.2 Measurement of Oven-Dried Weight of Biomass

Air-dried *Miscanthus* particles (**Figure 2- 1** (c)) (100 mg) was wrapped in a known weight of aluminum foil. The sample was heated in an oven at 105 °C overnight. Upon completion, the sample was transferred into a desiccator for 5 min and then weighed. The water content was calculated using **Equation 2.1** as reported previously.³

$$\text{moisture \%} = \frac{m_{\text{air dried}} \times m_{\text{oven dried}}}{m_{\text{air dried}}} \times 100 \quad \text{Equation 2.1}$$

$m_{\text{air-dried}}$: mass of air-dried *Miscanthus* particles, and; $m_{\text{oven-dried}}$ mass of *Miscanthus* particles after heating at 105 °C overnight.

2.2.3 Incubation of Biomass in Ionic Liquids and Separation of Polymers

An ionic liquid/water mixture (80/20 wt/wt, 10 g) was prepared in an Ace pressure tube (volume ~15 mL, L × O.D. 10.2 cm × 25.4 mm). The ground *Miscanthus* particles (1 g on oven-dry weight basis) (**Figure 2- 1 (c)**) were then transferred into the tube, giving a total biomass loading of 9 wt%. The tube was sealed tightly and incubated at 120 °C for 22 h without stirring. When the incubation had elapsed, a mixture containing the cellulose-rich-fraction, labelled as 'cellulose-rich-material' (CRM) and a liquor were obtained. After cooling the mixture to room temperature, a small quantity of the liquor (200 µL) was extracted for the analysis of solubilised sugars and degradation products (**Section 2.2.5**). The mixture was diluted with EtOH (10 ml) and subsequently separated through a 542-whatman filter paper (hardened, ashless, Sigma-Aldrich, UK) in a Büchner funnel. The recovered CRM was repeatedly rinsed with EtOH, while the EtOH-filtrate was collected for precipitation of Ionosolv-lignin. The recovered CRM was air-dried at room temperature.

2.2.4 Precipitation of Ionosolv-Lignin

The EtOH-filtrate (**Section 2.2.3**) was concentrated by evaporating the EtOH under reduced pressure using a rotavap. The concentrated liquid was mixed with deionised water (30 ml), whereupon, a black precipitate formed instantaneously. The resultant mixture was transferred into a 50 ml-Falcon tube, and was centrifuged at a speed of 4000 r.p.m for 20 min. The precipitate was washed with deionised water (2 x 30 ml) and then dried at 40 °C *in vacuo* overnight. The yield labelled as 'Ionosolv-lignin' was measured as described previously.

2.2.5 Quantification of Degradation Products, 5-HMF and furfural, and Solubilised Sugars.

The liquor, extracted from the mixture obtained after the incubation process (**Section 2.2.3**) was mixed with deionised water (600 µl) in a 1.5 ml vortex-plastic cup, and was vortexed and centrifuged at a speed of 13000 r.p.m for 10 min. The liquid layer was transferred into an HPLC vial and analysed on a Shimadzu Analytic system equipped with an Aminex HPX-87H column (Biorad) using the following method: 10 mM aqueous H₂SO₄ mobile phase, column oven temperature 55 °C, flow rate 0.6 ml min⁻¹ and an acquisition time of 45 min. 2-furaldehyde (furfural) and 5-(hydroxymethyl)-2-furaldehyde (5-HMF) standards were prepared in deionised water with the following concentrations: 0.1, 1.0, 2.0 and 4.0 mg ml⁻¹. **Equation 2.2** was applied to calculate the wt% of solubilised sugar monomers and degradation products (S).³

$$\text{wt\% (S)} = \frac{A_{\text{HPLC}} \times F_D \times V_{\text{PL}} \times F_T(\text{S})}{F_{\text{HPLC}}(\text{S}) \times m_{\text{biomass}} \times F_C} \times 100\% \quad \text{Equation 2.2}$$

A_{HPLC} : area of HPLC peak; $F_{\text{HPLC}}(\text{S})$: HPLC calibration factor for substance S; F_D : dilution factor; V_{PL} : volume of deconstruction liquor in ml; m_{biomass} : biomass (oven-dried) weight in mg; F_C : fraction of glucan or hemicellulose sugars in untreated biomass as determined by compositional analysis; $F_T(\text{S})$: transformation factor accounting for molecular mass differences between starting material and product, 1.37 (furfural), 1.28 (5-HMF), 0.91 (glucose) and 0.88 (hemicellulose sugars).³

2.2.6 Extensive Purification of Recovered CRM: Soxhlet Extraction Technique

Figure 2- 2 shows a schematic diagram of a typical experimental set-up for the Soxhlet extraction technique. A recovered CRM sample (**Section 2.2.3**) was transferred into a cellulose thimble and placed into the thimble-section. Absolute ethanol (100 mL) was poured into a 250 ml of single-neck round bottom flask. The set-up was constructed according to **Figure 2- 2**. The technique was performed at 80 °C for one

week. Upon completion, a solid fraction named 'Soxhlet-CRM' was recovered. The Soxhlet-CRM sample was then dried at room temperature.

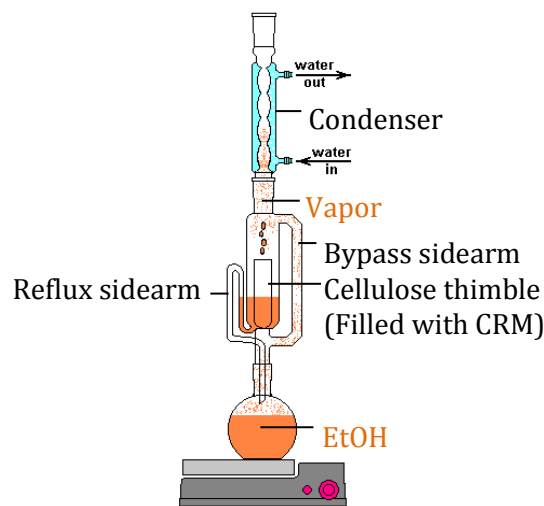


Figure 2- 2. Soxhlet extraction technique.

2.3 Treatment of Microcrystalline Sigmacell-Cellulose in Ionic Liquids

An ionic liquid/water mixture (80/20 wt/wt, 10 g) was prepared in an Ace pressure tube (volume ~15 mL, L × O.D. 10.2 cm × 25.4 mm). Into the tube, untreated MCC-particles (1 g on oven-dry weight basis, type 20, 20 µm, Sigma-Aldrich UK) were then transferred, giving a total MCC loading of 9 wt%. The tube was sealed tightly and incubated at 120 °C for 22 h without stirring. After cooling to room temperature, the resultant mixture was diluted with EtOH (10 ml) and later filtered through a 542-whatman filter paper in a Büchner funnel. This yielded a solid fraction labelled as 'Treated-MCC'. The Treated-MCC was repeatedly rinsed with EtOH and later dried at room temperature.

2.4 Characterisation of Cellulose Products

2.4.1 Compositional Analysis

The composition of lignin and carbohydrates in cellulose samples was measured by compositional analysis.⁴² **Figure 2- 3** provides a summary of the analysis. The analysis consists of three steps: (1) acid hydrolysis (**Section 2.4.1.1**), (2) separation and quantification of sugar products (**Section 2.4.1.2**), and (3) determination of ash and acid insoluble compositional analysis lignin (AICL) (**Section 2.4.1.3**).

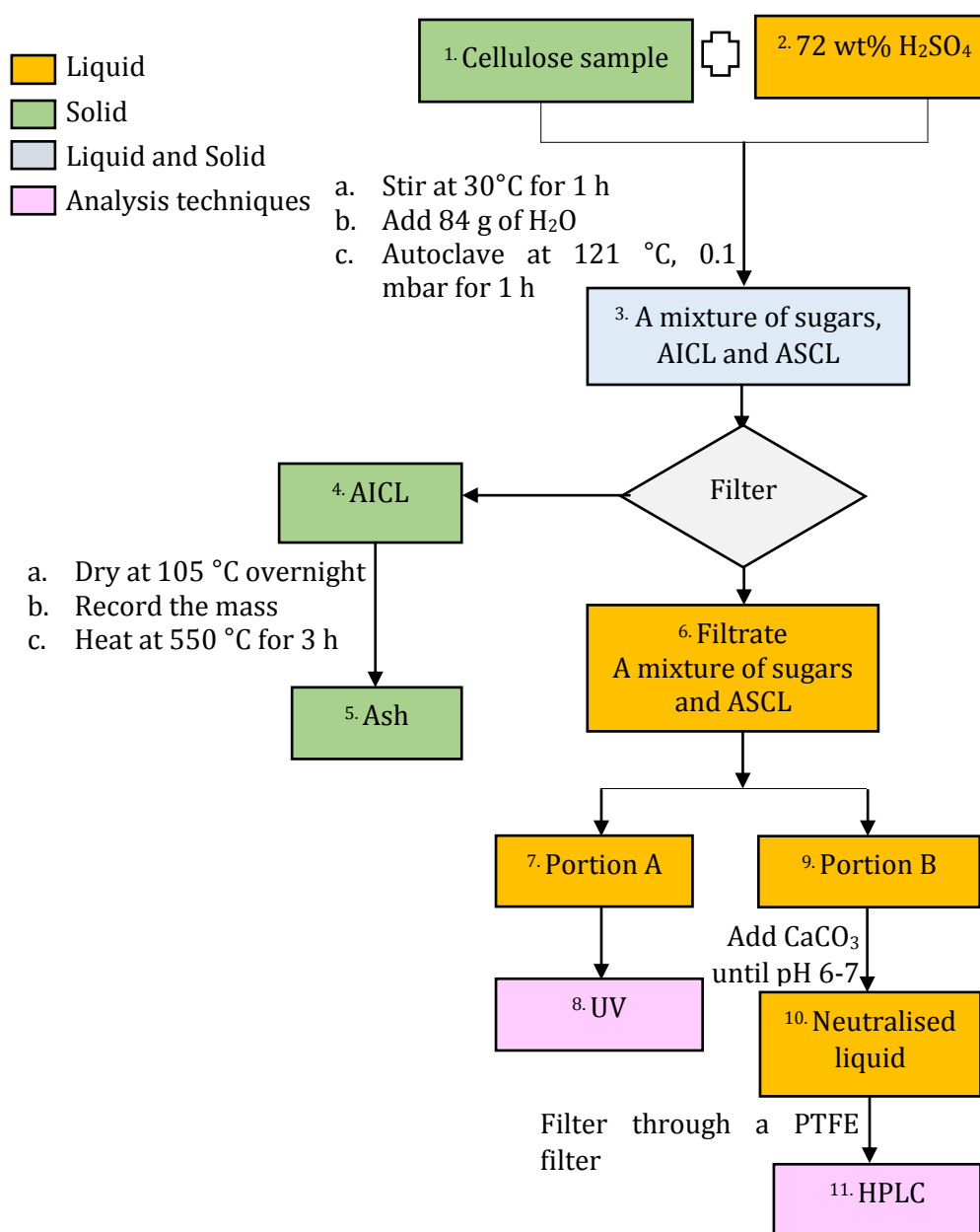


Figure 2- 3. Compositional analysis.

2.4.1.1 Acid Hydrolysis

A cellulose sample (300 mg, oven dried basis) was loaded into an Ace pressure tube. 72 wt% of H_2SO_4 (3 mL) was poured into the tube. The resultant mixture was stirred using a Teflon stir rod at room temperature for one minute. The tube was incubated in a water bath, allowing the mixture to react at 30 °C for 1 h. During incubation period, the mixture was stirred for every 10 min interval using the Teflon stir rod without removing the tube from the water bath. When the incubation period elapsed, the mixture was diluted to 4% concentration by addition of distilled water (84 g). The tube was sealed tightly, inverted several times and then autoclaved at 121 °C for one hour.

2.4.1.2 Separation and Quantification of Products

When the autoclave had completed, the resultant mixture (**Figure 2- 3 (3)**) was filtered through an ashed crucible under reduced pressure. The filtrate (**Figure 2- 3 (6)**) (40 mL) was transferred in a 50 mL-falcon tube. The solid trapped in the ashed crucible was dried in an oven at 105 °C overnight, yielding an acid insoluble compositional analysis lignin (AICL) sample (**Figure 2- 3 (4)**). The filtrate was divided into two portions (20 mL each). For Portion A (**Figure 2- 3(7)**), the filtrate was analysed using UV-VIS spectroscopy at a wavelength of 240 nm with distilled water as a solvent. This step was performed to detect acid soluble compositional analysis lignin (ASCL).

Portion B (**Figure 2- 3 (9)**) was used to analyse the sugars. The filtrate was neutralised by adding calcium carbonate (CaCO_3) until the pH reached 5-6. The mixture was allowed to stand, separating a solid and a liquid fractions. The liquid (**Figure 2- 3 (10)**) was taken (1 mL), filtered through a PTFE filter and later transferred into a HPLC vial. The HPLC analysis of sugars was conducted on a Shimadzu Analytical system equipped with an Aminex HPX-87H column (Biorad) using the following method: deionised water as mobile phase, column oven temperature 55 °C, flow rate 0.6 ml min⁻¹ and an acquisition time of 25 min.

2.4.1.3 Determination of Ash and Acid-Insoluble-Compositional-Lignin (AICL)

The crucible containing the AICL (**Section 2.4.1.2, Figure 2- 3(4)**) was placed in a muffle furnace and heated at 570 °C for 3 h. The ash (**Figure 2- 3 (5)**), leftover traces of the thermochemical process, was weighed and its mass recorded.

2.4.2 Enzymatic Saccharification

A method from the technical report NREL/TP-510-42629 was used.⁸⁹ A flow chart summarising the process is shown in **Figure 2- 4**.

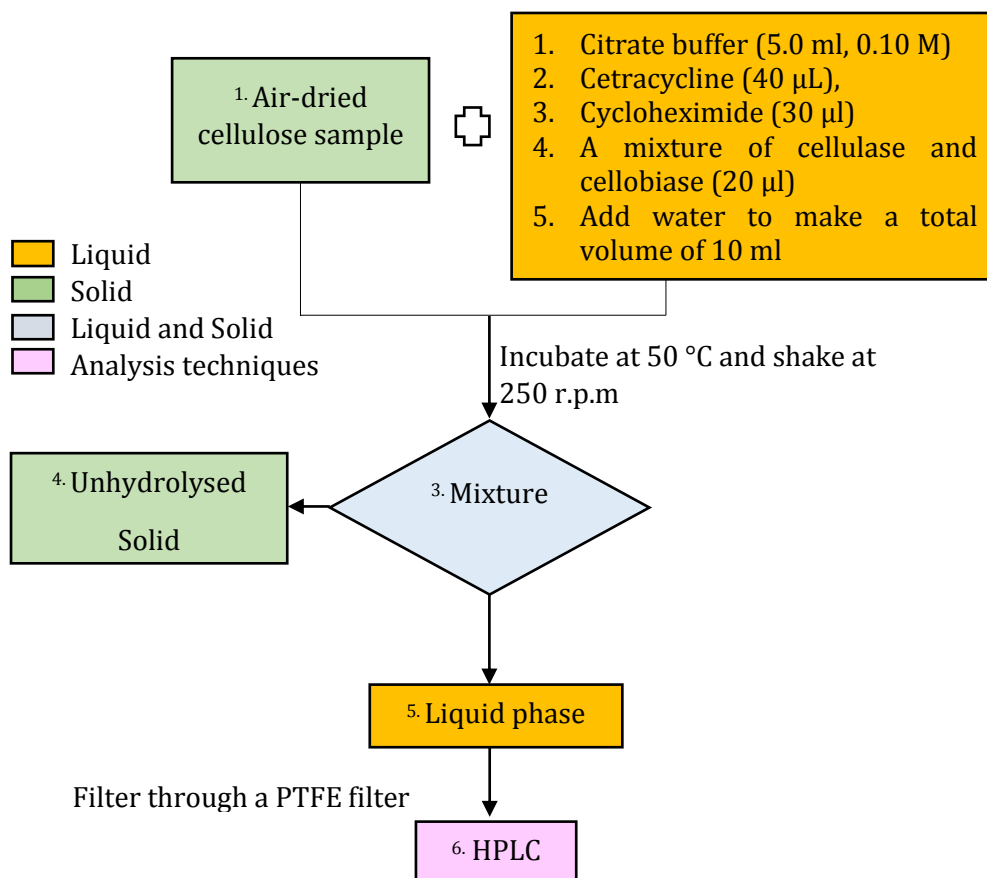


Figure 2- 4. Enzymatic Saccharification.

A cellulose sample (100 mg, oven-dried basis) was transferred into a culture tube. The following chemicals were then added into tube: citrate buffer (5.0 ml, 0.10 M), tetracycline (40 μ L), cycloheximide (30 μ L) and a mixture of cellulase and cellobiase enzymes (20 μ L). The cellulase and cellobiase enzymes were purchased from Novozymes Corp. A total volume of 10 ml was then made by adding distilled water. The tube was incubated in an incubator at 50 °C and shaken at 250 r.p.m. When the incubation had elapsed, the liquid phase (**Figure 2- 4 (5)**) (1.0 mL) was taken using a 1 mL syringe, filtered through a PTFE filter and poured into a HPLC vial. The liquid was analysed on a Shimadzu Analytical system equipped with an Aminex HPX-87H column (Biorad) and Refractive Index (RI) detector using the following method: deionised water as mobile phase, column oven temperature 55 °C, flow rate 0.6 ml min⁻¹ and an acquisition time of 25 min.

2.4.3 Infrared Spectroscopy

IR spectra were recorded using a Spectrum 100 IR machine (Perkin Elmer) equipped with a universal ATR sampling accessory with diamond crystal.

2.4.4 CHNS Elemental Analysis

A FlashEA 11122 Elemental Analyser was used to determine the composition of CHNS elements. The analysis was conducted at MEDAC Ltd, UK.

2.4.5 Matrix Assisted Laser Desorption/Ionisation

MALDI-TOF spectra were obtained using a Waters Micromass MALDI-TOF equipped with a Time-Of-Flight (TOF) detector and nitrogen laser (emitting at 337 nm). The spectra were recorded in negative ion mode.

2.4.6 Scanning Electron Microscopy and Energy Dispersed X-Ray

Prior to the analysis, the sample was coated with gold or chromium. The imaging process was conducted on a JSM 6400 equipped with a secondary electron (SE) detector using the following conditions: current voltage of 20 keV and a working distance of 15 mm. The elemental analysis was then performed using Oxford Instruments INCA energy dispersive analytical system (EDX) that is fitted with the SEM.

2.4.7 Kinematic Viscosity

Standard ASTM protocols were used to measure the viscosity of cellulose samples.^{83,84} A cellulose sample (0.25 g) and distilled water (25 mL) were transferred into a 100 ml of borosilicate jar. Three glass balls (4 to 6-mm diameter) were loaded into the jar. The jar was sealed tightly and shaken for 90 min using a mechanical stirrer. After the shaking process had elapsed, the jar was allowed to stand for 2 min and degassed. 0.5 M of bis(ethylenediamine)copper(II) hydroxide (CUEN) solution (25 ml) (diluted from 1 M in water as received, Sigma-Aldrich UK) was then pipetted into the jar. The mixture was mixed by shaking the jar for 15 min. At the end of the mixing process, the jar was allowed to stand for 2 min, degassed and flushed with nitrogen gas for 2 min.

Using a 1 mL-syringe, the resultant cellulose-CUEN mixture was poured into a miniature u-tube (type M2, nominal average length 250 mm, PSL Rheotek). The tube was immersed in an oil bath initially and heated at 30 °C. The tube was allowed to stand for 15 min, enabling the temperature to equilibrate. The efflux time for the cellulose-CUEN solution to travel from the beginning point to the end point marked on the u-tube was recorded. **Equation 2.3** was then applied to calculate the kinematic viscosity, ϵ , of the solution.

$$\epsilon = Ct$$

Equation 2.3

ϵ : kinematic viscosity (mm^2s^{-1}); C: calibration constant of the miniature u-tube, 0.005094 (mm^2s^{-1})/s (provided by the manufacturer), and; t: efflux time.

3 . Understanding the Process

3.1 Background of Study

Motivation

In general, deconstruction of biomass in ionic liquids *via* the Ionosolv approach, involves two main steps. The first step deals with incubating a biomass-ionic liquid mixture at a certain temperature and time period, separating lignin from a cellulose-rich fraction, the cellulose-rich-material (CRM). The second step involves recovering the CRM and the lignin, named 'Ionosolv-lignin', separately. To achieve an effective separation, the ionic liquids used must be:

1. A Brønsted acid catalyst that enables hydrolysis of ester bonds linking the lignin with the CRM.
2. An effective delignification agent; able to separate the lignin from the CRM by depolymerising and dissolving the lignin.

An ionic liquid's anion is known to significantly influence both hydrolysis and delignification.³ For deconstruction of *Miscanthus*, hydrogen sulfate, $[\text{HSO}_4]^-$, was found to be the most effective anion for this process.^{3,29} When deconstructing *Miscanthus* in a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture at 120 °C, the removal of hemicellulose and lignin was found to be time dependent; the longer the period of incubation, the more hemicellulose and lignin were removed.³ As a result, the CRM composition was enriched with cellulose.^{3,29} However, it was also reported that the $[\text{HSO}_4]^-$ anion could chemically interact with the CRM during the deconstruction process. A recent study used a series of alkylammonium hydrogen sulfate, $[\text{R}_n\text{NH}_m][\text{HSO}_4]$, ionic liquids to deconstruct *Switchgrass* under similar conditions to the aforementioned.²⁸ At the end of the deconstruction process, the $[\text{HSO}_4]^-$ anion was found to adsorb into recovered CRMs, exhibiting a negative impact on the production of glucose *via* enzymatic saccharification.

In line with this, the main motivation of this study is to understand the mechanism of interaction between cellulose and the $[\text{HC}_4\text{im}][\text{HSO}_4]$ ionic liquid, during the deconstruction of *Miscanthus*.

Problem Statement

It was hypothesised that the $[\text{HSO}_4]^-$ anion might be chemically bound into the recovered CRMs.²⁸ However, this hypothesis lacks supporting evidence. Thus, thorough investigation is required.

Hypothesis

In relation to deconstruction of *Miscanthus* in alkyl imidazolium hydrogen sulfate, $[\text{C}_n\text{C}_m\text{im}][\text{HSO}_4]$ ionic liquids, if cellulose fibrils become more exposed due to significant removal of hemicellulose and lignin, then $[\text{C}_n\text{C}_m\text{im}][\text{HSO}_4]$ ionic liquids will have a chance to adsorb onto the surface of the cellulose fibrils *via* either physisorption or chemisorption.

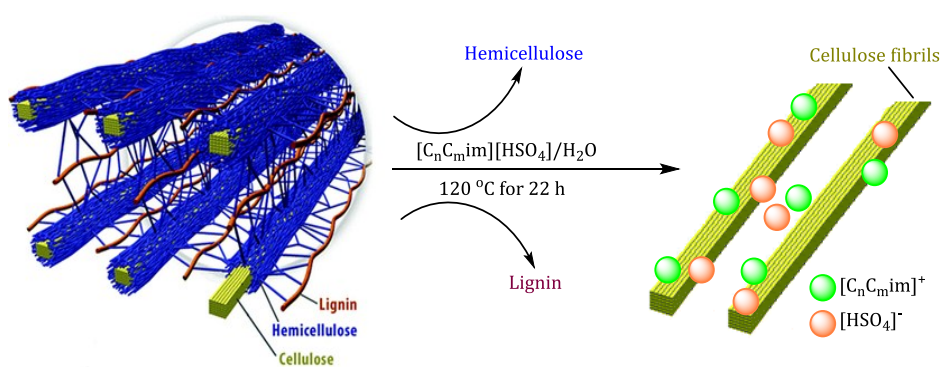


Figure 3- 1. Separation of biomass's polymers (cellulose-hemicellulose and lignin⁹) and surface reactions between cellulose and $[\text{C}_n\text{C}_m\text{im}][\text{HSO}_4]$ ionic liquids during the deconstruction process.

Objectives

The objectives of this study are to:

1. Understand the deconstruction process.
2. Understand adsorption phenomena that occur between cellulose fibrils and $[\text{C}_n\text{C}_m\text{im}][\text{HSO}_4]$ ionic liquids.

Strategies

A number of identified strategies are as follows:

1. Perform deconstruction of *Miscanthus* in a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture at 120 °C for 22 h.
2. Purify the recovered cellulose-rich-materials (CRM) *via* two techniques, Büchner-funnel filtration and Soxhlet extraction.
3. Analyse the products obtained from these purification techniques using a series of chemical and analytical methods.

3.2 Results and Discussion

3.2.1 Synthesis of Alkylimidazolium Hydrogen Sulfate, $[\text{C}_n\text{C}_m\text{im}][\text{HSO}_4]$, Ionic Liquids

The synthesis of 1-butylimidazolium hydrogen sulfate, $[\text{HC}_4\text{im}][\text{HSO}_4]$, followed an acid base neutralisation process. A previous study, that synthesised the $[\text{HC}_4\text{im}][\text{HSO}_4]$ ionic liquid *via* acid-base neutralisation suggested that a large ΔpK_a value between H_2SO_4 , pK_a -3, and 1-butylimidazole, $[\text{C}_4\text{im}]$, $pK_a \sim 7$, enabled complete proton transfer to occur.²⁹ Thus, the synthesis is most likely to follow the following mechanism (**Figure 3- 2**):

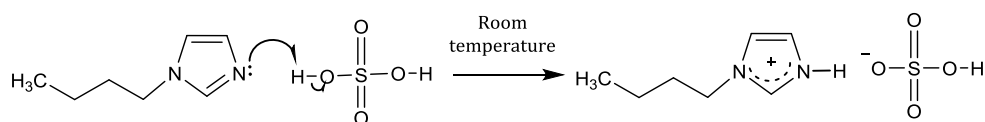


Figure 3- 2. Synthesis of the $[\text{HC}_4\text{im}][\text{HSO}_4]$ ionic liquid.

The synthesis of 1,3-dialkylimidazolium ionic liquids generally involves two steps: an alkylation followed by an anion metathesis or a hydrolysis step.²⁹ **Figure 3- 2** proposes the possible mechanism of the synthesis of the 1-butyl-3-methylimidazolium hydrogen sulfate $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ ionic liquid. It starts with *N*-methylation of $[\text{C}_4\text{im}]$ with dimethyl sulfate, $(\text{CH}_3)_2\text{SO}_4$, forming the 1-butyl-3-methylimidazolium methyl sulfate, $[\text{C}_4\text{C}_1\text{im}][\text{CH}_3\text{OSO}_3]$ ionic liquid (**Figure 3- 3**, Step 1).

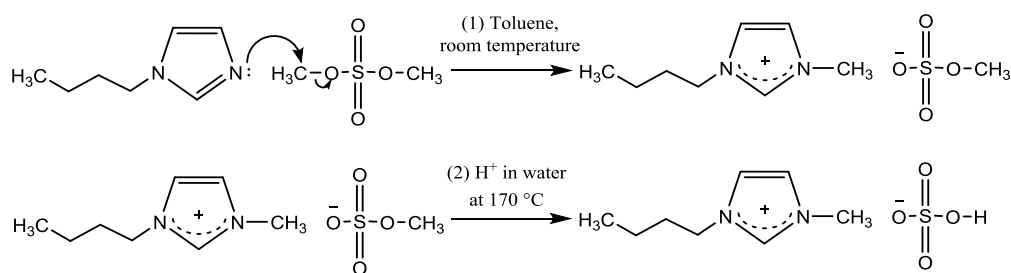


Figure 3- 3. Synthesis of the $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ ionic liquid.

Acid hydrolysis comprises the second step, where the methyl sulfate, $[\text{CH}_3\text{OSO}_3]^-$, anion is hydrolysed to the hydrogen sulfate, $[\text{HSO}_4]^-$, anion at 170 °C, eventually forming the $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ ionic liquid (**Figure 3- 3**, Step 2).

$(\text{CH}_3)_2\text{SO}_4$ is classified as a 'select carcinogen' according to the International Agency on Research, US.⁹⁰ Exposure to this chemical for a lengthened period can increase the risk of damaging liver and kidney systems. To minimise this risk when handling, neutralising solutions, such as dilute caustic or ammonia aqueous, should be used to neutralize the $(\text{CH}_3)_2\text{SO}_4$ spills.

3.2.2 Understanding the Process

Figure 3- 4 provides a flow chart of the deconstruction of *Miscanthus* in a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture at 120 °C for 22 h. The process was divided into four steps: (1) mixing and incubation, (2) recovery of CRM, (3) analysis of solubilised sugars and degradation products and (4) precipitation of Ionosolv-Lignin.

Deconstruction of Biomass in Ionic Liquids

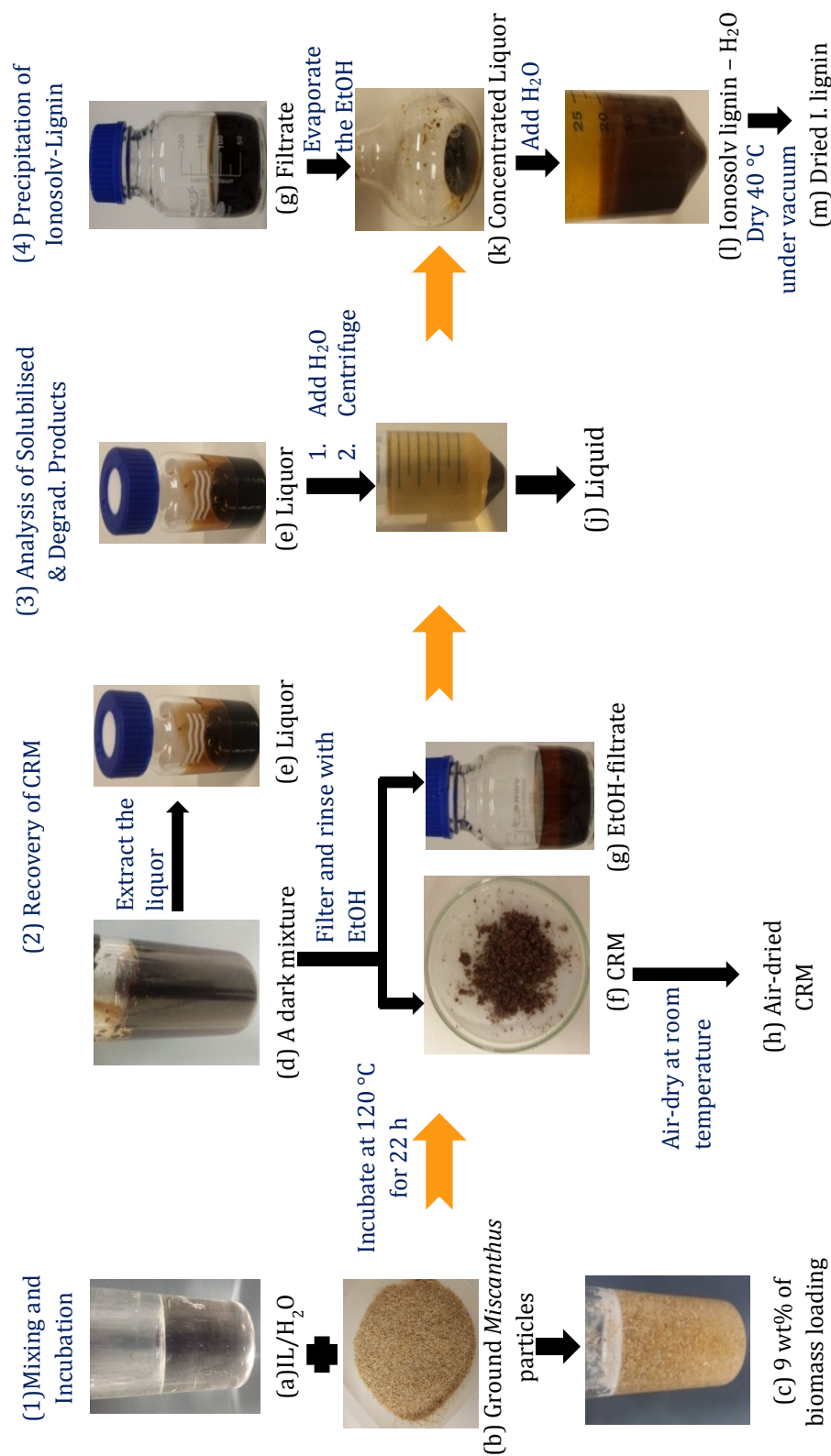


Figure 3- 4. Deconstruction of *Miscanthus* in a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h.

Step 1: Mixing and Incubation

Prior to the process, *Miscanthus* sticks were sliced and ground, forming smaller particles (**Figure 3- 4**, Step 1 (b)). Reducing the particle size of biomass increases not only the surface area to volume ratio²⁶, but also diffusion of the ionic liquid into the biomass structure.⁹¹ As a result, dissolution rates of biomass particles during the deconstruction process increase.⁹¹ Shaking of the pressure tube (**Figure 3- 4**, Step 1) was performed in order to enhance the surface contact between the biomass particles and the [HC₄im][HSO₄]/H₂O mixture.

Besides the ionic liquid's anion, the water content in deconstruction solvents and the period of incubation are two additional factors that influence the separation of biopolymers. Prolonging the incubation period of *Miscanthus* in [HC₄im][HSO₄]/H₂O (80/20 wt/wt) at 120 °C, from 4 to 22 h increased delignification and removal of hemicellulose, enhancing the purity of the cellulose.³ Meanwhile, reducing the amount of water of the [HC₄im][HSO₄]/H₂O from 20 to 5 wt/wt (incubation at 120 °C for 20 h) caused a substantial removal of the cellulose fraction.³

Step 2: Recovery of Cellulose-Rich-Material (CRM)

When the incubation period had completed, a dark mixture (**Figure 3- 4**, Step 2 (d)) was obtained. The dark mixture (**Figure 3- 4**, Step 2 (d)) was mixed with EtOH (30 mL) purposely to solubilise the [HC₄im][HSO₄]. The EtOH-[HC₄im][HSO₄] was separated from the CRM by Büchner filtration. As a result, 62.1±0.6 wt% of the CRM (relative to the mass of the untreated *Miscanthus* used) was recovered and labelled as 'Büchner-CRM'.

The impact of the deconstruction conditions on the surface morphology of the Büchner-CRM was analysed by Scanning Electron Microscopy (SEM). **Figure 3- 5** compares the micrographs of the untreated *Miscanthus* and the Büchner-CRM. Examination of the cross section of a *Miscanthus* particle (**Figure 3- 5** (a)-(b)) clearly showed linear-packed fibrils; one of the physical characteristics of the cellulose. They are well assembled and tightly packed, forming a bundle of fibrils wrapped in a sheath (**Figure 3- 5** (a)). The fibrils were extruded out of the sheath (**Figure 3- 5** (a)); a typical consequence of the grinding process applied to reduce the size of the *Miscanthus* particles

(Section 2.2.1, Chapter 2: Methodologies). The sheath exhibited a grainy texture, contrary to that of the core section (**Figure 3- 5 (a)**).

After the incubation process, the linear-rod structure of cellulose fibrils became more exposed and pronounced (**Figure 3- 5 (c)-(d)**). Hemicellulose acts as a bridge that connects cellulose and lignin. It also binds non-covalently to the surface of cellulose fibrils,⁹ interconnecting each fibril, eventually forming a rigid cellulose fibril network. Therefore, it was believed that under the incubation conditions, lignin and particularly hemicellulose were removed. This eventually loosened the rigid network, thus increasing the exposure of the cellulose fibrils (**Figure 3- 5 (c)-(d)**).

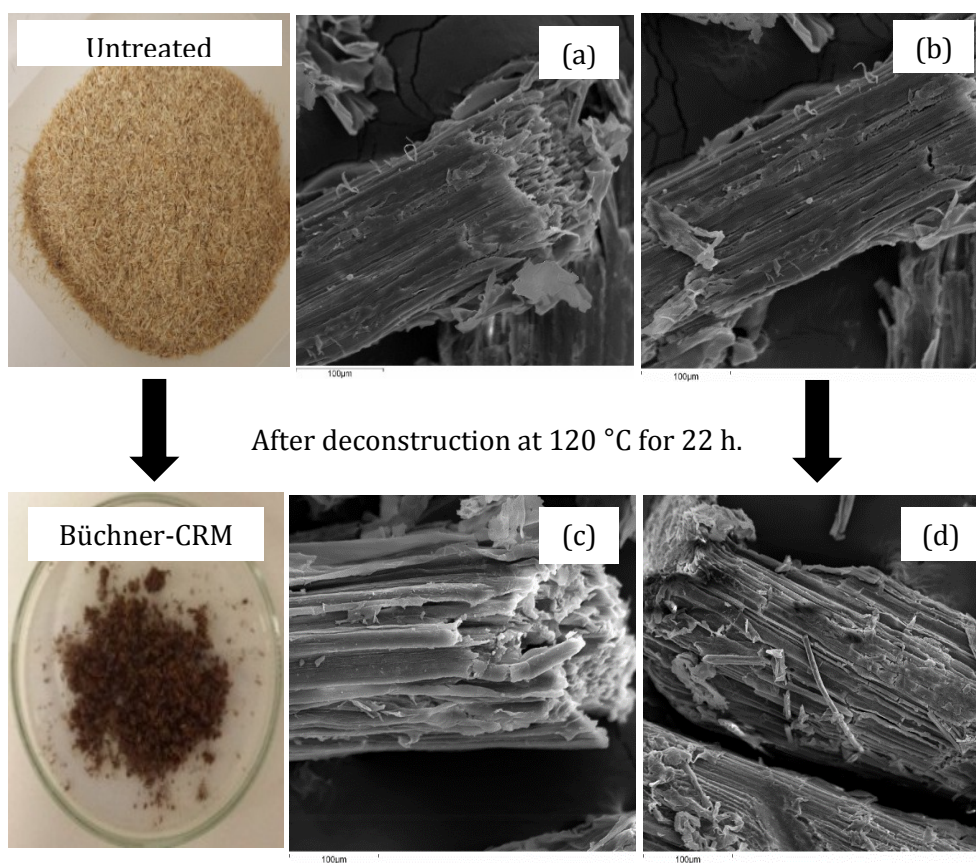


Figure 3- 5. SEM micrographs of (a)-(b) the untreated *Miscanthus*, and; (c)-(d) the Büchner-CRM. The Büchner-CRM was obtained after the incubation of *Miscanthus* a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h.

Attenuated Total Reflection-Infrared (ATR-IR) analysis was conducted on the Büchner-CRM to elucidate its chemical functional groups. The resultant spectrum was compared with the [HC₄im][HSO₄] ionic liquid and Microcrystalline Sigmacell-Cellulose (MCC) as shown in **Figure 3- 6**.

Table 3- 1 summarises the recorded IR-bands with their functional group assignment. Characteristic IR-bands of cellulose (**Figure 3- 6, (b)**), were observed in the Büchner-CRM (**Figure 3- 6 (a)**). However, the band at 897 cm^{-1} representing C-O-C glycosidic bonds linking glucose units in cellulose^{92,93}, had noticeably disappeared while some IR-bands in the $[\text{HC}_4\text{im}][\text{HSO}_4]$ (**Figure 3- 6 (c)**) had emerged. This might suggest that the Büchner-CRM was composed largely of cellulose, with both the $[\text{HC}_4\text{im}]^+$ and the $[\text{HSO}_4]^-$ adsorbed onto its surface.

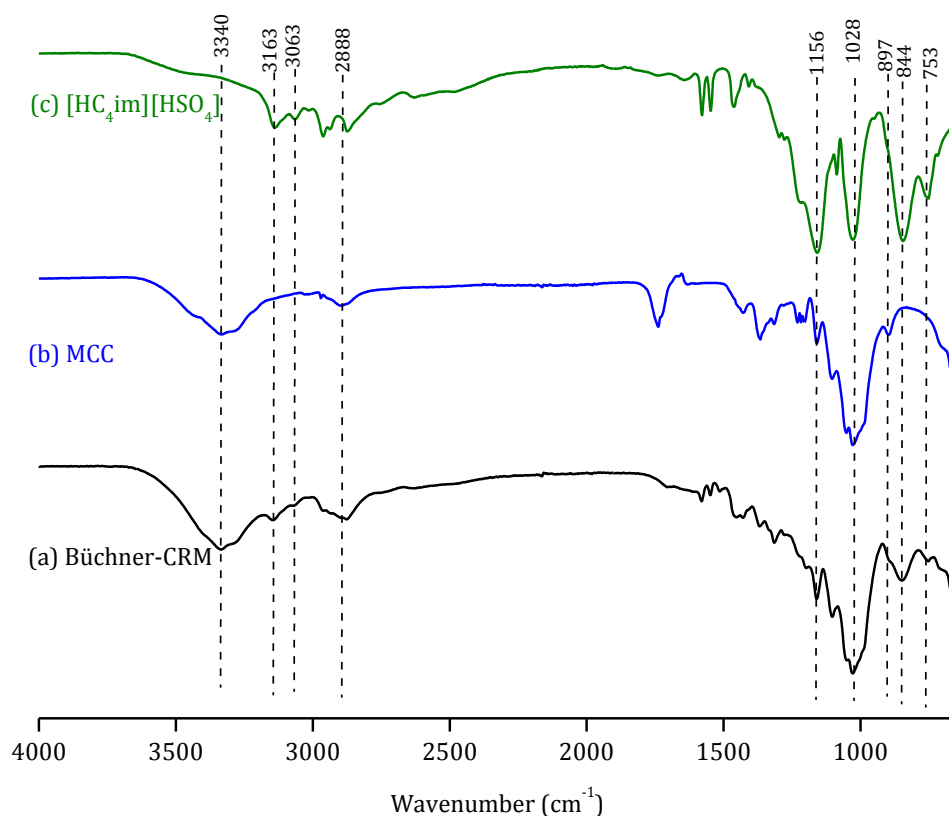


Figure 3- 6. ATR-IR spectra of the $[\text{HC}_4\text{im}][\text{HSO}_4]$ ionic liquid, Microcrystalline Sigmacell-Cellulose (MCC) and the Büchner-CRM obtained after the deconstruction of *Miscanthus* in a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture at $120\text{ }^\circ\text{C}$ for 22 h.

Table 3- 1. Functional groups of the Büchner-CRM obtained after the deconstruction of *Miscanthus* in a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h.

Wave number (cm ⁻¹)		Functional groups
This study	Literature	
3340	3600-3000 (Yang <i>et al.</i> , ⁹⁴)	O-H stretching
3146	3133 (Casford <i>et al.</i> , ⁹⁵)	Symmetric C-H _{ring} stretching from [HC ₄ im] ⁺
3021	3106 (Casford <i>et al.</i> , ⁹⁵)	Asymmetric C-H _{ring} stretching from [HC ₄ im] ⁺
2888	2860-2970 (Yang <i>et al.</i> , ⁹⁴) 2910(Serrano <i>et al.</i> , ⁹²)	C-H _n stretching of alkyl, aliphatic
1156	1169 (Serrano <i>et al.</i> , ⁹²)	C-O antisymmetric bridge stretching pyranose ring
1156	1300-1000 (Cha <i>et al.</i> , ⁹⁶)	SO ₄ symmetric stretching of [HSO ₄] ⁻
1028	1076-1023 (Serrano <i>et al.</i> , ⁹²)	C-O-C stretching vibration of pyranose ring
1028	1000-900(Cha <i>et al.</i> , ⁹⁶)	SO ₄ asymmetric stretching of [HSO ₄] ⁻
897	903 (Serrano <i>et al.</i> , ⁹²) 893 (Nelson <i>et al.</i> , ⁹³)	C-O-C glycosidic bond of pyranose ring
753	1000-900 (Cha <i>et al.</i> , ⁹⁶)	S-(OH) stretching of [HSO ₄] ⁻

Step 3: Analysis of Solubilised Sugars and Degradation Products

After the incubation process had completed, a small quantity of liquor (**Figure 3- 4**, Step 2 (e)) was extracted from the dark mixture (**Figure 3- 4**, Step 2 (d)). The liquor was analysed by High Performance Liquid Chromatography (HPLC) with Ultraviolet (UV) and Refractive Index (RI) detectors. **Figure 3- 7** summarises the results.

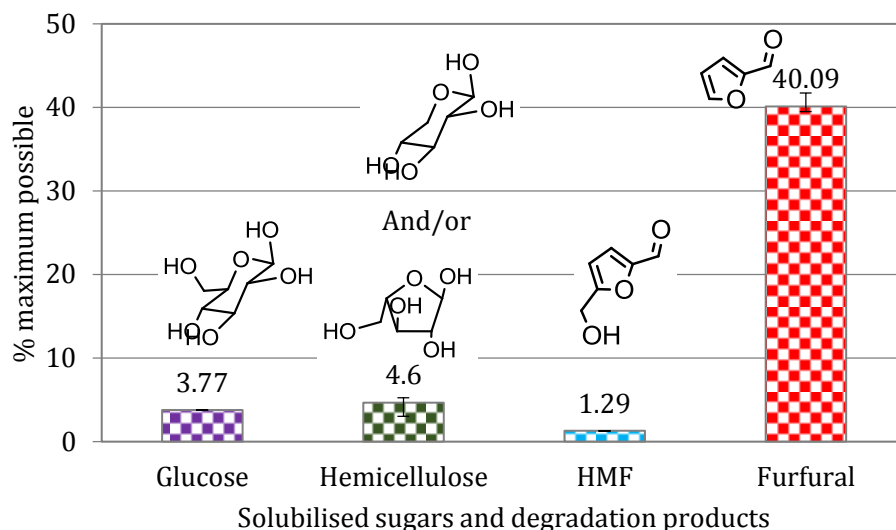


Figure 3- 7. Solubilised sugars and degradation products in the liquor. The liquor was extracted from the dark mixture after deconstruction of *Miscanthus* in a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture at 120 °C for 22 h. Error bars ($\pm$) are standard deviations of triplicate samples.

The liquor contained small quantities of glucose and hemicellulose sugars. This could be explained by the incubation conditions, $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture at 120 °C for 22 h, which have led to acid-catalysed hydrolysis. However, the amount of hemicellulose sugars was ca. 38% higher than that of glucose (**Figure 3- 7**). The likely reason is that depolymerisation of hemicellulose is easier than that of cellulose, due to its highly branched character.⁹ The glycosidic bonds in hemicellulose degrade 1500 times faster compared to those of cellulose.⁹⁷

It was apparent that during the incubation process, glucose, and hemicellulose sugars underwent dehydration reactions, forming 5-HMF and furfural (**Figure 3- 7**). The $[\text{HSO}_4]^-$ anion contains a mildly acidic proton.⁹⁸ A number of studies have demonstrated that $[\text{HSO}_4]^-$ -based ionic liquids degrade hexoses and pentoses, yielding 5-HMF and furfural, respectively.^{99,100} As an example, Lima *et al.* treated xylose in the ionic liquid 1-ethyl-3-methylimidazolium hydrogen sulfate, $[\text{C}_2\text{C}_1\text{im}][\text{HSO}_4]$, at 100 °C for 30 min; whereupon 86% of xylose degraded to furfural.⁹⁹ For comparative purpose, an ionic liquid 1-butyl-3-methylimidazolium chloride, $[\text{C}_4\text{C}_1\text{im}]\text{Cl}$ was used; no furfural was detected even after the reaction was extended to 4 h.⁹⁹ Thus, the study of Lima *et al.*⁹⁹ shows that Brønsted acidity possessed by the proton on the $[\text{HSO}_4]^-$ anion, is responsible for degradation reactions as observed in **Figure 3- 7**.

Furfural appeared to be the major product (**Figure 3- 7**). This could be related to the difference in stabilities of glucose and hemicellulose in an acidic medium, as reported previously.^{9,101} It was reported that in aqueous H₂SO₄ at pH 1.5, 160 °C for 30 min, xylose was more susceptible to degrade to furfural than glucose to 5-HMF.⁹ At lower pH values, glucose was inclined to decompose *via* fructose isomerisation followed by degradation of fructose to 5-HMF.¹⁰¹ However, a study on the kinetics of D-glucose conversion at 120 – 160 °C by *in situ* ¹³C-NMR concluded that conversion of D-glucose to D-fructose was a reversible reaction.¹⁰² The energy barrier for isomerisation of glucose to fructose was larger than that of converting fructose to 5-HMF *via* the dehydration process. The concentration of the open chain form of the D-glucose was extremely low, 0.04%.¹⁰² Moreover, the open chain form of glucose was reported to have a very short life time (<100 fs).¹⁰³ Unlike glucose, the degradation of xylose does not involve the isomerisation step. The 2,5-anhydride intermediate (furfural precursor) forms very rapidly where the reaction energy barrier is only 13 kcalmol⁻¹, thus increasing the rate of furfural production.

The presence of hemicellulose sugars and their main degradation product, furfural, in the liquor (**Figure 3- 7**) supported the initial assumption (Step 2: Recovery of Cellulose-Rich-Material, (CRM)) that hemicellulose was removed from the rigid cellulose fibrils network. As a result, the linear-rod structure of cellulose fibrils became more exposed (**Figure 3- 5(c)-(d)**).

Step 4: Precipitation of Ionosolv-Lignin

The filtrate (**Figure 3- 4**, Steps 2 and 4 (g)) was concentrated by evaporating the EtOH. When adding deionised water to the concentrated liquor (**Figure 3- 4**, Step 4 (k)), a black precipitate formed instantaneously (**Figure 3- 4**, Step 4 (k)-(l)); an indication of the formation of a lignin-rich precipitate. At the end of the process, 19.4±0.8 wt% (relative to the amount of the untreated *Miscanthus* used) of product was recovered and labelled as 'Ionosolv-lignin'. ATR-IR analysis of the Ionosolv-lignin (**Figure 3- 8** and **Table 3- 2**) recorded IR-bands that matched with those of lignin extracted from *Miscanthus*, as reported previously.⁹²

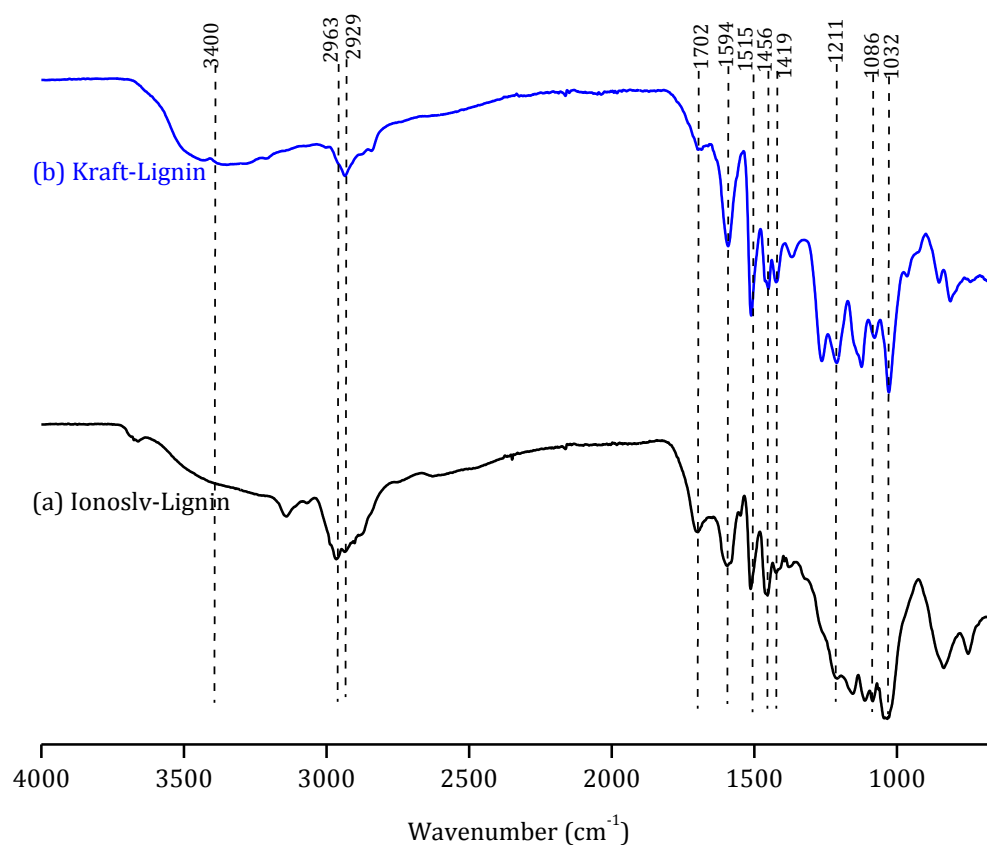


Figure 3- 8. IR spectra of Kraft-lignin and the Ionosolv-lignin obtained after deconstruction of *Miscanthus* in a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h.

Table 3- 2. Functional groups of the Ionosolv-lignin obtained after the deconstruction of *Miscanthus* in a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h.

Wavenumber (cm ⁻¹)		
This study	Literature (Serrano <i>et al.</i> , ⁹²)	Functional groups
3400	3400	Aromatic O-H group
2963	2960	C-H vibration of CH ₂ and CH ₃ groups
2929	2925	C-H vibration of CH ₂ and CH ₃ groups
1702	1705	Non-conjugated carbonyl groups
1594	1600	Aromatic ring vibrations of the phenyl propane
1515	1515	skeleton
1456	1460	C-H vibration of CH ₂ and CH ₃ groups
1419	1425	Aromatic ring vibrations of the phenyl propane
		skeleton
1211	1220	Ether bridges vibrations
1086	1080	Ether bridges vibrations
1032	1030	Aliphatic O-H group

Summary

In summary, under the deconstruction conditions of a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h, successful separation of CRM and lignin was achieved. Relative to the amount of untreated *Miscanthus* used the recovered CRM, Büchner-CRM, weighed 62.1±0.6 wt% while the recovered lignin, Ionosolv-Lignin, weighed 19.4±0.8 wt%. Under the SEM microscope, the Büchner-CRM exhibited more exposed rod-like particles (**Figure 3- 5** (c)-(d)). The Büchner-CRM was constituted of cellulose as suggested by the ATR-IR analysis (**Figure 3- 6, Table 3- 1**), however, its surface contained traces of [HC₄im][HSO₄]. Lignin was the main component in the Ionosolv-lignin as observed by the ATR-IR analysis (**Figure 3- 8 and Table 3- 2**).

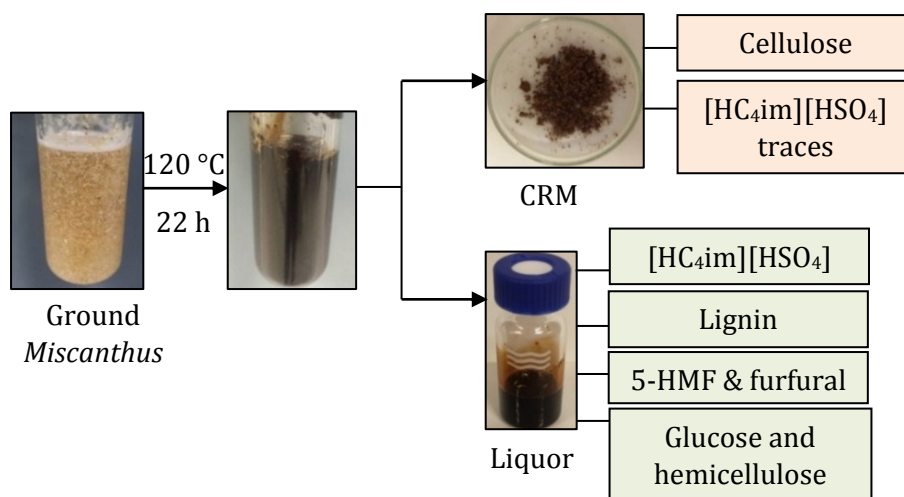


Figure 3- 9. Deconstruction of *Miscanthus* a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h.

3.2.3 Understanding Surface Interaction

Overview of Experiment

In the deconstruction of biomass using ionic liquids, the process will always leave a small residual quantity of ionic liquid on recovered CRMs.⁴⁷ Similarly, it was also observed (Section 3.2.2, Step 2) that [HC₄im][HSO₄] traces were on the surface of the Büchner-CRM. If the [HC₄im][HSO₄] is only subject to physisorption, then a comprehensive purification, such as Soxhlet extraction, seems to be the best option to significantly remove the [HC₄im][HSO₄] traces from the CRM. Therefore, an experiment was designed to examine this in detail (Flow chart in Figure 3- 10).

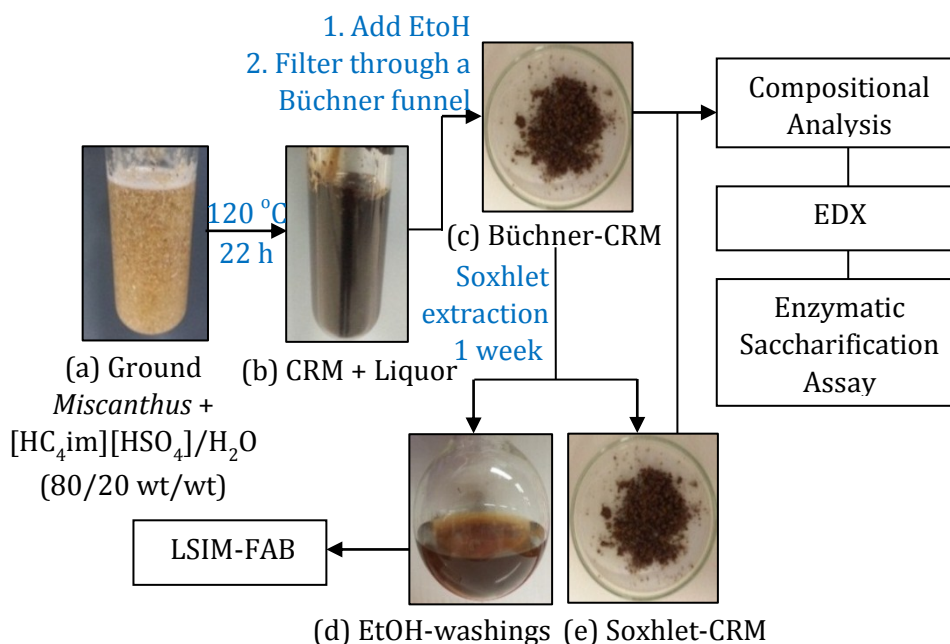


Figure 3- 10. Interaction of CRM with the [HC₄im][HSO₄] ionic liquid.

A small quantity of the Büchner-CRM (**Figure 3- 10 (c)**) was continuously purified with EtOH in a Soxhlet extractor for one week. This resulted in a purified CRM labelled as ‘Soxhlet-CRM’ (**Figure 3- 10 (e)**). The Büchner-CRM and the Soxhlet-CRM were then both characterised using the following techniques: Compositional Analysis, composition of biopolymers, cellulose, hemicellulose, and lignin; surface chemical identification; Energy-Dispersed X-ray (EDX), surface elemental composition; and enzymatic saccharification assay for production of glucose. The EtOH, collected at the end of the Soxhlet extraction (**Figure 3- 10 (d)**), was labelled as ‘EtOH-washings’. Liquid Secondary Ionisation Mass Spectroscopy – Fast Atomic Bombardment (LSIMS-FAB) was employed to analyse chemical constituents in the EtOH-washings.

Results and Discussion

Mass Recovery and Composition of Biopolymers

The masses of CRMs recovered from different purification techniques (**Figure 3- 10**) are recorded in **Table 3- 3**. The Soxhlet-CRM (**Figure 3- 10 (e)**) recovered 6.7 wt% less than the Büchner-CRM (**Figure 3- 10 (c)**). Compositional analysis measures, gravimetrically, the composition of biopolymers, cellulose, hemicellulose, xylose and arabinose,

and lignin, in the recovered CRMs. **Table 3- 3** also tabulates the results of this analysis performed on the Büchner-CRM (**Figure 3- 10 (c)**) and the Soxhlet-CRM (**Figure 3- 10 (e)**).

Table 3- 3. Effect of different purification techniques on the composition (wt%^a) of the recovered CRMs. Error values are standard deviations of triplicate samples.

Components	Untreated	Büchner-CRM	Soxhlet-CRM
Recovered CRM ^b	-	62.1±0.6	55.4±0.2
Glucan	47.4±0.2	40.9±0.2	43.7±0.6
Xylan	23.8±0.2	0.294±0.04	-
Arabinan	0.644±0.04	-	-
CA-Lignin ^c	24.1±0.4	18.1±0.7	10.8±0.7
Extractives	4.30	-	-
Ash	0.594±0.1	2.77±1.0	1.09±1.0
Mass Loss	-	37.9	44.3

^a Composition (wt%) is expressed as amount of product (mg) obtained upon completion of compositional analysis, per amount of CRM used (300 mg).

^b Recovered CRM (wt%) is expressed as weight of CRM recovered after deconstruction (g) per amount of the untreated *Miscanthus* used (g).

^c CA-Lignin refers to lignin recovered from samples or CRMs upon completion of compositional analysis, per amount of samples or CRMs used (300 mg).

The removal of hemicellulose from *Miscanthus* was reported to be time dependent.^{3,29} For example, Brandt *et al.*, extended the period of incubation from 4 to 22 h, as a result, the removal of hemicellulose from *Miscanthus* was improved from 84% to 92%.³ A similar trend was observed in this study where the incubation conditions of a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h completely removed hemicellulose (**Table 3- 3**). This appears consistent with the observations in **Section 3.2.2**. Higher removal of hemicellulose might indicate complete disruption of interpolymer linkages connecting hemicellulose-cellulose and hemicellulose-lignin, which in turn enhanced the exposure of the cellulose fibrils, as seen in **Figure 3- 5 (Section 3.2.2)**. However, the removal of lignin seemed not to be very affected (**Table 3- 3**).

Table 3- 3 also highlights the importance of purification technique on the composition of the recovered CRMs. Relative to the untreated *Miscanthus*, the Büchner-CRM (**Figure 3- 10 (c)**) had 25% less lignin, while the ash content increased by two orders of magnitudes (**Table 3- 3**). After purifying the Büchner-CRM using the Soxhlet extraction, the resultant Soxhlet-CRM (**Figure 3- 10 (e)**) had the following composition: relative to the Büchner-CRM, hemicellulose was not detected by gravimetric means; lignin and ash contents further decreased by ca. 40% and ca. 60%, respectively.

The removal of lignin in this experiment (Soxhlet-CRM, **Table 3- 3**) was much lower than that observed by Brandt *et al.* where ca. 80% of lignin was removed from *Miscanthus* using a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture at 120 °C for 20 h.³ An increase in lignin content was previously observed when the deconstruction used Brønsted acid catalysts, including $[\text{HSO}_4]$ -ionic liquids.^{28,29} Two factors could possibly be associated with the observed higher lignin content in this experiment (**Table 3- 3**): formation of pseudo-lignin, a lignin-free compound possessing similar characteristics as lignin^{28,29}; and formation of carbon-carbon bonds induced by condensation reactions.^{104,105} According to a previous study, the pseudo-lignin was obtained when treating free lignin-holocellulose in an acidic medium.¹⁰⁴ The production of the pseudo-lignin increased as more holocellulose degraded.¹⁰⁴ Retention of lignin in CRMs could also be caused by condensation reactions of lignin under an acidic environment, forming carbon-carbon bonds. As a result, the condensed lignin traps in cellulose fibrils, making the condensed lignin less soluble in organic solvents.¹⁰⁵ Condensation might also occur from an *in situ* reaction between degradation products, 5-HMF and furfural, with benzyl carbonium ions, highly reactive intermediates of lignin.¹⁰⁵ Brandt *et al.* observed an increase in lignin content of 8% when hemicellulose was further degraded by half, as a result of extending the period of incubation from 4 to 20 h.³ However, an explanation was not provided.³

Besides measuring the composition of biopolymers by gravimetric means, compositional analysis also indirectly provides an indication of the presence of ionic liquid traces.²⁸ In general, according to the NREL protocol⁴², the compositional analysis could be divided into two major steps: (1) acid hydrolysis; and (b) thermochemical treatment. The former step hydrolyses: interpolymer-ester bonds, connecting lignin and cellulose-hemicellulose

fractions; and intrapolymer-ether bonds, within cellulose and hemicellulose. As a result, two products are obtained: a soluble product contains glucose and hemicellulose sugars, which are then analysed by HPLC; and an insoluble product comprises mostly lignin. Subsequently, the latter step, thermochemical treatment, is conducted where the insoluble product is heated at a high temperature, removing lignin. Any leftover traces of the thermochemical treatment are called 'ash'. George *et al.* analysed CRMs obtained from deconstruction of *Switchgrass* using alkylammonium hydrogen sulfate, $[R_nNH_m][HSO_4]$ ionic liquids. The ash produced at the end of the compositional analysis, exhibited a strong correlation with sulfur content; an indication of incomplete removal of the $[R_nNH_m][HSO_4]$ traces.²⁸ Based on these observations, the production of ash in this experiment (**Table 3- 3**) therefore denoted the $[HC_4im][HSO_4]$ traces in the recovered CRMs. As expected, the Soxhlet-CRM produced lesser ash than the Büchner-CRM (**Table 3- 3**). Reduced surface retention of the $[HC_4im][HSO_4]$ traces in the Soxhlet-CRM, obtained from continuous purification, was believed to be the main contributing factor. Interestingly, despite being purified for one week, the Soxhlet-CRM still produced some quantities of ash, even much higher than that of the untreated *Miscanthus*. George *et al.* also washed their CRMs in Soxhlet extractors for one week, which then suggested that sulfur might be chemically bound on the surface of their CRMs.²⁸

Identification of Chemical Constituents in the EtOH-Washings

The EtOH-washings (**Figure 3- 10(d)**) were concentrated and then analysed by LSIMS-FAB to identify the chemical constituents. The resultant spectra are shown in **Figure 3- 14**. The $[HC_4im]^+$ cation and the $[HSO_4]^-$ anion were both detected at $m/z = 125$ (**Figure 3- 11 (a)**) and at $m/z = 97$ (**Figure 3- 11 (b)**), respectively. These ions were removed from the Büchner-CRM into the EtOH solvent during the Soxhlet extraction process; a strong indication of physisorption. This corroborates with the compositional analysis whereby the Soxhlet-CRM produced much lower ash content than the Büchner-CRM (**Table 3- 3**).

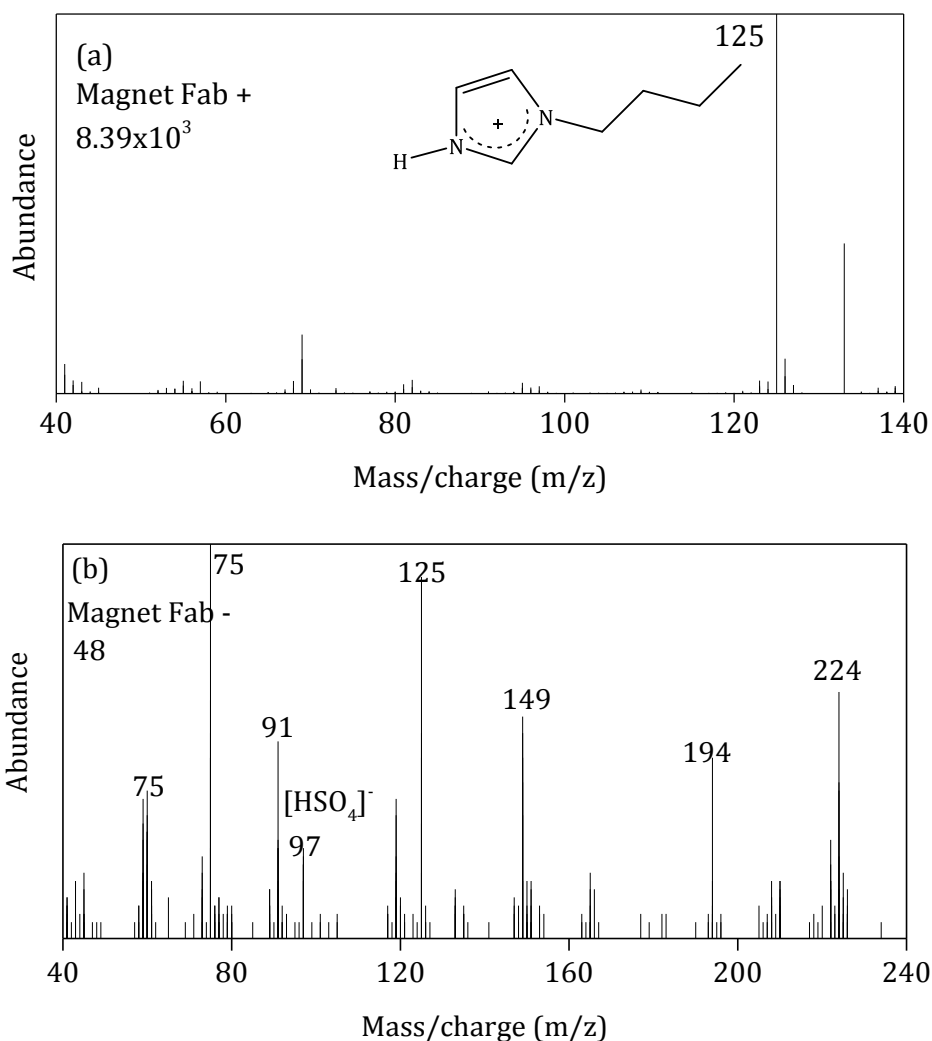


Figure 3- 11. LSIMS-FAB spectra of (a) positive ion mode, and; (b) negative ion mode of the EtOH-washings obtained after continuous purification of the Büchner-CRM with Soxhlet extraction for one week (**Figure 3- 10** (c)-(e)).

Besides the [HSO₄]⁻ anion, other ion peaks were also detected. The EtOH-washings appeared darker (**Figure 3- 10**) than the colourless initial EtOH. As observed in **Section 3.2.2**, the colour of the liquor, obtained after the incubation had completed, was noticeably dark (**Figure 3- 10**, Step 3 (e)), due to the presence of lignin (**Section 3.2.2**, Step 4) glucose, hemicellulose sugars, 5-HMF and furfural (**Figure 3- 4**, **Section 3.2.2**, Step 3). Additionally, the compositional analysis (**Table 3- 3**) demonstrated the loss of hemicellulose and particularly lignin from the CRM (Büchner-CRM) following the Soxhlet extraction (Soxhlet-CRM). Thus, the presence of extracted hemicellulose and lignin was believed to be the explanation for the darkening of the EtOH-washings.

During the Soxhlet extraction (**Chapter 2, Figure 2- 2**), a collector flask was heated at 80 °C throughout the period of one week. This was performed to evaporate EtOH, enabling purification process. However, this condition might be too harsh for the extracted lignin and hemicellulose. As a result, both extracted polymers might have degraded, leading to the formation of other compounds detected in the negative ion mode (**Figure 3- 11 (b)**).

Surface Composition of Recovered CRMs

The impact of purification on the surface composition of the resultant CRMs was further examined using EDX. **Figure 3- 12** shows the obtained sulfur mapping micrographs. Sulfur is one of the chemical elements of the $[\text{HC}_4\text{im}][\text{HSO}_4]$ ionic liquid. The surface of Büchner-CRM appeared to be enriched with sulfur (**Figure 3- 12 (b)**). The presence of sulfur was far less visible on the surface of the Soxhlet-CRM (**Figure 3- 12 (d)**). **Table 3- 4** summarises the data obtained from the EDX analysis.

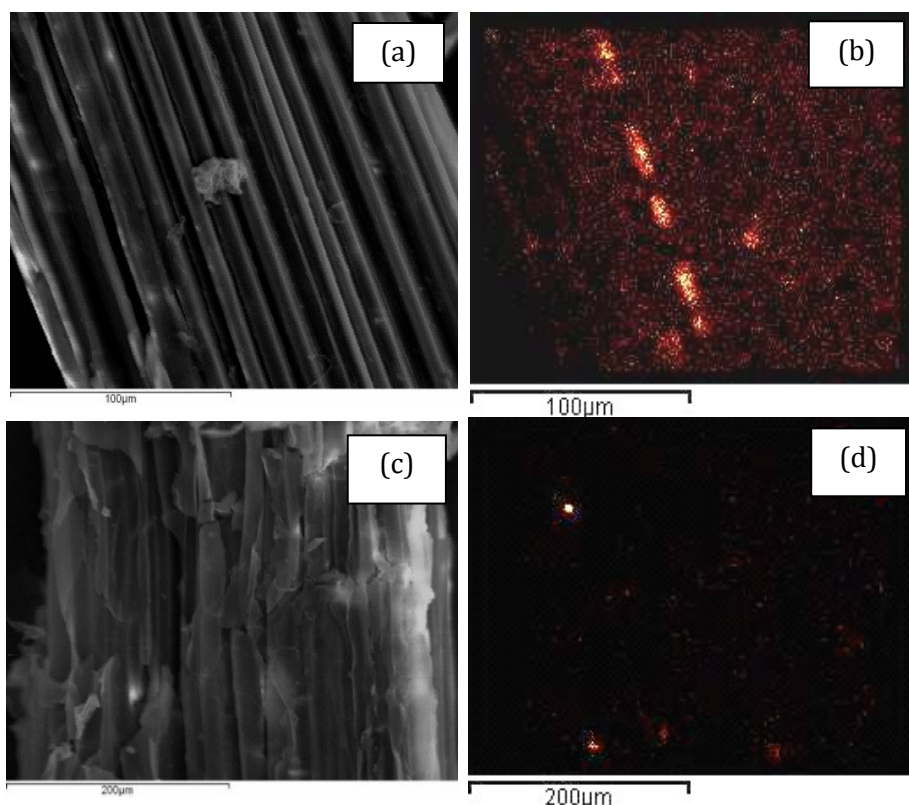


Figure 3- 12. EDX sulfur mapping of: (a)-(b) the Büchner-CRM, and, (c)-(d) Soxhlet-CRM. Red colouration indicates sulfur.

Table 3- 4. EDX surface elemental composition of the untreated *Miscanthus*, the Büchner-CRM and the Soxhlet-CRM. Error values are standard deviations from values obtained from several focal points.

Chemical Elements	Atomic % (K shell)		
	Untreated	Büchner-CRM	Soxhlet-CRM
Carbon, C	56.8±4.0	76.0±2.0	53.6±3.0
Oxygen, O	42.8±4.0	19.8±3.0	46.2±5.0
Silicon, Si	0.0193±0.02	0.0311±0.03	0.0463±0.05
Sulfur, S	0.0245±0.04	2.07±0.06	0.0547±0.02
Chlorine, Cl	0.136±0.02	-	-
Potassium, K	0.162±0.02	0.0166±0.07	-

The theoretical C/O value for cellulose and hemicellulose is 1:1. For the untreated *Miscanthus*, the C/O value was closer to that of cellulose or hemicellulose, rather than lignin. Lignin has far higher % carbon and far lower % oxygen. However, a small increase in % carbon with a small reduction in % oxygen in the untreated *Miscanthus* (relative to theoretical C/O of cellulose or hemicellulose) might be due to a small fraction of lignin bound to the cellulose-hemicellulose on the surface. The untreated *Miscanthus* also contained a small quantity of sulfur, chlorine, and potassium.

Relative to the untreated *Miscanthus*, the surface of the Büchner-CRM contained a much higher % of carbon than % of oxygen. This is most likely to indicate disintegrated lignin and the [HC₄im][HSO₄], which were not removed during the Büchner filtration (**Figure 3- 10**). However, it is very difficult to distinguish these compounds using EDX. This is because each chemical element only produces a specific x-ray photon energy, regardless of differences in its chemical states. As an example, carbon and oxygen will emerge at energies (K_α) of 0.277 keV¹⁰⁶ and 0.523 keV¹⁰⁶ in EDX spectra, irrespective of whether the analysed sample is either cellulose or lignin. However, mass recovery and compositional analysis (**Table 3- 3**) provided a clear indication of the presence of disintegrated lignin and the [HC₄im][HSO₄] on the surface of the Büchner-CRM. An increase in sulfur content (Büchner-CRM, **Table 3- 4**) indicates a trace quantity of [HC₄im][HSO₄], which in turn increased the production of ash during the compositional analysis, as observed previously (**Table 3- 3**).

For the Soxhlet-CRM, the C/O value was considerably closer to that of the untreated *Miscanthus* (**Table 3- 4**). The compositional analysis (**Table 3- 3**) demonstrates the loss of lignin after purifying the Büchner-CRM in a Soxhlet extraction for one week. Thus, a decrease in C/O value (Soxhlet-CRM, and **Table 3- 4**) was likely due to the removal of lignin. The compositional analysis (**Table 3- 3**) shows that Soxhlet-CRM produced much lower ash than the Büchner-CRM, due to the extraction of the physisorbed $[\text{HC}_4\text{im}][\text{HSO}_4]$ traces by the EtOH (LSIM-FAB, **Figure 3- 11**). Thus, less sulfur content was detected in the Soxhlet-CRM by EDX (**Figure 3- 12** and **Table 3- 4**). Interestingly, despite being continuously purified for one week, sulfur content in the Soxhlet-CRM was nevertheless higher than that of the untreated *Miscanthus* (**Table 3- 4** and **Figure 3- 12**). In accordance with the compositional analysis (Soxhlet-CRM, **Table 3- 3**), the $[\text{HSO}_4]^-$ anion was therefore speculated to chemically bind onto the surface of the CRM. The surface of the Soxhlet-CRM was estimated to have one sulfur atom per 163 glucose monomer units.

Examination of Glucose released

An enzymatic saccharification assay evaluates the release of glucose by deconstructed biomass in ionic liquids.⁹ The assay is known to be less tolerant towards lignin¹⁰⁷ and ionic liquid traces¹⁰⁸; these inhibitors block the access of hydrolases to cellulose, which in turn suppresses the release of glucose. Hydrolases are enzymes that hydrolyse β -1,4 glycosidic bonds in cellulose, for example cellulase and cellobiase. The enzymatic saccharification assay was conducted on the Büchner-CRM and the Soxhlet- CRM. The results are illustrated in **Figure 3- 13**.

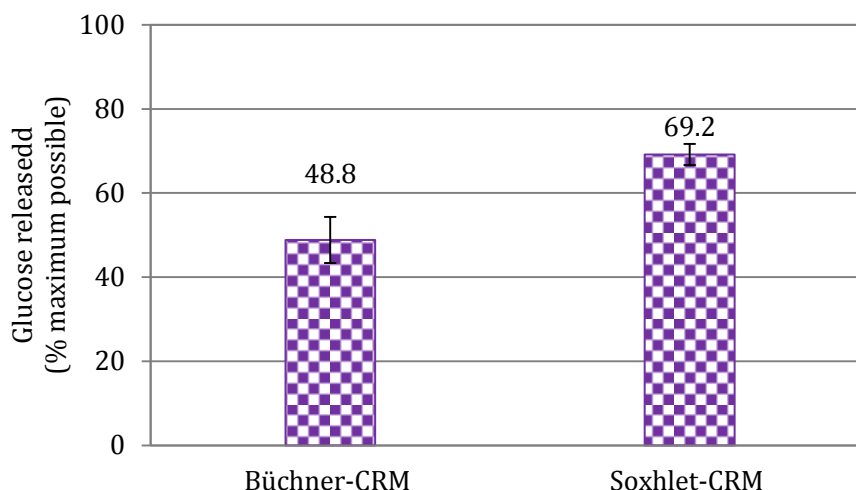


Figure 3- 13. Glucose released (%) from the Büchner-CRM and the Soxhlet-CRM *via* enzymatic saccharification. The assay employed a mixture of cellulose and cellobiase enzymes, conducted at 50 °C for 72 h. The data was corrected with the glucose content found in the enzyme mixture. The glucose released was calculated relative to 100 mg of CRM used for the assay. Error bars ($\pm$) are standard deviations of triplicate samples.

As anticipated, CRM with a much cleaner surface released a far greater quantity of glucose; total glucose released by the Soxhlet-CRM was ca. 40% greater compared to that of the Büchner-CRM (**Figure 3- 13**). During the assay, the physisorbed residuals, disintegrated lignin, hemicellulose and the $[\text{HC}_4\text{im}][\text{HSO}_4]$ trace may have been desorbed from the Büchner-CRM into the assay's buffered media. This was believed to result in an inactivation of cellulase and cellobiase enzymes, inhibiting the release of glucose. Based on previous literature^{107,108}, the inactivation of the enzymes was believed to occur *via*: (1) non-productive binding of the enzymes to lignin, and (2) coordination of the $[\text{HSO}_4]^-$ anion to positively charged residues on the enzymes. To demonstrate that the $[\text{HSO}_4]^-$ anion can impair the enzymatic activity of the enzymes, assay mixtures were contaminated with the $[\text{HC}_4\text{im}][\text{HSO}_4]$. Microcrystalline Sigmacell-cellulose (MCC) was used to mimic CRM. The glucose released as a result of adding contaminant is shown in **Figure 3- 13**.

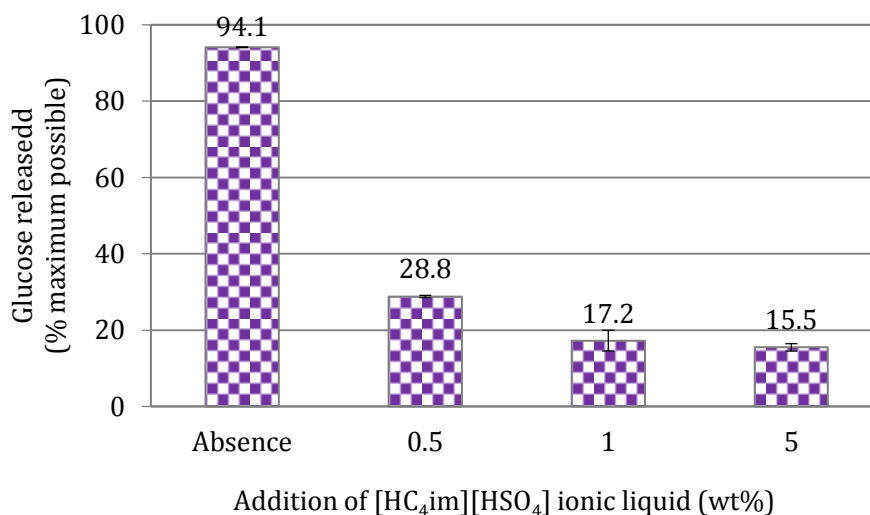


Figure 3- 14. Glucose released (%) from the Untreated-MCC as a function of quantity of [HC₄im][HSO₄] added. The assay employed a mixture of cellulose and cellobiase enzymes, conducted at 50 °C for 72 h. The data was corrected with the glucose content found in the enzyme mixture. The glucose released was calculated relative to 100 mg of CRM used for the assay. Error bars ($\pm$) are standard deviations of triplicate samples.

From **Figure 3- 14**, it can be observed that the amount of glucose released markedly dropped even with addition of a small quantity of contaminant (0.5 wt%). Increasing the quantity of [HC₄im][HSO₄] steadily decreased the glucose released from the Untreated-MCC. The Soxhlet-CRM had a far cleaner surface, thus, the inactivation of the enzymes was minimised. This is likely to be the best explanation for an increase in the glucose released by the Soxhlet-CRM (**Figure 3- 13**).

Summary

The overall findings of this investigation are summarized **Figure 3- 15**. The purification step had an impact on the composition of the recovered CRMs. As demonstrated by mass recovery (**Table 3- 3**), compositional analysis (**Table 3- 3**) and EDX analysis (**Figure 3- 12**, and **Table 3- 4**) inadequate purification led to the retention of the following residues on the surface of recovered CRM (Büchner-CRM): disintegrated lignin, hemicellulose and $[\text{HC}_4\text{im}][\text{HSO}_4]$ traces. Continuous purification by Soxhlet extraction enhanced the removal of these residuals, yielding the CRM (Soxhlet-CRM) with a significantly cleaner surface. This subsequently reduced non-productive binding of cellulase and cellobiase enzymes, enabling more glucose to be released during the enzymatic saccharification assay (**Figure 3- 13**). Besides physisorption as the predominant mechanism for the CRM- $[\text{HC}_4\text{im}][\text{HSO}_4]$ interaction (LSIMS-FAB, **Figure 3- 11**; and EDX analysis, **Figure 3- 12**), several observations were made suggesting that chemisorption might have also been involved. Despite being continuously purified for one week in a Soxhlet extractor, the Soxhlet CRM still had $[\text{HC}_4\text{im}][\text{HSO}_4]$ traces on the surface: sulfur colourisation was still visible, albeit at a far lower intensity, with an estimation of one sulfur atom per 100 glucose monomer units (EDX analysis, **Figure 3- 12** and **Table 3- 4**). Moreover, the production of ash had increased by ca. 50% relative to that of the untreated *Miscanthus* (compositional analysis, **Table 3- 3**). Chemisorption was speculated to involve the $[\text{HSO}_4]^-$ anion; however, additional investigation is necessary to confirm this analysis.

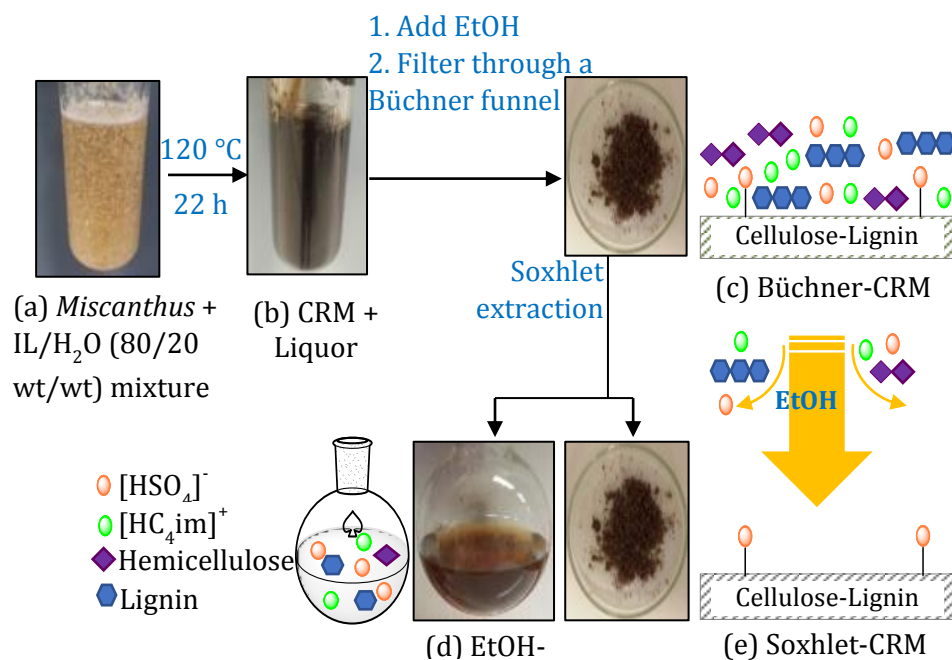


Figure 3- 15. Surface interactions between the CRM and the [HC₄im][HSO₄] ionic liquid during the deconstruction process.

4. Understanding the Nature of Adsorption

4.1 Background of Study

Motivation

The study in **Chapter 3** demonstrated that separation of CRMs and lignin in *Miscanthus* was successfully achieved under incubation conditions of [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h. What is more interesting is that this study shows there is a likelihood of the [HC₄im][HSO₄] chemically interacting with CRMs. Unfortunately, it is still unclear how CRMs and the [HC₄im][HSO₄] could chemically interact throughout the course of incubation process.

Cellulose, a major constituent of CRMs (**Table 3- 3**), was believed to likely undergo esterification with the [HSO₄]⁻ anion in this study. Sulfur containing solvents, such as sulfuric acid, H₂SO₄, and sulfamic acid, NH₂SO₃, are active towards esterifying hydroxyl groups on cellulose. Two types of esterification reaction have been reported: sulfation forming a carbon-oxygen-sulfur, -C-O-S-, bond, and; sulfonation forming a carbon-sulfur-oxygen, -C-S-O- bond.^{15,55-57,65,109} Interestingly, a recent study found that cellulose not only underwent a partial hydrolysis but also a sulfation reaction in an alkylimidazolium hydrogen sulfate, [C_mC_nim][HSO₄] ionic liquid. This was observed by Mao *et al.* when incubating Microcrystalline Sigmacell-cellulose, MCC in an ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate, [C₄C₁im][HSO₄] ionic liquid under conditions of a [C₄C₁im][HSO₄]/H₂O (75/25) mixture at 100 °C for 12 h.¹¹⁰ Mao *et al.*'s claim took account of a small quantity of sulfur detected by CHNS analysis; however, this is the only strong evidence provided. It is known that CHNS analysis is not able to identify chemical bonds; therefore, the assumption made by Mao *et al.* might be erroneous.

Thus, this study was designed to verify that the interaction of CRMs with the [HC₄im][HSO₄], particularly the [HSO₄]⁻ anion, involved chemisorption, in addition to physisorption. To exclude any possible interference arising from either lignin or hemicellulose, Microcrystalline Sigmacell-Cellulose (MCC) was used to mimic cellulose in *Miscanthus*. A series of characterization techniques were employed; this is to identify the most sensitive technique to detect the presence of sulfur-containing cellulose compounds.

Problem Statement

No previous studies have well characterised cellulose samples treated in alkylimidazolium hydrogen sulfate, $[C_nC_mim][HSO_4]$, ionic liquids to unveil possibilities of chemisorption.

Hypothesis

If it happens that cellulose is chemically interacted with the $[HSO_4]$ anions, any analytical techniques must be able to detect the presence of either sulfated or sulfonated compounds.

Objectives

This study has the following objectives:

1. To understand the nature of adsorption (physisorption or chemisorption) of cellulose and the $[HC_4im][HSO_4]$. MCC was used as a model compound of cellulose in CRM during incubation process.
2. To identify the most sensitive technique that can identify the chemisorption interaction between MCC and the $[HC_4im][HSO_4]$.
3. To examine the impact of synthesis routes concerning the $[HC_4im][HSO_4]$ and 1-butyl-3-methylimidazolium hydrogen sulfate, $[C_4C_1im][HSO_4]$, on chemisorption interaction with MCC.

4.2 Overview of Experiment

This experiment attempted to understand the nature of adsorption between cellulose and imidazolium hydrogen sulfate, $[C_nC_{mim}][HSO_4]$, ionic liquids during the course of the deconstruction process. Using similar conditions as applied to the deconstruction of *Miscanthus* (**Chapter 3**), the experiment was designed according to **Figure 4- 1**.

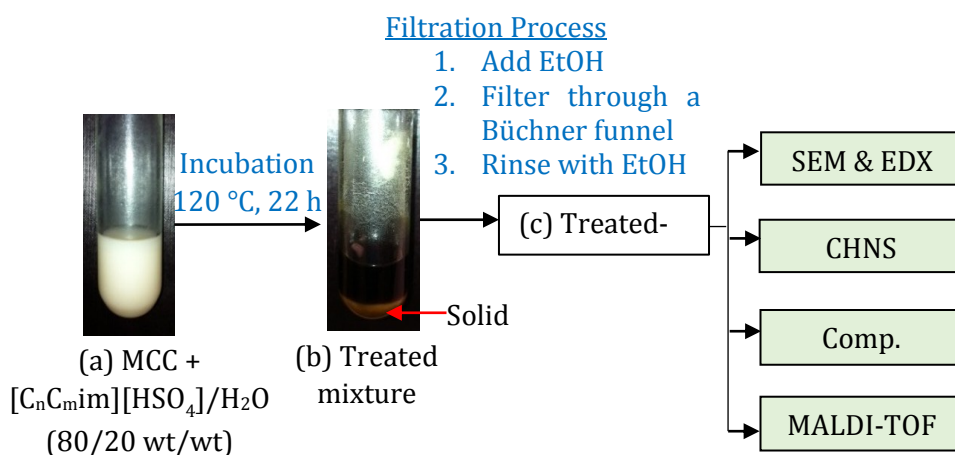


Figure 4- 1. Treatment of MCC in a $[C_nC_{mim}][HSO_4]/H_2O$ (80/20 wt/wt) mixture at 120 °C for 22 h.

The Untreated-MCC was added to a $[C_nC_{mim}][HSO_4]/H_2O$ (80/20 wt/wt) mixture giving a total MCC loading of 9 wt% (**Figure 4- 1** (a)), followed by incubation at 120 °C for 22 h. When the incubation period had elapsed, a 'treated mixture', contained a liquor and a solid fraction, was obtained (**Figure 4- 1** (b)). The treated mixture was then subjected to a filtration process (**Figure 4- 1**, Filtration Process), resulting in a solid fraction denoted as 'Treated-MCC'.

Two $[C_nC_{mim}][HSO_4]$ ionic liquids were employed: 1-butylimidazolium hydrogen sulfate, $[HC_4im][HSO_4]$, and 1-butyl-3-methylimidazolium hydrogen sulfate, $[C_4C_1im][HSO_4]$. The resultant solid fractions (**Figure 4- 1** (d)) were labelled as 'Treated MCC- $[HC_4im][HSO_4]$ ' and 'Treated MCC- $[C_4C_1im][HSO_4]$ '.

The physical and chemical properties of the resultant Treated-MCC samples were examined using the following techniques:

1. Compositional analysis: actual composition of cellulose.
2. Scanning Electron Microscopy and Energy Dispersed X-Ray (SEM & EDX, respectively): surface morphologies and surface chemical composition, respectively.
3. Infrared spectroscopy: chemical functional groups.
4. CHNS elemental analysis: total elemental composition.
5. Matrix Assisted Laser Desorption Ionisation combined with Time of Flight (MALDI-TOF): mass spectroscopy, masses of repeating units and end-group, and polymer molecular weight distribution.

4.3 Results and Discussion

4.3.1 Detection of Sulfur-Containing Oligosaccharides

Composition of the Untreated MCC

The composition of cellulose in the Untreated-MCC was initially measured using compositional analysis.⁴² In addition to cellulose as the primary polymer, other constituents were also detected by gravimetric methods (relative to the initial amount of Untreated-MCC, ca. 300 mg): 2 ± 0.1 wt% of hemicellulose comprising arabino-xylans, gluco-mannans, and galactans¹⁰ (derived from xylose and mannose monomers), and; 1.21 wt% of ash. The Untreated-MCC is obtained from cotton liners (Sigma Aldrich UK, CAS number: 9004-34-6); it typically contains nearly 95% of pure cellulose.¹¹

Examination of Surface Morphology

The treatment began by employing the ionic liquid $[\text{HC}_4\text{im}][\text{HSO}_4]$, yielding Treated MCC- $[\text{HC}_4\text{im}][\text{HSO}_4]$. Morphological changes, which might potentially be experienced by the MCC following treatment with $[\text{HC}_4\text{im}][\text{HSO}_4]$, were investigated using SEM. **Figure 4-2** compares the obtained micrographs of the Untreated-MCC and the Treated MCC- $[\text{HC}_4\text{im}][\text{HSO}_4]$.

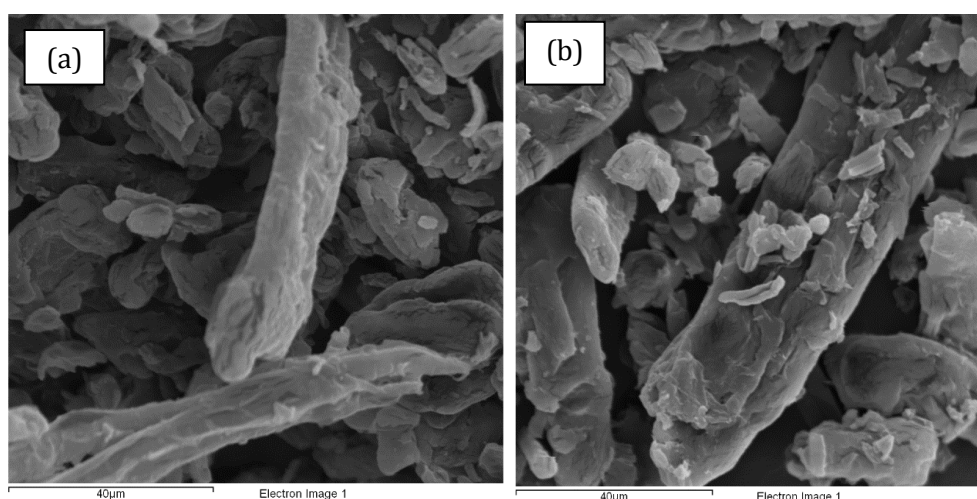


Figure 4-2. SEM micrographs of (a) Untreated-MCC, and; (b) Treated MCC- $[\text{HC}_4\text{im}][\text{HSO}_4]$. The Treated MCC- $[\text{HC}_4\text{im}][\text{HSO}_4]$ was prepared by mixing the untreated MCC with a $[\text{HC}_4\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) mixture, incubated at 120 °C for 22 h.

The Untreated-MCC consisted of particles of irregular and inhomogeneous shape with different particle sizes (**Figure 4-2 (a)**); this is in contrast with the morphology of the Büchner-CRM, where the cellulose fibrils were observed to be well assembled and tightly packed, forming a linear-rod structure (SEM micrograph, **Figure 3- 5, Chapter 3**). This morphological dissimilarity was due to the fact that the samples were obtained from different sources: MCC is derived from cotton linters, while the Büchner-CRM was obtained from deconstruction of *Miscanthus*.

The treatment appeared not to cause any physical alteration to the Treated-MCC's particles (**Figure 4-2 (b)**). No appearance of particle swelling was observed in the micrograph (**Figure 4-2 (b)**), as typically observed when treating cellulose with cellulose-dissolving solvents, such as the ionic liquid 1-butyl-3-methylimidazolium chloride $[\text{C}_4\text{C}_1\text{im}]\text{Cl}$.³ Under the SEM microscope, Ang *et al.* noticed that rice husk residues had swollen as a result of treatment with $[\text{C}_4\text{C}_1\text{im}]\text{Cl}$.¹¹² The hydrogen bond basicity, β , of solvents has been suggested as an indicator of effectiveness when attempting to swell and dissolve cellulose.^{2,9} The Kamlet-Taft empirical polarity scales can be used to measure polarity of solvents. Two dyes, *N,N*-diethyl-4-nitroaniline and 4-nitroaniline, are used to determine a β value of a given solvent. The β value indicates the ability of the solvent to donate its electron density to protons of the 4-nitroaniline, forming a hydrogen bond. Detailed mechanisms and procedures of the measurement can be found elsewhere.¹¹³ According to Brandt *et al.*, dialkylimidazolium ionic liquids with β values higher than 0.6, are effective for cellulose dissolution.⁹ The β values of 1-butyl-3-methylimidazolium hydrogen sulfate, $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ and 1-butyl-3-methylimidazolium chloride, $[\text{C}_4\text{C}_1\text{im}]\text{Cl}$, ionic liquids were previously measured to be 0.67 and 0.83, respectively.³

Gräsvik *et al.* explained that the increased acidity in the $[\text{HC}_4\text{im}][\text{HSO}_4]$ could be linked to the presence of an additional acidic N-H proton on the imidazolium ring.¹¹⁴ This is evidenced by a study of To which used the Hammett method to compare the acidities of $[\text{HC}_4\text{im}][\text{HSO}_4]$ and $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$.¹¹⁵ It was found that the acidity (H_0) values of concentrated $[\text{HC}_4\text{im}][\text{HSO}_4]$ and $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ were measured to be 1.73 and 1.96, respectively.¹¹⁵

In an imidazolium-based ionic liquid, the cation and the anion can interact with each other *via* H-bonding. The anion forms H-bonding with C2-H proton and, to a lesser extent, C4-H and C5-H protons on the imidazolium ring.¹¹⁶ Because the [HC₄im]⁺ cation contains an acidic N-H proton, an additional strong H-bonded site is thus available. As a result, the [HC₄im]⁺ cation forms stronger H-bonding to the [HSO₄]⁻ anion, compared to [C₄C₁im][HSO₄]. When relating to the Kamlet-Taft β value, stronger H-bonding within the [HC₄im][HSO₄] might retard the [HSO₄]⁻ anion to form H-bonding with the protons of the 4-nitroaniline, thus decreasing the β value.

The above explanations might justify the decreased β value of the [HC₄im][HSO₄] mixtures as expected. In addition, the incubation process (**Figure 4- 1**) used 20 wt% of water; this was believed to significantly decrease the β value of the mixture. The negative impact of water on β values of ionic liquids has been previously reported.² Thus, the decreased β value reduces the ability of [HC₄im][HSO₄] to either swell or dissolve the cellulose; the best explanation for the intact particles observed in the Treated MCC-[HC₄im][HSO₄] (**Figure 4-2 (b)**).

Surface Chemical Composition

The Untreated-MCC and Treated MCC-[HC₄im][HSO₄] samples were analysed by EDX to determine their surface composition. The results are summarised in **Table 4- 1**. The theoretical ratio of C/O is 1:2. The C/O value of Untreated-MCC (**Table 4- 1**) was closer to that of the theoretical cellulose. The EDX also detected calcium, but at a far lower concentration (Untreated-MCC, **Table 4- 1**). A small quantity of sulfur was detected; such amount was believed to be associated with the retention of the [HSO₄]⁻ anions on the surface of the Treated MCC-[HC₄im][HSO₄] following the incubation process.

Table 4- 1. Surface elemental composition of the Untreated MCC and the Treated MCC-[HC₄im][HSO₄]. The Treated MCC-[HC₄im][HSO₄] was prepared by mixing the untreated MCC with a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture, incubated at 120 °C for 22 h. Error values are standard deviations from values obtained from several focal points.

Chemical Elements	Atomic % (K shell)		
	Theoretical of cellulose	Untreated MCC	Treated MCC-[HC ₄ im][HSO ₄]
Carbon, C	54.5	57.2±3.0	56.0±4.0
Oxygen, O	45.4	42.7±5.0	43.8±6.0
Sulfur, S	-	-	0.09±0.5
Calcium, Ca	-	0.05±0.07	-

Figure 4- 3 shows the distribution of sulfur on the surface of the Treated MCC-[HC₄im][HSO₄] obtained by EDX surface elemental mapping.

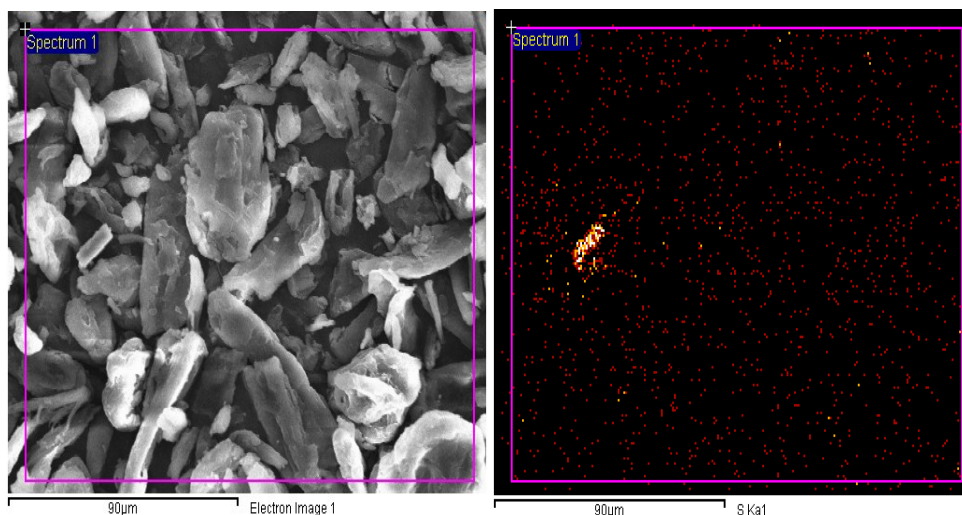


Figure 4- 3. EDX sulfur mapping micrograph of the Treated MCC-[HC₄im][HSO₄]. The Treated MCC-[HC₄im][HSO₄] was prepared by mixing the Untreated-MCC with a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture, incubated at 120 °C for 22 h.

Total Chemical Composition

CHNS analysis provides quantitative information on carbon, hydrogen, nitrogen and sulfur contents in the obtained samples. **Table 4- 2** provides a comparison of elemental composition (carbon, hydrogen, nitrogen and sulfur) of the Untreated-MCC and the Treated MCC-[HC₄im][HSO₄] samples. The wt% of C and the wt% of H detected in both samples were close to those of theoretical cellulose: 44 wt% of C and 7 wt% of H.

Table 4- 2. CHNS elemental composition of the Untreated-MCC and the Treated MCC-[HC₄im][HSO₄]. Treated MCC-[HC₄im][HSO₄] was prepared by mixing the untreated MCC with a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture, incubated at 120 °C for 22 h. Error values are standard deviations of triplicate samples.

Chemical Elements	Wt%		
	Theoretical of cellulose	Untreated MCC	Treated MCC
Carbon, C	44	41.5±0.006	41.7±0.02
Hydrogen, H	7	6.12±0.01	6.12±0.007
Nitrogen, N	-	-	0.54±0.02
Sulfur, S	-	-	0.54±0.03

The analysis indicates that the treatment process resulted in retention of [HC₄im]⁺ cation and [HSO₄]⁻ anions, which were not completely removed following the purification step (Filtration process, **Figure 4- 1**). This is evidenced by the detection of a small quantity of sulfur and nitrogen in the Treated MCC-[HC₄im][HSO₄] (**Table 4- 2**). Treated MCC-[HC₄im][HSO₄] was estimated to have one sulfur atom per 60 glycosyl monomer units.

The results of EDX and CHNS analyses are contradicted. It is clear that there is more sulfur in the bulk (CHNS analysis, **Table 4- 2**) than on the surface (EDX, **Table 4- 1**). This contradiction is rather expected because EDX has a high detection limit, around 1% or higher (**Chapter 2, Section 1.8.1**).⁶³ In addition, the technique is not able to analyse the entire surface of a given sample. In contrast, CHNS elemental analysis involves a complete combustion of entire sample at a very high temperature, producing combusted gases containing a total CHNS composition of the sample. It has a detection limit of 3%.

Elucidation of Chemical Functional Groups

IR spectroscopy was successfully applied previously to analyse sulfonated, -C-SO₃H, and sulfated, -C-OSO₃H, cellulose products.^{55-57,65,109} Thus, it was decided to characterise the Treated MCC-[HC₄im][HSO₄] using ATR-IR. The resultant spectrum was compared with the spectra of the Untreated-MCC and the neat [HC₄im][HSO₄] ionic liquid (**Figure 4- 4**)

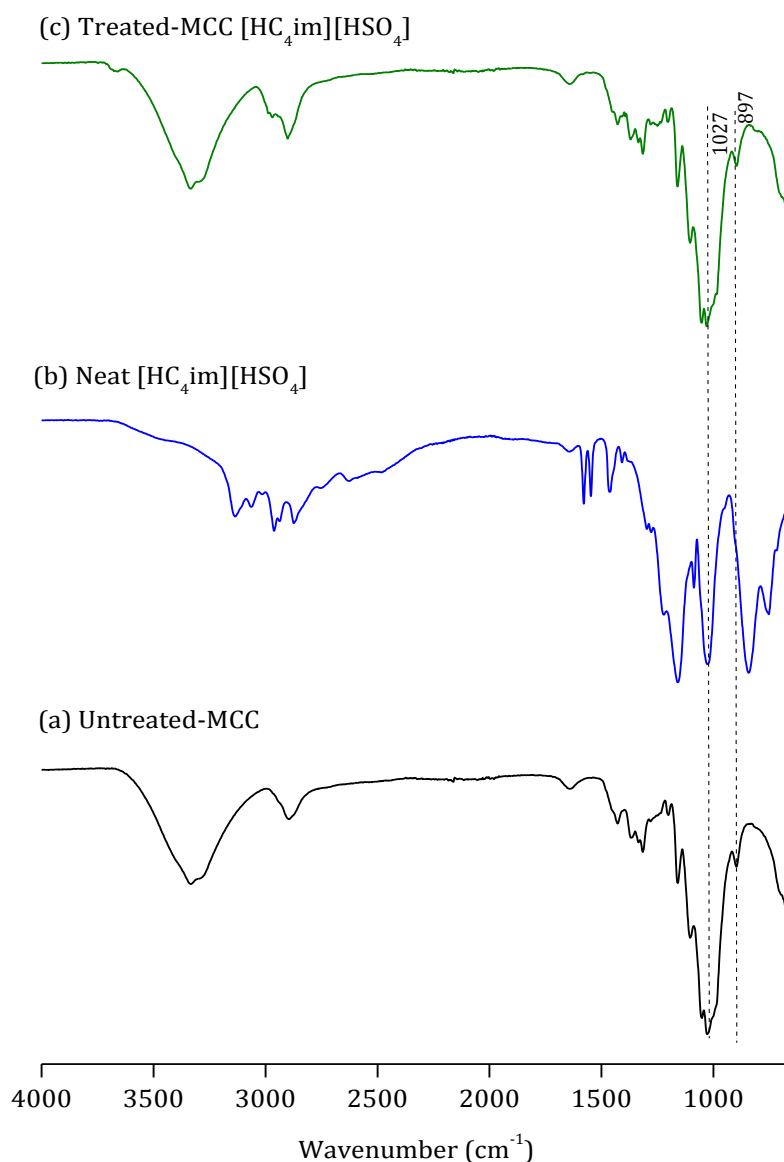
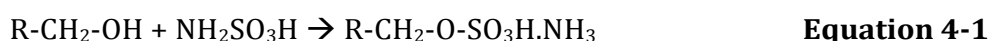


Figure 4- 4. AT-IR spectra of (a) Untreated-MCC (b) neat [HC₄im][HSO₄] ionic liquid and (c) Treated MCC-[HC₄im][HSO₄]. The Treated MCC-[HC₄im][HSO₄] was obtained by incubating the Untreated-MCC in a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture at 120 °C for 22 h.

There is no real difference between the spectra of the Treated MCC-[HC₄im][HSO₄] (**Figure 4- 4 (c)**) and the Untreated-MCC (**Figure 4- 4 (a)**). IR bands at 897 cm⁻¹ and 1027 cm⁻¹ could be attributed to a C-O-C stretching vibration and a vibration of C-O-C glycosidic bond, respectively, of pyranose rings.^{93,117} In addition, IR bands indicating asymmetric stretching of [HSO₄]⁻ anion (as observed in **Figure 4- 4 (b)**) were not recorded in the Treated MCC-[HC₄im][HSO₄] spectrum (**Figure 4- 4 (c)**). A previous study investigated sulfation of C6-hydroxyl group of cotton

cellulose by $\text{NH}_2\text{SO}_3\text{H}$; the formation of a $-\text{C}-\text{O}-\text{S}-$ bond was confirmed by the emergence of a strong IR band at 810 cm^{-1} , indicating a $\text{C}-\text{O}-\text{SO}_3$ group.^{55,56} However, the band of interest was apparently absent in the Treated MCC- $[\text{HC}_4\text{im}][\text{HSO}_4]$'s spectrum (**Figure 4- 4 (c)**), suggesting that the primary alcohol of the MCC did not react with $[\text{HSO}_4]^-$ anion during the incubation process. Sulfation of primary alcohols requires a strong nucleophile, such as hydrogen sulfite, HSO_3^- , in order to form the $-\text{C}-\text{O}-\text{S}$ bond. The sulfation reaction is irreversible when employing the $[\text{HSO}_3]^-$ anion as shown in **Equation 4-1**.¹¹⁸



The $[\text{HSO}_4]^-$ anion is a far weaker nucleophile than $[\text{HSO}_3]^-$ anion; therefore, the sulfation of the C6-hydroxyl group of MCC was believed to be less favourable.

Understanding Chemical Characteristics of Treated-MCC

Aldehydes containing α -hydrogen atoms can be sulfonated with sulfur trioxide¹¹⁹; thus, it was believed that aldehydes of the reducing-ends of MCC particles could possibly have undergone an esterification reaction in the presence of $[\text{HSO}_4]^-$ anions during the incubation process. Due to its capability of providing valuable information, for example masses of repeating units and end-groups, MALDI-TOF has been actively employed to analyse several polymers,¹²⁰ including cellulose.⁶⁹ Thus, this technique was used to characterise Treated-MCC samples.

Figure 4- 5 compares negative ion MALDI spectra of the Treated MCC- $[\text{HC}_4\text{im}][\text{HSO}_4]$ and the Untreated-MCC samples, which were co-crystallised with a 6-aza-2-thiothymine, AZA, matrix (the chemical structure is shown in **Figure 4- 7 (h)**). Only background ion signals were observed for the Untreated-MCC (**Figure 4- 5 (a)**), believed to be derived from the fragmentation of Untreated-MCC particles. This is a typical observation when analysing many oligosaccharides, generally associated with the lack of functional groups to be ionised by matrix molecules;^{69,72} as a result, no ion signals are desorbed and later detected by TOF detector.

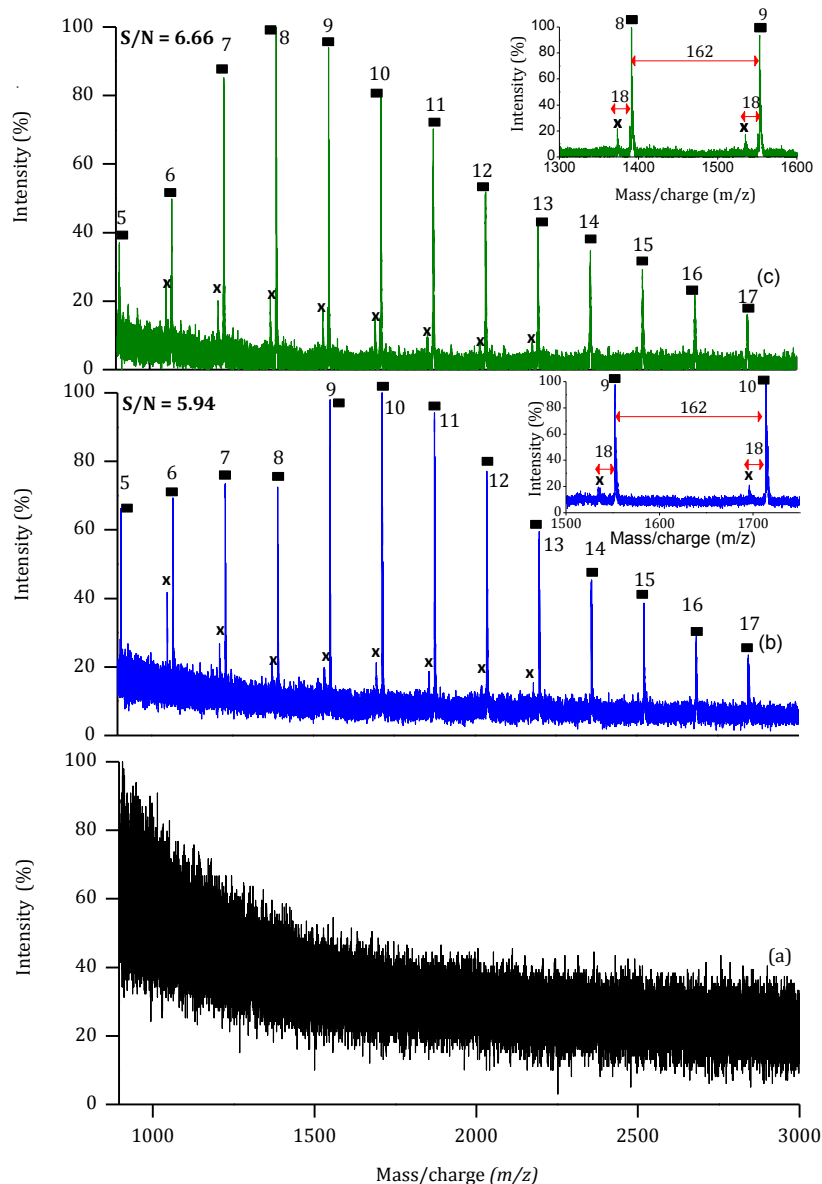


Figure 4- 5. Negative ion MALDI spectra of (a) Untreated-MCC; (b) Treated MCC-[HC₄im][HSO₄], and; (c) Treated MCC-[C₄C₁im][HSO₄]. All samples were co-crystallised with a 6-Aza-2-thiothymine (AZA) matrix. Treated-MCC samples were prepared by mixing the Untreated-MCC with a [C_nC_mim][HSO₄]/H₂O (80/20 wt/wt) mixture, incubated at 120 °C for 22 h. (■) denotes anions of sulfur-containing oligosaccharides, [((C₆H₁₀O₅)_nOSO₃H)-H]⁻, (x) denotes anions of deprotonated dehydrated-sulfur-containing oligosaccharides, [((C₆H₁₀O₅)_nOSO₃H)-H₂O)-H]⁻ (n = degree of polymerisation).

Interestingly, a series of ion signals (■) emerged in the Treated MCC-[HC₄im][HSO₄] spectrum (**Figure 4- 5** (b)), indicating the presence of different chain lengths of cellulose oligosaccharides. Most importantly, the oligosaccharides contained labile protons, H⁺, which were successfully deprotonated by the AZA matrix. This eventually produced varying oligosaccharide anions, later desorbed and detected by the TOF detector.

The mass interval between adjacent signals was calculated to be m/z 162, representing one glycosyl, unit, (C₆H₁₀O₅), the monomer of cellulose.⁶⁹ The DP (n) of the recorded signals was determined to be in the range of 5 to 17. Each DP had an additional mass of $m/z=96$, probably representing one unit of deprotonated bisulfate species ($m/z=97$). All ion signals (■) represented deprotonated sulfur-containing oligosaccharide, $[(C_6H_{10}O_5)_nOSO_3H-H]^-$ anions (n = 5 to 17), consistent with theoretical values of sulfated-oligosaccharides, as summarised in **Table 4- 3**. A series of minor signals (x) exhibiting a mass of m/z 18 lower than the major signals, were also recorded, indicating the absence of one molecule of water. This means that one of the hydroxyl groups on the cellulose had experienced dehydration reactions during the incubation process.

Table 4- 3. Deprotonated sulfur-containing oligosaccharides, $[(C_6H_{10}O_5)_nOSO_3H]^-$, with differing DP values observed in the Treated MCC-[HC₄im][HSO₄]. Treated MCC-[HC₄im][HSO₄] was prepared by mixing the Untreated-MCC with a [HC₄im][HSO₄]/H₂O (80/20 wt/wt) mixture, incubated at 120 °C for 22 h.

Degree of polymerisation, DP ^a (n)	$[(C_6H_{10}O_5)_nOSO_3H]^-$ (■)		
	Theoretical <i>m/z</i>	Observed <i>m/z</i>	Intensity (%)
5	907	905	72
6	1069	1068	75
7	1231	1229	78
8	1393	1391	77
9	1555	1553	98
10	1717	1714	100
11	1880	1876	95
12	2042	2039	81
13	2204	2201	66
14	2365	2362	55
15	2528	2524	49
16	2691	2686	41
17	2853	2847	37

^a Degree of polymerisation refers to number of glucose units in deprotonated sulfur-containing oligosaccharides, $[(C_6H_{10}O_5)_nOSO_3H]^-$.

In general, signal intensity gives an indication of the maximum range of analyte concentration, which may be detected by the mass spec detector in a given sample.¹²¹ Regarding the analysis of the Treated MCC-[HC₄im][HSO₄] (**Figure 4- 5 (b)** and **Table 4- 3**), it can be observed that the intensity of $[(C_6H_{10}O_5)_nOSO_3H]^-$ anions steadily increases as DP increases from 5 to 9, reaches a maximum at a DP of 10, representing the highest concentration of the oligosaccharide, and steadily decreases as DP increases from 11 to 17. The results suggest that the MCC did chemically react with [HSO₄]⁻ anions, yielding (C₆H₁₀O₅)_nOSO₃H oligosaccharides during the incubation process at 120 °C for 22 h.

In this section, it has been demonstrated that the Treated MCC-[HC₄im][HSO₄] had the [HSO₄]⁻ anions adsorbed onto its surface, as shown by EDX (**Figure 4- 3** and **Table 4- 1**) and CHNS analysis (**Table 4- 2**). When analysing the sample using MALDI-TOF (**Figure 4- 5**, **Table 4- 3**), only one unit of “[HSO₄]” was chemically bonded to each cellulose oligosaccharide. However, ATR-IR did not detect an IR band indicating a C-O-SO₃ group (**Figure 4- 4**), indicating that the chemisorption did not occur through the formation of -C-O-SO₃- bond. These observations suggest that the chemisorption might have involved a reaction between the [HSO₄]⁻ anion and an end-group of oligosaccharide, probably with aldehyde of reducing end.

4.3.2 Sulfur-Containing Oligosaccharides: Impact of Acidity of Ionic Liquids

The ionic liquid [HC₄im][HSO₄] was synthesised by direct addition of sulfuric acid, H₂SO₄, acid to 1-butyylimidazolium, [C₄im]. It was possible that [HC₄im][HSO₄] might have contained a relatively small quantity of undissociated H₂SO₄, potentially facilitating chemisorption reaction during the incubation process. To exclude the impact of undissociated H₂SO₄, treatment of MCC was replicated using ionic liquid [C₄C₁im][HSO₄], which was synthesised by hydrolysis of [C₄C₁im][MeOSO₃]. The resultant sample was labelled as “Treated MCC-[C₄C₁im][HSO₄]”. Interestingly, the resultant spectrum (shown in **Figure 4- 5** (c)) exhibited a similar pattern to that of the Treated-MCC-[C₄C₁im][HSO₄] sample (**Figure 4- 5** (b)). Masses of major (■) and minor (x) signals matched with the theoretical values of (C₆H₁₀O₅)_nOSO₃H (**Table 4- 3**) and ((C₆H₁₀O₅)_nOSO₃H)-H₂O oligosaccharides. This demonstrates that the presence of [HSO₄]⁻ anions was likely the key determining factor for chemisorption reaction.

Data from **Figure 4- 5** (b) and **Figure 4- 5** (c) were compared. A histogram comparing the intensities of (C₆H₁₀O₅)_nOSO₃H oligosaccharides as a function of DP is shown in **Figure 4- 6**. **Figure 4- 6** demonstrates that [HC₄im][HSO₄] led to a greater degree of chemisorption than [C₄C₁im][HSO₄].

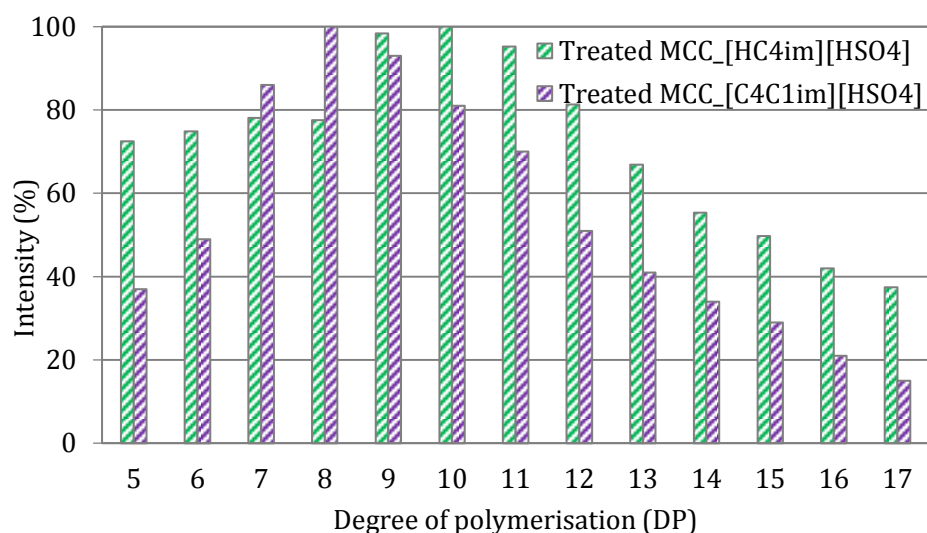


Figure 4- 6. Deprotonated sulfur-containing oligosaccharides, $(C_6H_{10}O_5)_nOSO_3H$, with DP=5 to 17 of Treated MCC-[HC₄im][HSO₄] and Treated MCC-[C₄C₁im][HSO₄]. The samples were co-crystallised with a 6-Aza-2-thiothymine (AZA) matrix. The Treated MCC samples were prepared by mixing the Untreated-MCC with a [C_nC_mim][HSO₄]/H₂O (80/20 wt/wt) mixture, incubated at 120 °C for 22 h.

4.3.3 MALDI-TOF Analysis: Understanding Sensitivity of Matrixes

The detection of $(C_6H_{10}O_5)_nOSO_3H$ oligosaccharides in the Treated-MCC samples was successfully achieved using a 6-aza-2-thiothymine (AZA) matrix. In order to establish that AZA is the most sensible choice of matrix, other promising compounds were also examined. Thus, several matrixes with varying acidic and basic character were selected; among them were: sinapinic acid (SA); 2,5-dihydroxybenzoic acid (2,5-DHB); 3-indoleacrylic acid (IAA), and; 6-aza-2-thiotymine (AZA) (chemical structures are illustrated in Figure 4- 7).

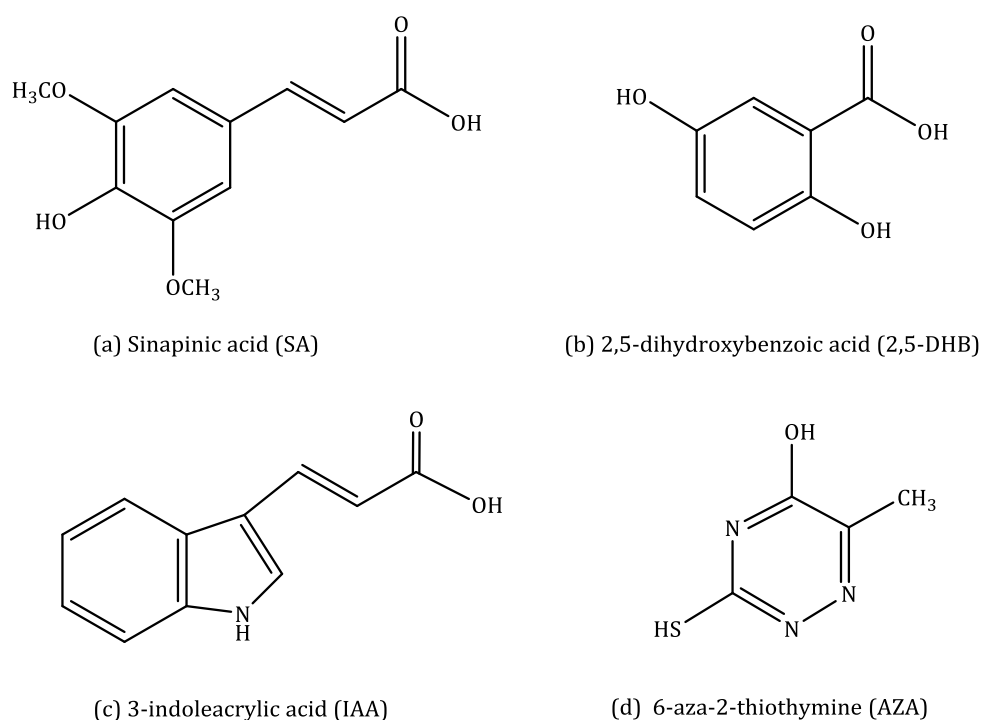


Figure 4- 7. Matrixes used to analyse the Treated-MCC-[C₄C₁im][HSO₄] sample using MALDI-TOF.

The performance of the studied matrixes was evaluated based on two aspects: (1) intensity of the recorded ion signals and (2) the signal to noise (S/N) ratio of obtained spectra. The S/N ratio represents the ratio of signal to ion background (height) of each ion signal; a sum of S/N ratio is then obtained, followed by division by the number of ion signals.^{122,123} An example of a calculation is included in the Appendix (A-1). Analysis of each spectrum provides justification for selecting AZA as the best matrix for (C₆H₁₀O₅)_nOSO₃H oligosaccharides. **Figure 4- 8** highlights the MALDI-TOF spectra in negative ion mode of the Treated MCC-[C₄C₁im][HSO₄] sample co-crystallised with different matrixes.

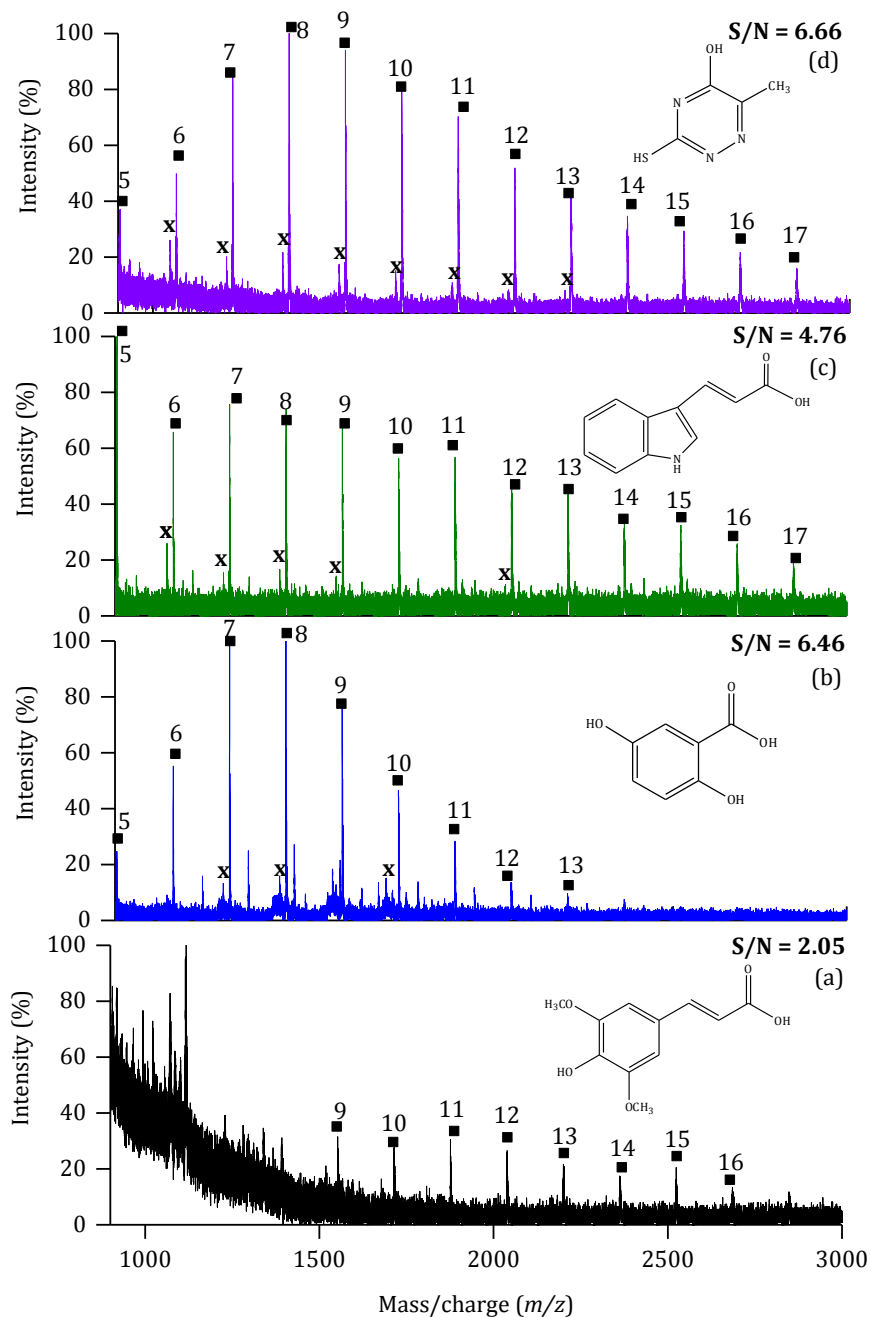


Figure 4- 8. Negative ion MALDI-TOF spectra of Treated MCC-[C₄C₁im][HSO₄] co-crystallised with different matrixes: (a) SA (b) 2,5-DHB (c) IAA and (d) AZA. The Treated MCC-[C₄C₁im][HSO₄] was prepared by mixing the Untreated-MCC with a [C₄C₁im][HSO₄]/H₂O (80/20 wt/wt) mixture, incubated at 120 °C for 22 h. (■) denotes anions of deprotonated sulfur-containing oligosaccharides, [((C₆H₁₀O₅)_nOSO₃H)-H]⁻, (x) denotes anions of deprotonated dehydrated-sulfur-containing oligosaccharides, [((C₆H₁₀O₅)_nOSO₃H)-H₂O)-H]⁻ (n = degree of polymerisation).

Sinapinic Acid (SA), and 2,5-Dihydroxybenzoic Acid (2,5-DHB)

An array of ion signals were recorded and their masses were in good agreement with theoretical values of $(C_6H_{10}O_5)_nOSO_3H$ and $((C_6H_{10}O_5)_nOSO_3H) \cdot H_2O$ oligosaccharides, as tabulated in **Table 4- 3**. Matrixes incorporating carboxylic acid ($-COOH$), functional groups, such as 2,5-DHB and α -cyano-4-hydroxycinnamic acid, α -CHCA, have been reported to be able to deprotonate several compounds.^{124,125} Kong *et al.* analysed thymine ($m/z=126$), which was co-crystallised with the 2,5-DHB and the α -CHCA. Both matrixes yielded an ion signal with $m/z=125$ in the negative ion spectra, indicating loss of a proton.¹²⁴

A compound incorporating a sulfonic acid ($-SO_3H$) functional group can also be deprotonated by the 2,5-DHB and the α -CHCA matrixes. Taurochenodeoxycholic acid ($m/z = 499$) was observed to lose a proton when the acid was co-crystallised with either the 2,5-DHB or the α -CHCA matrixes, indicating that the acidic proton on the $-SO_3H$ group of the acid had been abstracted.¹²⁵ Kong *et al.* investigated that the 2,5-DHB and the α -CHCA matrixes had ionised during laser ablation, forming carboxylate (COO^-) anions. One possible mechanism leading to ionisation of the 2,5-DHB matrix during laser ablation was proposed (illustrated in Figure 4- 9).¹²⁴ Hydroxyl groups on the benzene ring of the 2,5-DHB were not ionised¹²⁴, possibly due to the fact that the energy provided by the nitrogen laser (ca. 335 kJ/mole) is inadequate to overcome the bond enthalpy of hydroxyl group (467 kJ/mole). In addition, a hydroxyl substituent is an electron-donating group, which in turn increases the stability of the benzene ring.

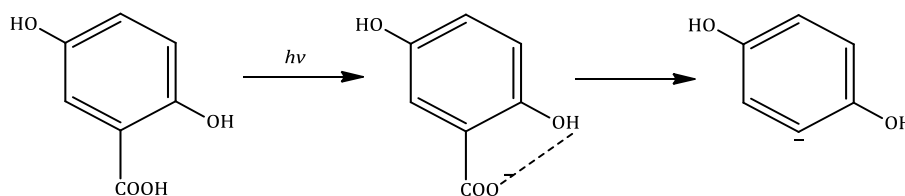


Figure 4- 9. Ionisation of the 2,5-dihydroxybenzoic acid, DHB, matrix during the laser ablation process.¹²⁴

Based on the report of Kong *et al.*¹²⁴, ionisation involving a sinapinic acid (SA) matrix was believed to follow the mechanism as proposed in **Figure 4- 10**.

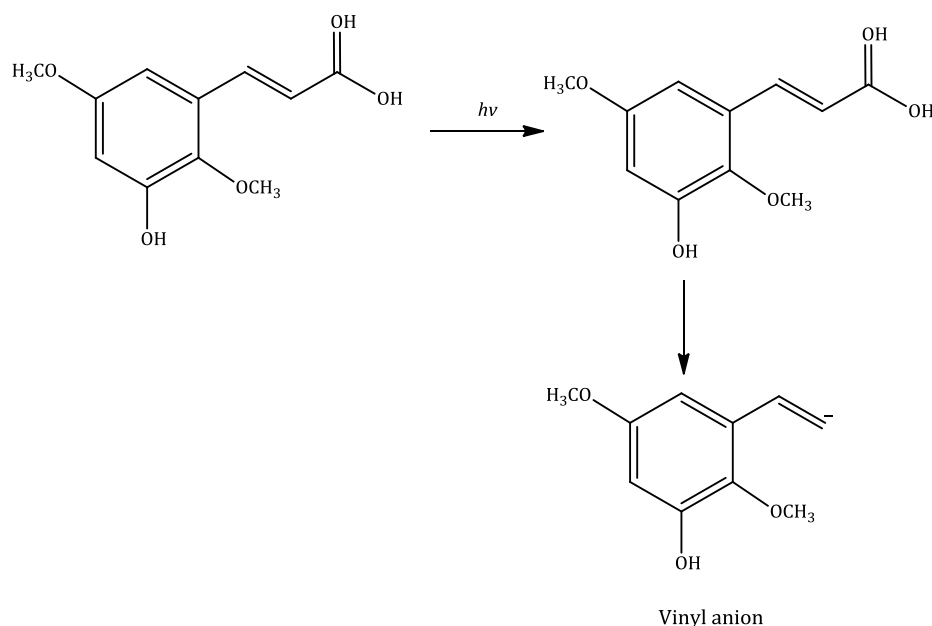


Figure 4- 10. Ionisation of the sinapinic acid (SA) matrix during the laser ablation process.

Referring to these explanations, it was believed that the production of the 2,5-DHB- and SA-derived anions (**Figure 4- 9** and **Figure 4- 10**) might be responsible for the deprotonation of acidic protons of $(C_6H_{10}O_5)_nOSO_3H$ and $(C_6H_{10}O_5)_nOSO_3H-H_2O$, producing a number of oligosaccharide ion signals.

Relative to 2,5-DHB, the use the SA (**Figure 4- 8 (a)**) matrix only produced $[(C_6H_{10}O_5)_nOSO_3H-H]^-$ ion signals of DP higher than 8. However, the intensities of these ion signals were considerably lower than those of the 2,5-DHB spectrum (**Figure 4- 8 (b)**). In addition, the region of m/z lower than 1500 was noticeably dominated by background ion signals. The S/N ratio of the SA matrix spectrum was calculated to be 2.05, far lower than the value of the 2,5-DHB spectrum ($S/N=6.46$). A matrix with an olefinic side chain, for example sinapinic acid, is favoured to undergo oligomerisation in the gas phase, in contrast to 2,5-DHB.¹²⁶ According to the proposed ionisation mechanism of the SA matrix (**Figure 4- 10**), the hydroxyl group at meta position of benzene ring is unable to undergo H-bonding with the $-COO^-$ anion. As a result, the COO^- anion becomes less stable, eventually being fragmented to yield a vinyl anion. The vinyl anion stabilises its negative charge through charge delocalization. In this study, the vinyl anion substituents in the SA matrix were thought to undergo an anionic addition polymerisation; this decreases the number of available SA anions to deprotonate $(C_6H_{10}O_5)_nOSO_3H$

oligosaccharides. This is a likely explanation for the poor performance of the SA matrix. In relation to 2,5-DHB matrix, the hydroxyl group at ortho position of the benzene ring undergoes H-bonding with the -COO^- anion (**Figure 4- 9**); this enhances the stability of the 2,5-DHB anion. In addition, the 2,5-DHB matrix does not have vinyl substituents, which are active initiators for polymerisation. As a result, the deprotonation of $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ oligosaccharides may have been increased. This was evidenced by a significant increase in the intensity of $[(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3]^-$ anions ($n = 5$ to 14), yielding a S/N ratio of 6.46.

3-Indoleacrylic Acid (IAA) and 6-Aza-2-Thiothymine (AZA)

Compared to the 2,5-DHB matrix (**Figure 4- 8 (b)**), an increase in the desorption of high molecular weight $[(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3]^-$ anions ($n = 9$ to 17) was observed with the IAA matrix (**Figure 4- 8 (c)**). This could possibly be due to enhanced solid-phase solubility. Solid-phase solubility measures the level of incorporation of analyte molecules within matrix molecules in the resultant solid sample; a homogenous incorporation enables the analyte molecules to be well separated from one and another.⁷⁹ The solid sample, the final product of co-crystallisation, is used for the laser ablation process. Co-crystallisation involves mixing the matrix and analyte (at a high matrix-to-analyte ratio) in a suitable solvent. The resultant mixture is deposited on a target plate, and the solvent is allowed to evaporate, yielding a solid sample.⁷⁹ Intermolecular interactions, such as H-bonding, influence the solid-phase solubility. For example, polyethylene glycol (PEG) co-crystallised with the α -CHCA matrix, produced the best spectrum; this was believed to be due to H-bonding occurring between highly oxygenated PEG chains and the carboxylic acid group of the α -CHCA matrix.⁷⁹ Besides the -COOH functional group, the IAA matrix contains an aromatic pyrrole ring (**Figure 4- 7(c)**); this provides two available H-bonded sites in the IAA matrix (NH and COOH), which may have enhanced solid-phase solubility.

The AZA matrix appeared to be the most suitable matrix for $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ oligosaccharides, based on the following observations: an enhancement in the desorption of both $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ and $[(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}]\cdot\text{H}_2\text{O}$ oligosaccharides, particularly at higher molecular masses ($n = 9$ to 17), and; the highest S/N ratio compared to other matrixes (**Figure 4- 8**).

A matrix incorporating an aromatic nitrogen heterocycle, particularly with a nitrogen lone pair which does not participate in the six- π electron aromatic system, has been demonstrated to be suitable for compounds of different acidic character. For example, Hercules and co-workers found that, in addition to the 9-aminoacridine (9AA) matrix deprotonating the acidic proton on the $-\text{SO}_3\text{H}$ group of taurodeoxycholic acid, the less acidic proton on temazepam was also deprotonated.¹²⁵

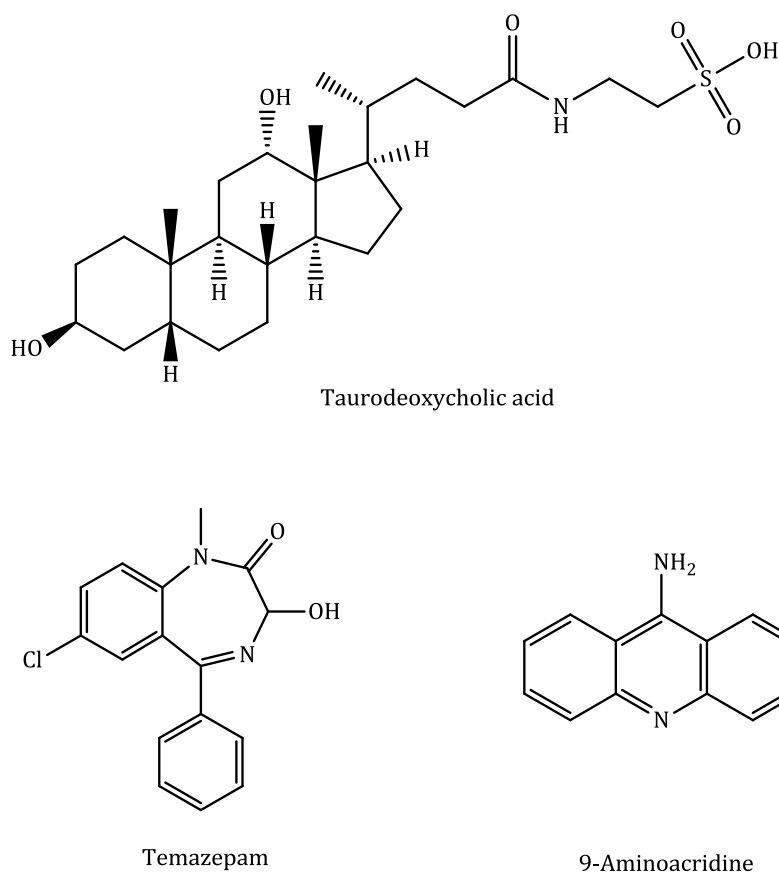


Figure 4- 11. Taurodeoxycholic acid, 9-aminoacridine (9AA) matrix and temazepam.¹²⁵

Regarding this study, the AZA matrix incorporates three highly basic sites, afforded by three nitrogen atoms with lone pairs not involved in the six π electron aromatic system; this significantly increases the basicity of the AZA matrix. As a result, the solid phase solubility was believed to be significantly enhanced, increasing the ionisation of both $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ and $((\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H})\cdot\text{H}_2\text{O}$ oligosaccharides. In conclusion, the performance of matrixes in deprotonating $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ and $((\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H})\cdot\text{H}_2\text{O}$ oligosaccharides could be ranked as follows: AZA>IAA>DHB>SA.

Summary

Two significant findings emerged from this study. Firstly, incubating cellulose in a $[\text{C}_n\text{C}_{\text{m}}\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) at 120 °C for 22 h resulted in the $[\text{HSO}_4]^-$ anion interacting with cellulose *via* chemisorption mechanism. Secondly, MALDI-TOF was the most promising technique in revealing chemisorption. The technique detected a series of deprotonated sulfur-containing oligosaccharides, $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$. This was confirmed by an additional mass of m/z 96 representing a deprotonated bisulfate species, recorded for each negative ion signal recorded in MALDI-TOF spectra (**Figure 4- 5**). The $[\text{HSO}_4]^-$ anion was the actual active species in forming $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ oligosaccharides, regardless different routes in synthesising the $[\text{HC}_4\text{im}][\text{HSO}_4]$ and the $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ (**Figure 4- 6**). Choosing a suitable matrix was essential in interpreting MALDI-TOF spectra properly. In this study, 6-aza-2-thiothymine (AZA) matrix was the best choice, giving the highest signal to noise (S/N) ratio compared to other studied matrixes (**Figure 4- 8**).

Unlike MALDI-TOF, other techniques, including EDX (**Figure 4- 3**), CHNS analysis (**Table 4- 2**) and AT-IR (**Figure 4- 4**) were only able to provide information, which mostly suggested a physisorption mechanism.

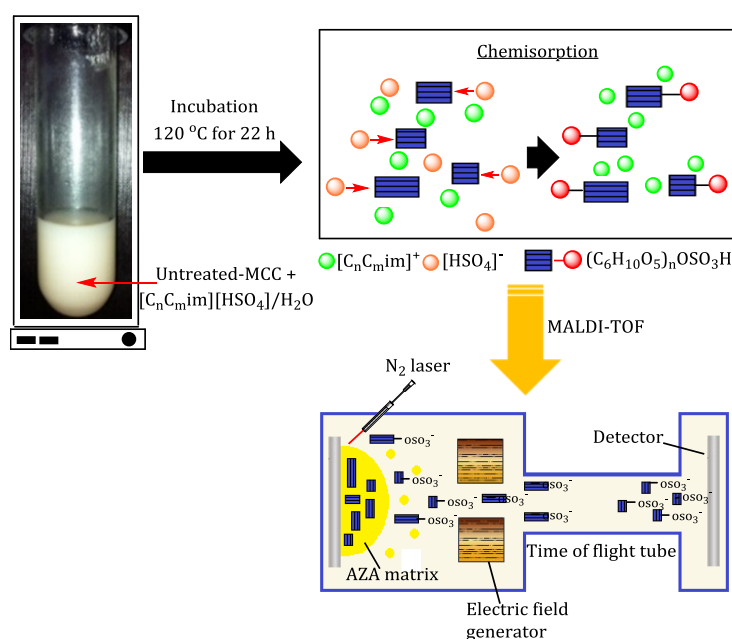


Figure 4- 12. Desorption of deprotonated $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ oligosaccharides during MALDI-TOF analysis.

5. Chemisorption and Depolymerisation

5.1 Background of Study

Motivation

Recently, several reports in the chemical literature have demonstrated the ability of dialkylimidazolium hydrogen sulfate, $[C_nC_mim][HSO_4]$, ionic liquids to catalyse the hydrolysis of Microcrystalline Sigmacell-Cellulose (MCC).⁶¹ According to a study of Man *et al.*, a decrease in the particle size of Treated-MCC samples was observed following treatment of Untreated-MCC samples in the ionic liquid 1-butyl-3-methylimidazolium hydrogen sulfate, $[C_4C_1im][HSO_4]$, at elevated temperatures.⁶¹ The $[HSO_4]^-$ anions had physically adsorbed onto the surface of Treated-MCC samples, as observed using the Energy Dispersed X-Ray (EDX) technique.⁶¹

Further to the study of Man *et al.*⁶¹, the investigation in **Chapter 4**, performed in an analogous manner to the deconstruction of *Miscanthus* process (**Chapter 3**), indicated that the $[HSO_4]^-$ anions not only physically absorb on to the surface, but also chemically bond to cellulose particles. Chemisorption was strongly evidenced by the detection of $(C_6H_5O_5)_nOSO_3H$ oligosaccharides using MALDI-TOF mass spectroscopy (**Chapter 4, Figure 4- 6**).

In consideration of the aforementioned findings, the purpose of this study was to investigate if there is a relationship between chemisorption and depolymerisation when incubating cellulose in $[C_nC_mim][HSO_4]/H_2O$ mixtures. Water was anticipated to have a significant influence on this relationship; thus, examining this aspect is worth exploring.

Problem Statement

The relationship between chemisorption and depolymerisation during incubation of cellulose in $[C_nC_mim][HSO_4]/H_2O$ mixtures has yet to be previously studied. Thereby, investigating the interplay of these two pathways, particularly in the presence of water, is of great interest.

Hypothesis

The correlation between chemisorption and depolymerisation reactions is varied depending on water content of $[C_nC_mim][HSO_4]/H_2O$ mixtures.

A positive correlation is more prevalent in the presence of lower water content. In contrast, increasing the water content inclines to result in a negative correlation.

Objectives

This primary objective of this study is to understand the interplay between chemisorption and depolymerisation, and how water effects this relationship.

Strategies

The outlined strategies are as follows:

1. Perform treatments using Untreated-MCC, a model polymer cellulose, in the neat ionic liquid $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ at 120 °C.
2. Examine the impacts of the following variables on chemisorption and depolymerisation: acid-to-water ratio and incubation period.

5.2 Overview of Experiments

The experimental procedures in **Chapter 3 (Figure 3.1)** were also applied to this study. The study employed $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ to treat the Untreated-MCC; the incubation process was conducted at 120 °C. Two variables were investigated:

1. Varying water contents for $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ mixtures; four mixtures were investigated (**Table 5- 1**).

Table 5- 1. Several $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ mixtures used in treating the Untreated-MCC sample at 120 °C.

Mixtures	Components (wt/wt)	
	$[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]$ (wt%)	H_2O (wt%)
I	96	4
II	80	20
III	50	50
IV	10	90

2. Different incubation periods; each mixture (**Table 5- 1**) was incubated for periods of 10 h and 1 week.

The resultant Treated-MCC samples were analysed using several methods:

1. Matrix Assisted Laser Desorption Ionisation combined with Time of Flight (MALDI-TOF) mass spectroscopy;
2. Viscosity measurement;
3. Enzymatic hydrolysis.

5.3 Results and Discussion

5.3.1 Significance of Analysis Methods

For each of the investigated conditions, the obtained results were discussed according to the following protocols:

(a) MALDI-TOF Mass Spectrometry

The intensity of $[(C_6H_5O_5)_nOSO_3H-H]^-$ ion signals represents the abundance of $(C_6H_5O_5)_nOSO_3H$ oligosacharides in a given Treated-MCC sample. Thus, cumulative intensities of the ion signals indicate the maximum range of concentrations of $(C_6H_5O_5)_nOSO_3H$ oligosacharides. This information could demonstrate the prevalence of chemisorption (and possibly depolymerisation) reactions. In this study, the degree of chemisorption was defined as the abundance of $(C_6H_5O_5)_nOSO_3H$ oligosacharides in a Treated-MCC sample, detected by MALDI-TOF. The analysis employed a 6-Aza-2-thiothymine (AZA) matrix; the most sensitive matrix for $(C_6H_5O_5)_nOSO_3H$ oligosacharides as observed in **Chapter 4 (Section 4.3.3, Figure 4- 8)**.

(b) Viscosity Measurement

To demonstrate that partial depolymerisation, catalysed by the $[HSO_4]^-$ anions, had occurred following the incubation process, the Treated-MCC samples were dissolved in a derivatizing solvent, 0.5 M aqueous bis(ethylenediamine) copper (II) hydroxide (CUEN). The fluidity of resultant mixtures to travel through a capillary in an Ostwald's viscometer was measured at 30 °C. The measured viscosity of a standard CUEN solution was $1.05 \text{ mm}^2\text{s}^{-1}$.

A polymer of low-molecular-weight is likely to exhibit a high fluidity (lower viscosity)¹²⁷, as observed by Kes *et al.*⁸⁵ The decrease in viscosity values is

likely to reflect an increase in the abundance of small cellulose particles that coordinate to Cu^{2+} ions in the CUEN solutions.

(c) Enzymatic Saccharification

In addition to the viscosity measurement, enzymatic saccharification conducted for a short period (2 h) could be an additional analysis for detecting the presence of low DP cellulose oligosaccharides in a given Treated-MCC sample.

Glucose is produced when cellobiose and soluble cellodextrins (obtained when cellulose is hydrolysed by cellulase enzymes, exoglucanase and endoglucanase) are completely hydrolysed by the cellobiase enzyme.¹²⁸ However, the action of cellobiase enzymes at hydrolysing cellobiose is slow, which in turn is slowest step in the assay (rate-limiting step).⁴⁴

For the cellulase enzymes, the enzymatic activity of exoglucanase and endoglucanase appeared to be influenced by the particle size of the cellulose substrate: the smaller the size of the particle (increase in surface area), the higher the production of cellobiose.¹²⁹ As a result, the glucose released also increases.

In this analysis, the production of glucose was examined *via* enzymatic saccharification, using a mixture of cellulase and cellobiase enzymes. The assay was conducted at 50 °C. The data was corrected with the glucose content found in the enzyme mixture. The glucose released was calculated relative to 100 mg of the Treated-MCC used for the assay.

5.3.2 Mixture I. [C₄C₁im][HSO₄]/H₂O (96/4 wt/wt)

The Treated-MCC samples were then analysed using MALDI-TOF, and their negative ion mode spectra are shown in **Figure 5- 1**. A set of (C₆H₁₀O₅)_nOSO₃H oligosaccharides (**■**) were detected. The incubation also produced several dehydration products: ((C₆H₁₀O₅)_nOSO₃H)-H₂O (**x**), ((C₆H₁₀O₅)_nOSO₃H)-3H₂O (**#**), and ((C₆H₁₀O₅)_nOSO₃H)-6H₂O (**○**).

Prolonging the incubation period from 10 h to 4 days enhanced the degree of chemisorption, as evidenced by an increase in the desorption of [((C₆H₁₀O₅)_nOSO₃H)-H]⁻ and [(((C₆H₁₀O₅)_nOSO₃H)-mH₂O)-H]⁻ (m=1 and 6) ions (**Figure 5- 1(a)-(c)**). Interestingly, the maximum range of anion concentrations gradually moved to high molecular weight regions (**Figure 5- 1 (a)-(c)**), indicating enhanced abundance of high DP (C₆H₁₀O₅)_nOSO₃H oligosaccharides. This eventually contributed to an increase in the S/N ratios (**Figure 5- 1(a)-(c)**).

The incubation lasting for one week significantly reduced the formation of both (C₆H₅O₅)_nOSO₃H and dehydrated ((C₆H₅O₅)_nOSO₃H)-mH₂O (m = 1 and 6) oligosaccharides. The spectrum was instead dominated by background ion signals (**Figure 5- 1 (d)**). This was believed to be due to extensive dehydration reactions occurring.

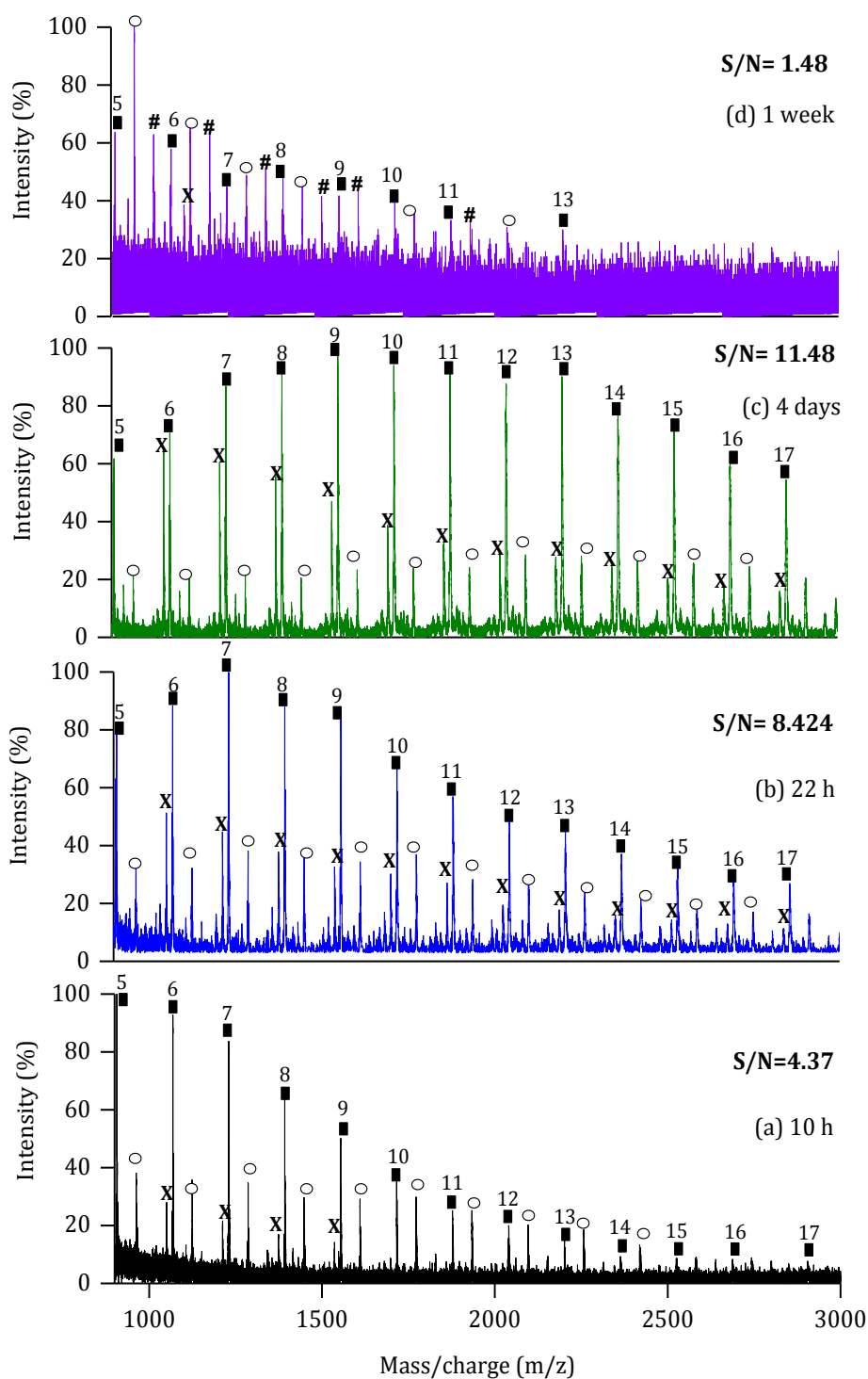


Figure 5- 1. Impact of incubation period on the profile of negative ion MALDI-TOF spectra of the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC in a $[C_4C_{1im}][HSO_4]/H_2O$ (96/4 wt/wt) mixture at 120 °C. Symbols: (■) denotes $[((C_6H_{10}O_5)_nOSO_3H)-H]^-$, (x) denotes $[((C_6H_{10}O_5)_nOSO_3H)-H_2O)-H]^-$, (#) denotes $[((C_6H_{10}O_5)_nOSO_3H)-3H_2O)-H]^-$, and; (o) denotes $[((C_6H_{10}O_5)_nOSO_3H)-6H_2O)-H]^-$ oligosaccharide anions (n = degree of polymerisation).

Figure 5- 2 highlights the viscosities of the Treated-MCC samples. As proposed in **Section 5.3.1** (Viscosity Measurement), a decreasing-trend observed in this analysis would indicate that the abundance small cellulose particles have increased. This would strongly suggest that more β -1,4-glycosidic bonds in cellulose have been hydrolysed over the incubation period.

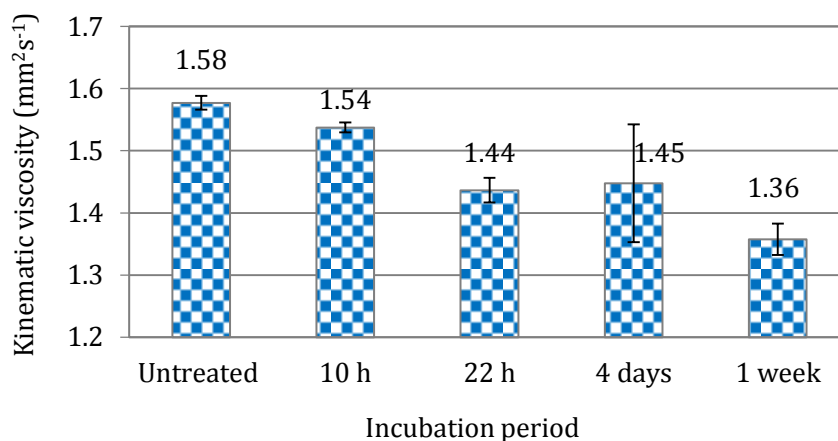


Figure 5- 2. Impact of prolonging incubation period on the viscosities of Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC in a $[\text{C}_4\text{C}_{1\text{im}}][\text{HSO}_4]/\text{H}_2\text{O}$ (96/4 wt/wt) at 120 °C. Error bars ($\pm$) are standard deviations of triplicate samples.

The Treated-MCC samples were then saccharified for 2 h, and the results are illustrated in **Figure 5- 3**. A decreasing-trend in glucose released was observed, contrary to the earlier proposition in **Section 5.3.1** (Enzymatic Saccharification).

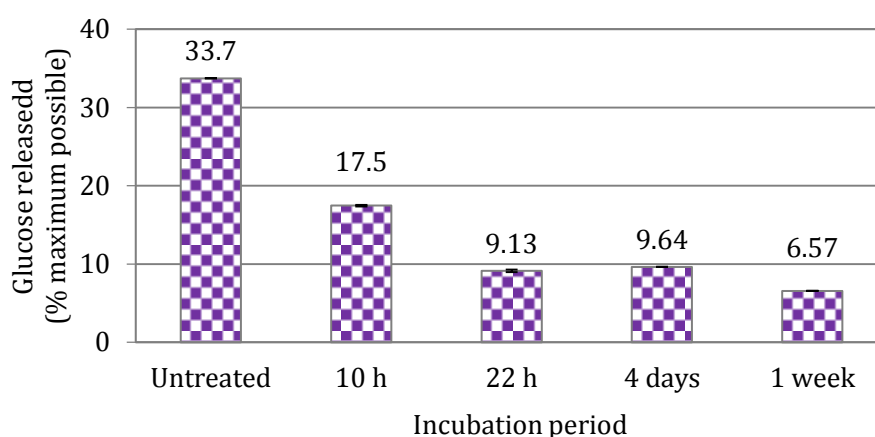


Figure 5- 3. Glucose released (after 2 h of saccharification) from Treated-MCC samples obtained from different incubation periods. The samples were obtained following incubation of the Untreated-MCC in a $[\text{C}_4\text{C}_{1\text{im}}][\text{HSO}_4]/\text{H}_2\text{O}$ (96/4 wt/wt) at 120 °C. Error bars ($\pm$) are standard deviations of triplicate samples.

Extensive dehydration occurs when exposing cellulose to a dehydrating agent, such as sulfuric acid, H_2SO_4 . A similar reaction was thought to occur as a result of exposing MCC particles to the $[\text{HSO}_4]^-$ anions for an extended period, decreasing the number of cellulose oligosaccharides to be enzymatically hydrolysed, thereby reducing the release of glucose. The enzymatic saccharification was then extended to 72 h, and the results are shown in **Figure 5- 4**.

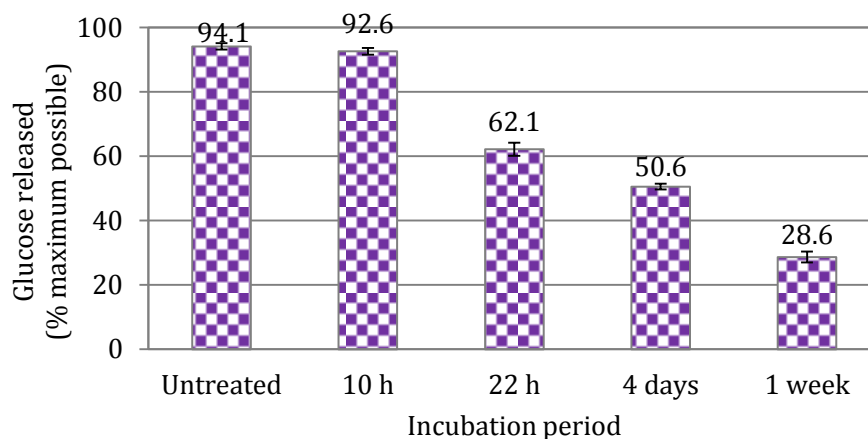


Figure 5- 4. Glucose released (after 72 h of saccharification) from the Treated-MCC samples obtained from different incubation period. The samples were obtained following incubation of the Untreated-MCC in the neat $[\text{C}_4\text{C}_{1\text{im}}][\text{HSO}_4]/\text{H}_2\text{O}$ (96/4 wt/wt) at 120 °C. Error bars ($\pm$) are standard deviations of triplicate samples.

The glucose released consistently decreased as the MCC particles were treated from 10 hours to 1 week (**Figure 5- 4**), consistent with the results of the two hours assay (**Figure 5- 3**). A visual examination on the resultant assay mixtures (photographed after the 72 h-period had elapsed) (**Figure 5- 5**) indicated that particles had accumulated. These particles appeared not to be enzymatically hydrolysed. The colour of these particles became darker as the MCC samples were incubated for longer periods (**Figure 5- 5**). These observations suggest that extensive dehydration had occurred when exposing cellulose to the $[\text{HSO}_4]^-$ anions for an extended period, forming compounds which could not be enzymatically hydrolysed.

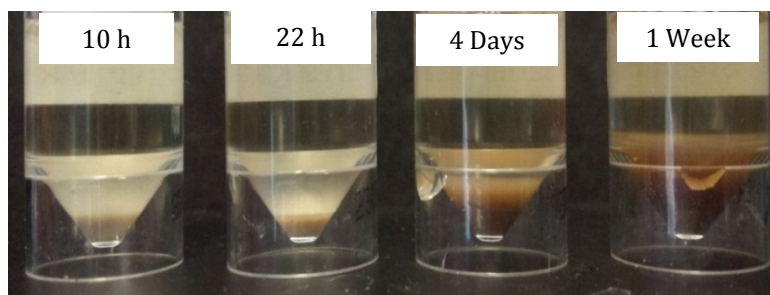


Figure 5- 5. Assay mixtures (upon 72 h of assay) containing the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC in the neat $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (96/4 wt/wt) at 120 °C.

5.3.3 Influence of Increasing Water Content on Chemisorption

The Treated-MCC samples were analysed using MALDI-TOF for the remaining ionic liquid-water systems: 80/20, 50/50 and 10/90 $[[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (wt/wt)). The recorded spectra are shown in **Figure 5- 6** to **Figure 5- 8**. Two types of sulfur-containing oligosaccharides were detected: $(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}$ (■) and $[(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}]\cdot\text{H}_2\text{O}$ (x).

Mixture II: $[\text{C}_4\text{C}_1\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt)

Figure 5- 6 shows MALDI-TOF spectra of the Treated-MCC samples obtained employing **Mixture II**. Prolonging the incubation period from 10 h to 4 days yielded a trend similar to that observed with **Mixture I** (**Figure 5- 1**). The addition of 20 wt% of water inhibited dehydration; the $[(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}]\cdot\text{H}_2\text{O}$ ions (x) were detected (**Figure 5- 6**), but at far lower intensities compared to those recorded from the samples treated using **Mixture I** (**Figure 5- 1**). In **Figure 5- 6** (a)-(c), it can also be seen that the intensity of background ion signals increases as the MCC particles were treated for 10 h to 4 days. This directly reduced the S/N ratios to a far greater extent than those calculated from the samples treated in **Mixture I** (**Figure 5- 1** (a)-(c)).

The Treated-MCC sample incubated for one week (**Figure 5- 6** (d)) exhibited a dissimilar trend to other samples (**Figure 5- 6** (a)-(c)). The highest intensity concentrations were found in the low molecular weight region (**Figure 5- 6** (d)). The background ion signals were highly intense, resulting in

a low S/N ratio (**Figure 5- 6 (d)**), relative to other samples (**Figure 5- 6(a)-(c)**). This observation may indicate the formation of either (or both) one two products: (1) dehydrated compounds derived from extensive dehydration of cellulose, or (2) cellulose oligosaccharides with the absence of readily ionised functional groups.

Mixture III: [C₄C₁im][HSO₄]/H₂O (50/50 wt/wt)

The recorded MALDI-TOF spectra of Treated-MCC samples for **Mixture III** are shown in **Figure 5- 7**. Surprisingly, the highest degree of chemisorption was achieved following incubation for 10 h, as evidenced by a significant increase in the number of desorbed $[(C_6H_{10}O_5)_nOSO_3H-H]^-$ (■) and $[(C_6H_{10}O_5)_nOSO_3H-H_2O-H]^-$ (x) ions. When the incubation period was prolonged to 22 h, the intensity of $[(C_6H_{10}O_5)_nOSO_3H-H]^-$ and $[(C_6H_{10}O_5)_nOSO_3H-H_2O-H]^-$ ions significantly diminished (**Figure 5- 7 (b)**). Following the incubation period of 22 h, neither $(C_6H_{10}O_5)_nOSO_3H$ nor $(C_6H_{10}O_5)_nOSO_3H-H_2O$ oligosaccharides were detected (**Figure 5- 7 (c)-(d)**), in direct contrast to the MALDI-TOF analysis for **Mixture I** (**Figure 5- 1 (c)-(d)**) and **Mixture II** (**Figure 5- 6(c)-(d)**).

Mixture IV: [C₄C₁im][HSO₄]:H₂O (10/90 wt/wt)

From the obtained MALDI-TOF spectra (**Figure 5- 8**) of **Mixture IV**, $[(C_6H_{10}O_5)_nOSO_3H-H]^-$ (■) (DP (n) = 5 to 17) ions were recorded only from the Treated-MCC sample incubated for 10 h (**Figure 5- 8 (a)**). The spectrum showed the lowest degree of chemisorption compared to the spectra of other samples (**Figure 5- 1, Figure 5- 6, and Figure 5- 7**). This is unsurprising, considering the low molar ratios of [HSO₄]-containing ionic liquid in the solvent mixture. When prolonging the incubation period over 10 h, ion signals indicating sulfur-containing oligosaccharides were not detected (**Figure 5- 8 (b)-(d)**). The spectra (**Figure 5- 8 (b)-(d)**) appeared almost identical to the Untreated-MCC spectrum (**Chapter 3, Figure 3.5 (a)**), likely indicating non-chemisorbed cellulose oligosaccharides.

In summary, enhanced degree of chemisorption with better S/N ratio was obtained at higher IL ratios in [C₄C₁im][HSO₄]/H₂O solvent systems. Relatively no detectable chemisorption of [HSO₄]⁻ anion was observed when raising quantity of water (decreasing quantity of [C₄C₁im][HSO₄]).

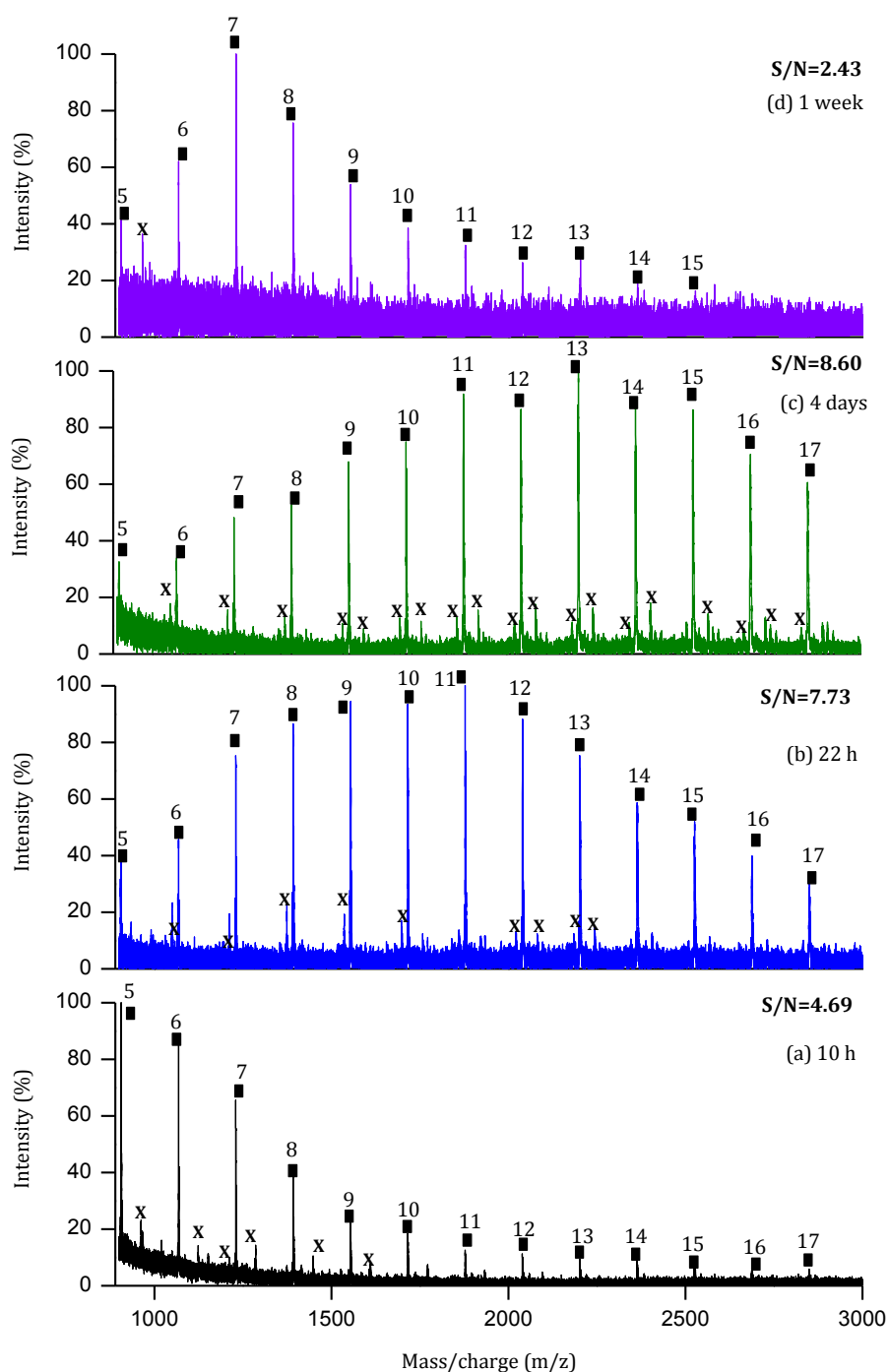
Mixture II: [C₄C₁im][HSO₄]/H₂O (80/20 wt/wt)

Figure 5- 6. Impact of incubation period on the profile of negative ion MALDI-TOF spectra of the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC (1 g per incubation) in **Mixture II** ([C₄C₁im][HSO₄]/H₂O (80/20 wt/wt)) at 120 °C. Symbols: (■) denotes $[(C_6H_{10}O_5)_nOSO_3H]^-$, and (x) denotes $[(C_6H_{10}O_5)_nOSO_3H-H_2O]^-$ oligosaccharide anions (n = degree of polymerisation).

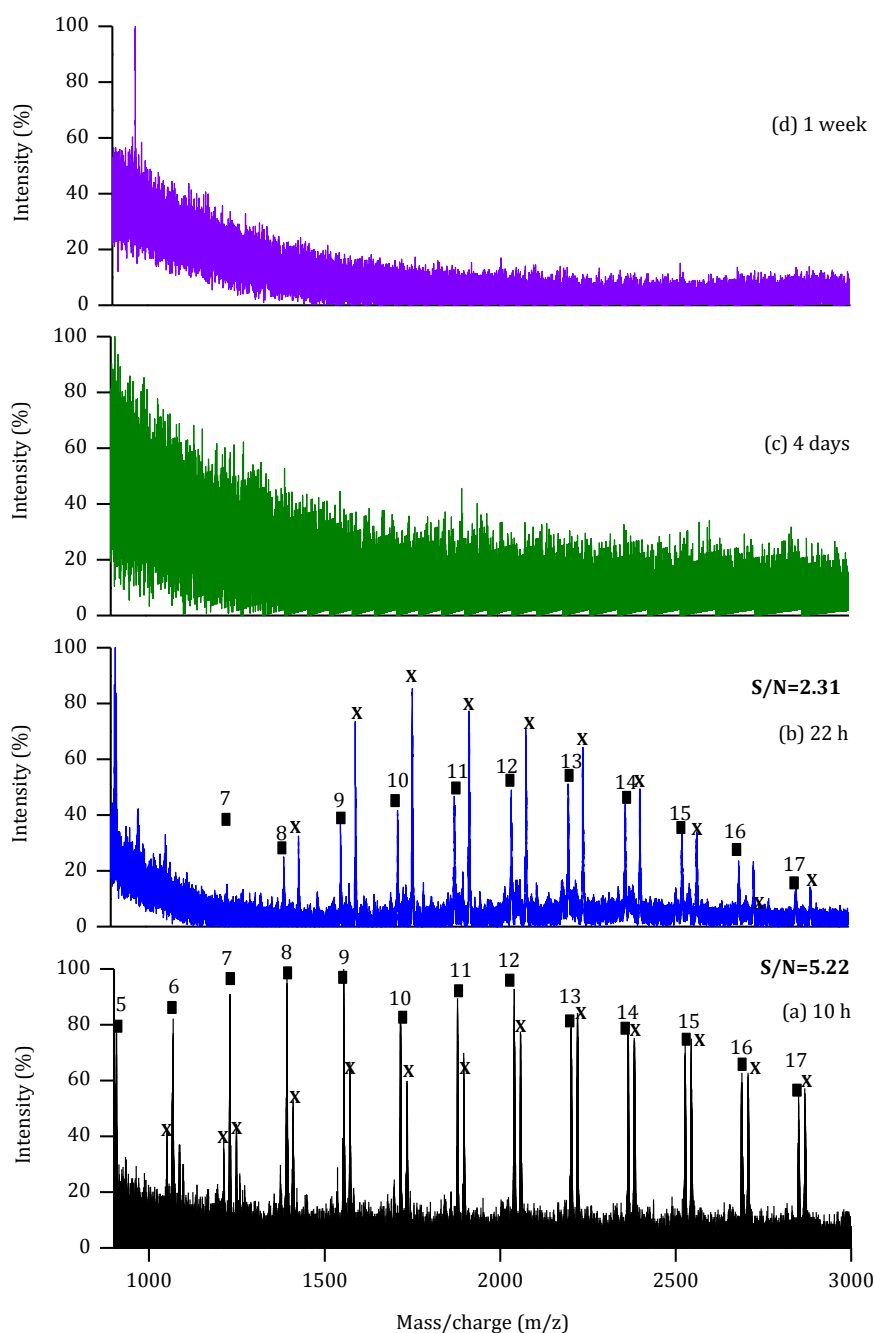
Mixture III: [C₄C₁im][HSO₄]/H₂O (50/50 wt/wt)

Figure 5- 7. Impact of incubation period on the profile of negative ion MALDI-TOF spectra of the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC (1 g per incubation) in **Mixture III** ([C₄C₁im][HSO₄]/H₂O (50/50 wt/wt)) 120 °C. Symbols: (■) denotes $[(C_6H_{10}O_5)_nOSO_3H]-H]^+$, and (x) denotes $[(C_6H_{10}O_5)_nOSO_3H-H_2O)-H]^+$ oligosaccharide anions (n = degree of polymerization).

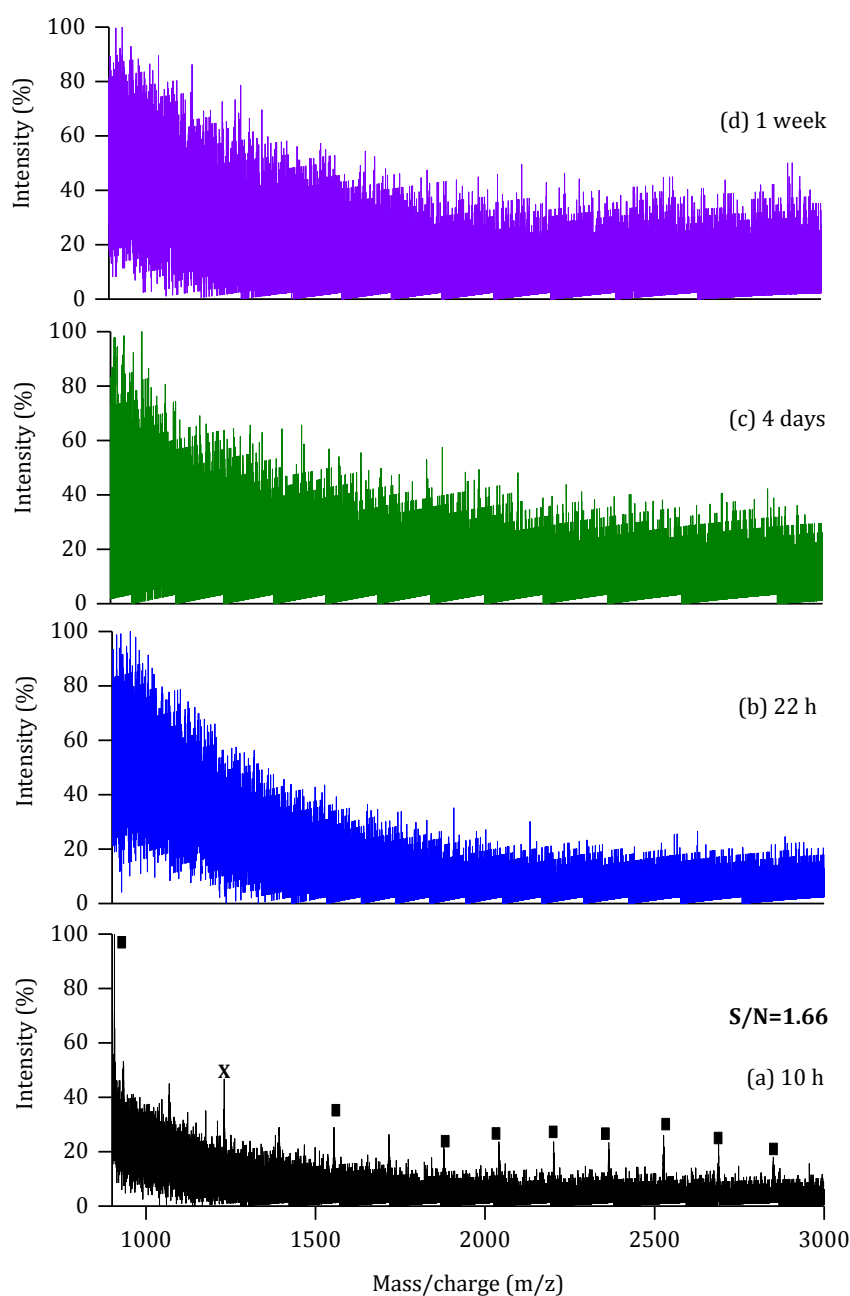
Mixture IV: [C₄C₁im][HSO₄]/H₂O (10/90 wt/wt)

Figure 5- 8. Impact of incubation period on the profile of negative ion MALDI-TOF spectra of the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC in (1 g per incubation) **Mixture IV** ([C₄C₁im][HSO₄]/H₂O (10/90 wt/wt)) at 120 °C. Symbols: (■) denotes $[((C_6H_{10}O_5)_nOSO_3H)-H]^-$, and (x) denotes $[((C_6H_{10}O_5)_nOSO_3H)-H_2O)-H]^-$ oligosaccharide anions (n = degree of polymerization).

5.3.4 Influence of Increasing Water Content: Depolymerisation of Cellulose

Viscosity Measurement

The viscosities of the Treated-MCC samples in 0.5 M of bis(ethylenediamine) copper (II) hydroxide CUEN solution were then measured, and the values are illustrated in **Figure 5- 9**.

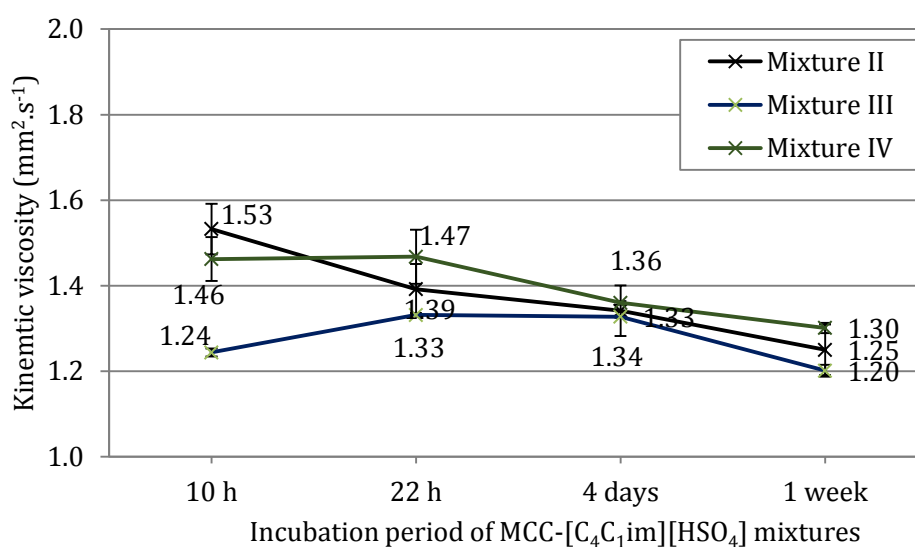


Figure 5- 9. Influence of increasing water content and increasing incubation period on the viscosities of Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC in varying $[C_4C_{1im}][HSO_4]/H_2O$ mixtures at 120 °C. The viscosities of the standard CUEN solution and the Untreated-MCC in CUEN solution were $1.05 \pm 0.01 \text{ mm}^2\text{s}^{-1}$ and $1.58 \text{ mm}^2\text{s}^{-1}$, respectively. Error bars ($\pm$) are standard deviations of triplicate samples.

In **Figure 5- 9**, it can be observed that the viscosity, to some extent, decreases with increasing water content of solvent mixtures. This indicates an increase in abundance of small cellulose particles, as proposed in **Section 5.3.1**. It is also apparent that the partial hydrolysis of cellulose in the $[C_4C_{1im}][HSO_4]/H_2O$ mixture occurred at a very slow rate (**Figure 5- 9**). As an example, the reaction took one week to obtain the highest abundance of small cellulose particles, as indicated by the highest reduction in viscosity (**Figure 5- 9**, 1 week).

The most essential step in acid-catalysed hydrolysis of cellulose is protonation of oxygen atoms on glycosidic sites (and possibly on pyranose rings) (**Figure 1- 11, Section 1.7.2, Chapter 1**). However, the accessibility of the oxygen atoms to $[\text{H}_3\text{O}]^+$ ions is restricted by the presence of strong inter- and intramolecular H-bonds (**Figure 1- 4, Section 1.3.4**); a structural impediment to the hydrolysis. Thus, it is required firstly to disrupt these molecular bonds using a solvent with a relatively strong H-bonding acceptor ability (a β value above than 0.67).⁹ The β value of $[\text{C}_4\text{C}_{1\text{im}}][\text{HSO}_4]$ was previously measured to be 0.83;³ such a value is below the cut-off point for dissolving cellulose.

The hydrolysis of cellulose is also influenced by an exo-anomeric effect, which in turn lowers the basicity of glycosidic oxygens.^{130,131} The exo-anomeric effect arises from delocalisation of electron as a result of donation from O(1) electron pair into the antibonding C(1)-O(5) orbital ($n(\text{O}(1)) \rightarrow \sigma^*(\text{C}(1)-(\text{O}(5)))$). This shortens C(1)-O(1) bond, increasing the bond order.¹³⁰ This explains the need to perform cellulose hydrolysis at high temperatures with the presence of strong acid catalysts ($\rho K_a < -3$). In fact, the use of highly concentrated H_2SO_4 ($\rho K_a -3$) is not able to completely depolymerise the biopolymer at room temperature. For example, only a reduction of 40 to 50% in DP values was obtained when an Untreated-MCC sample was treated in 65 wt% aqueous H_2SO_4 at room temperature for 1 h.¹³² Although the $[\text{HSO}_4]^-$ anion is a weak acid catalyst (ρK_a 1.99, $K_{\text{eq}} = 1 \times 10^{-2}$ at 25 °C), the anion was still able to initiate the partial hydrolysis of MCC particles as observed in this study (**Figure 5- 9**); this has also been reported by Mao *et al.*¹¹⁰

Employing an equivalent quantity (wt/wt) of the $[\text{C}_4\text{C}_{1\text{im}}][\text{HSO}_4]$ and water (**Mixture III, Table 5- 1**) yielded the greatest impact on depolymerisation (**Figure 5- 9**), particularly for the incubation period of 10 h. As described in **Section 1.7.2 (Depolymerisation of Cellulose, Chapter 1)**, the presence of $[\text{H}_3\text{O}]^+$ ions is essential for depolymerising cellulose; the ion not only rapidly protonates the oxygen on β -1,4-glycosidic bonds but also, to a lesser extent, oxygen on the pyranose ring. The $[\text{H}_3\text{O}]^+$ ions are likely to form when H_2O molecules are protonated by the acidic protons of $[\text{HSO}_4]^-$ anions (**Equation 5-1**). Tickle *et al.*¹³³ investigated the impact of H_2SO_4 -to-water ratio (wt/wt) on the Hammett acidity, H_0 , (analogues to pH measurement) of the resultant solutions. The concentration of $[\text{H}_3\text{O}]^+$ ions (as indicated by an

increase in H_0 values) increased with increasing water (decreasing H_2SO_4) quantities.¹³³ This concept could possibly be related to the observations for **Mixture I, II and III (Figure 5- 9)**: the higher the concentration of $[H_3O]^+$ ions produced (as more H_2O was added to the $[C_4C_{1im}][HSO_4]$ mixture), the more the glycosidic oxygen atoms were protonated. As a result, the abundance of low DP cellulose oligosaccharides increased. Compared with **Mixture I, Mixture II** and **Mixture III**, it appeared that **Mixture IV** had the smallest impact on depolymerisation (as determined viscometrically in **Figure 5- 9**).

Enzymatic Saccharification

Unlike **Mixture I (Figure 5- 3)**, the results obtained from **Mixture II** appeared to largely agree with the proposition in **Section 5.3.1 (c)** (Enzymatic Saccharification). A reduction in particle size of cellulose enhances the production of cellobiose. As the concentration of cellobiose substrate increases, the active sites of the cellobiase enzyme becomes saturated with substrate within a short period of assay. This leads to slight a reduction in the glucose released.

Interestingly, the glucose released plateaued regardless of how long the MCC particles were incubated (**Figure 5- 10**). This is indirect contradiction to the samples treated in **Mixture I**, which exhibited a decreasing trend in glucose released (**Figure 5- 3**) due to the formation of dehydration products (as observed in **Figure 5- 5**).

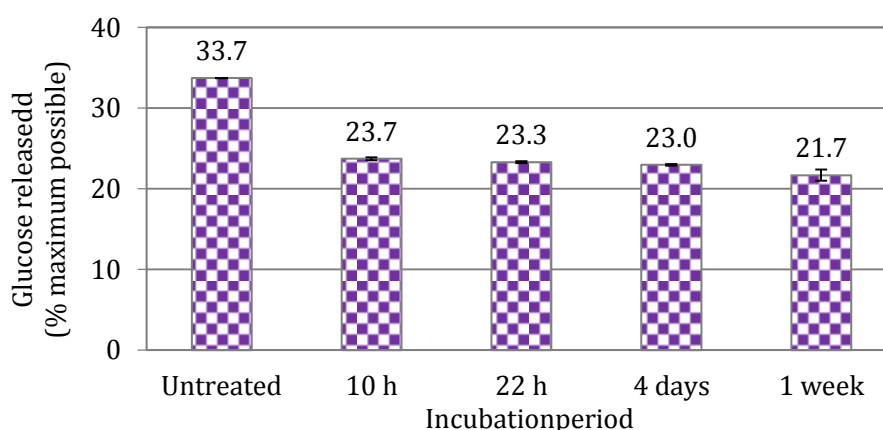


Figure 5- 10. Impact of incubation period on glucose released (2 h of assay) from the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC (1 g per incubation) in **Mixture II** ($[C_4C_{1im}][HSO_4]/H_2O$ (80/20 wt/wt)) at 120 °C. Error bars ($\square$) standard deviations of triplicate samples.

To confirm that dehydration products had not been formed during the incubation process using **Mixture II**, the assay was then extended to 72 h. The results are shown in **Figure 5- 11**. Interestingly, the release of glucose remains approximately constant for samples beyond 2 h of assay. The maximum quantity of glucose released was similar to that of the Untreated-MCC.

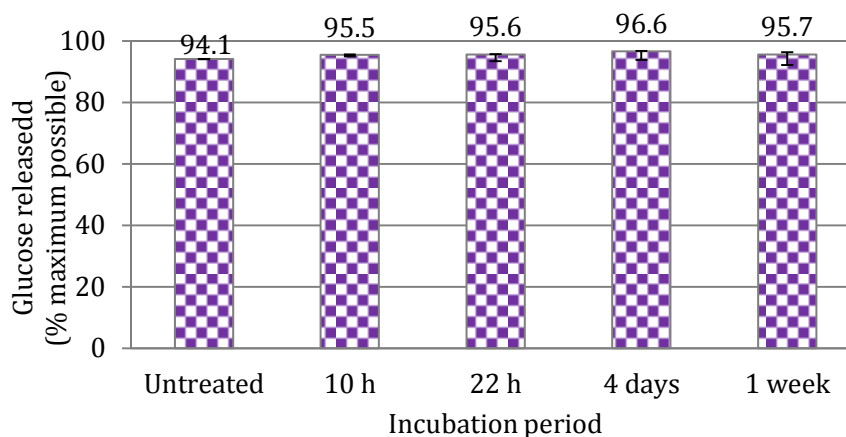


Figure 5- 11. Impact of incubation period on the glucose released (72 h of assay) from the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC (1 g per incubation) in **Mixture II** ($[\text{C}_4\text{C}_{11}\text{im}][\text{HSO}_4]/\text{H}_2\text{O}$ (80/20 wt/wt) at 120 °C. Error bars ($\pm$) are standard deviations of triplicate samples.

The Treated-MCC samples obtained by employing **Mixture III** and **Mixture IV** were also subjected to enzymatic saccharification for 2 hours. The results can be found in the Appendix (A- 2 and A- 3). Both mixtures, **Mixture III** and **Mixture IV**, exhibited a similar pattern to **Mixture II** (Figure 5- 10).

5.3.5 Correlation between Chemisorption and Depolymerisation

The MALDI-TOF analysis (**Figure 5- 1**, **Figure 5- 6**, **Figure 5- 7** and **Figure 5- 8**) suggested that the chemisorption is likely to occur at the reducing end of each cellulose oligosaccharide chain. This is supported by the fact that each ion signal had an additional mass of $m/z=96$, matching with the theoretical value of the corresponding $[(\text{C}_6\text{H}_{10}\text{O}_5)_n\text{OSO}_3\text{H}]\text{-H}^+$ ion. In addition, it is known that MALDI-TOF generally yields singly charged positive and negative signals.

In this study, the correlation between chemisorption and depolymerisation reactions is interesting. Both reactions are apparently influenced by the $[C_4C_{1im}][HSO_4]/H_2O$ ratios.

A Positive Correlation

A positive correlation between chemisorption and depolymerisation was observed when less water was incorporated into the $[C_4C_{1im}][HSO_4]/H_2O$ mixtures. This can be observed in the case of **Mixture I (Section 5.3.2)**. Prolonging the incubation period increased the following aspects: the depolymerisation of MCC particles (as determined viscometrically in **Figure 5- 2**), and the abundance of low DP $(C_6H_{10}O_5)_nOSO_3H$ oligosaccharides, particularly at high-molecular-weight region (MALDI-TOF analysis, **Figure 5- 1**). Increasing depolymerisation enhances the abundance of low DP cellulose oligosaccharides. In theory, this directly increases the number of reducing end groups. Due to a relatively small quantity of water in **Mixture I** (4 wt%), the solvation of $[HSO_4]^-$ anions by water molecules, likely *via* H-bonding, is minimized, and the intrinsic concentration of the $[HSO_4]^-$ anion is high. This is believed to enhance the interaction between reducing end groups and the $[HSO_4]^-$ anions, favoring chemisorption

A Negative Correlation

Increasing water content of $[C_4C_{1im}][HSO_4]/H_2O$ mixtures resulted in two opposing responses: positive for depolymerisation, and negative for chemisorption. The positive response on depolymerisation was observed when incubating the Untreated-MCC in **Mixtures II, III and IV** (viscosity measurement, **Figure 5- 9**). This has been discussed in detail earlier.

However, water appeared to impede the chemisorption, despite the increase in the number of reducing end groups in oligosaccharide chains (as indicated by the viscosity measurement, **Figure 5- 9**). From the MALDI-TOF analysis (**Figure 5- 1**, **Figure 5- 6**, **Figure 5- 7** and **Figure 5- 8**), it can be observed that the abundance of detected $(C_6H_{10}O_5)_nOSO_3H$ oligosaccharides significantly decreased as the water content was increased from 4 to 50 wt% (**Mixtures I, II, III and IV**). A plausible explanation to this may be that increasing water content enhanced intermolecular H-bonding of water with not only the $[HSO_4]^-$ anions, but also hydroxyl groups of MCC particles. This might

eventually have prevented interaction between reducing end groups and the $[\text{HSO}_4]^-$ anions, thus decreasing the extent of chemisorption. As overall, the water content of the solvent system plays a crucial role in determining both the extent of depolymerisation and chemisorption reactions.

5.3.6 Possible Active Sites of Chemisorption

Due to a limited number of studies, a mechanism describing a reaction between cellulose and $[\text{C}_n\text{C}_{\text{m}}\text{im}][\text{HSO}_4]$ ionic liquids remains unclear. Despite claiming to have obtained ester-sulfate functionalised nanocrystals, Mao *et al.* did not explain which functional groups on cellulose had chemically reacted with the $[\text{HSO}_4]^-$ anion, yielding the products.¹¹⁰

Cellulose consists of functional groups, which are likely to undergo an esterification reaction *via* both sulfation ($-\text{C}-\text{O}-\text{S}-$) and sulfonation ($-\text{C}-\text{S}-\text{O}-$). These functional groups are hydroxyl, and the aldehyde located on the reducing end group of each cellulose chain.

Hydroxyl Groups

The most established esterification involves a reaction between hydroxyl groups on cellulose, primarily $\text{OH}-\text{C}_6$, and sulfur trioxide (SO_3), a highly reactive electrophile.¹³⁴ This in turn yields a sulfated-cellulose compound containing $-\text{C}-\text{O}-\text{S}-$ bonds. A likely mechanism of this reaction is shown in **Figure 5- 12**.¹³⁴

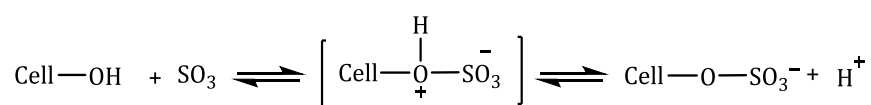


Figure 5- 12. A reaction of cellulose and SO_3 .¹³⁴

SO_3 is highly reactive. It exists as a gas rapidly reacting with water to yield H_2SO_4 . A fuming H_2SO_4 solution can be used as an option to acquire the SO_3 electrophile based on the following mechanism (**Figure 5- 13**).¹³⁴

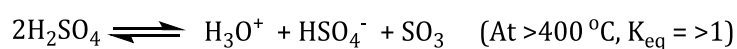


Figure 5- 13. Generation of SO_3 from fuming H_2SO_4 .¹³⁴

Figure 5- 14 shows a likely mechanism of sulfation of cellulose in the presence of fuming sulfuric acid, as proposed by Sjöström.¹³⁴

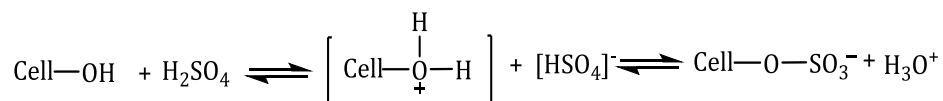


Figure 5- 14. A reaction of cellulose with fuming sulfuric acid.¹³⁴

In general, esterification reactions employing fuming H_2SO_4 requires high temperatures. As an example, preparing sugar-derived carbon catalysts from sucrose and glucose used 98 wt% H_2SO_4 ; the resultant mixtures were heated at 200 °C for 15 h.⁶⁵

The demand for high temperatures is due to the fact that decomposition of fuming H_2SO_4 to SO_3 gas is predominantly favoured at temperatures of 127 to 427 °C.¹³⁵ At room temperature, the fuming H_2SO_4 is prone to undergo autoprotolysis according to **Figure 5- 15**.¹³⁶



Figure 5- 15. Autoprotolysis of fuming H_2SO_4 .¹³⁶

Thus, with respect to this study, it seems very unlikely that the $[\text{HSO}_4]^-$ anion has decomposed to yield SO_3 , which then reacted with cellulose under the incubation conditions of $[\text{C}_4\text{C}_{1\text{im}}][\text{HSO}_4]/\text{H}_2\text{O}$ mixtures at 120 °C. This is strongly evidenced by the ATR-IR spectrum of the Treated MCC- $[\text{HC}_4\text{im}][\text{HSO}_4]$ (**Figure 4- 4 (c)**, **Chapter 4**), which did not record an IR band indicating a -C-O-S- bond at C6-cellulose. Thus, sulfation of OH-C6 on MCC particles during the incubation process is very likely not occurring.

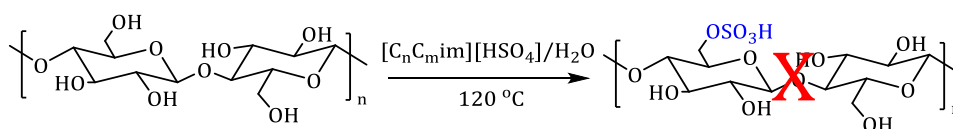


Figure 5- 16. A sulfation reaction of MCC particles with $[\text{C}_n\text{C}_{\text{mim}}][\text{HSO}_4]/\text{H}_2\text{O}$ mixtures.

However, the reaction in **Figure 5- 16** could potentially happen, to some extent, only when a highly concentrated H_2SO_4 is added to

MCC/[C₄C₁im][HSO₄] mixtures and the reaction is conducted at a temperature above 127 °C in an anhydrous system. However, this may lead to extensive dehydration of cellulose, which in turn impacts the enzymatic activity of cellulase and cellobiase enzymes, reducing the production glucose.

Aldehyde on Reducing End Group

An aldehyde with an α -proton can react with SO₃ *via* the proposed mechanism as shown in **Figure 5- 17**.¹³⁷

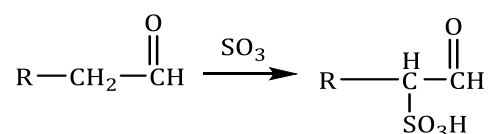


Figure 5- 17. Sulfonation of an aldehyde with SO₃.¹³⁷

Is believed that the reaction in **Figure 5- 17** is possible to occur on cellulose. However, it needs to be conducted in an inert environment. This is because SO₃ is very reactive. It produces white fumes when exposed to air; an indicator of the formation of H₂SO₄. In addition, SO₃ also reacts violently with water to produce H₂SO₄ (**Figure 5- 17**). However, the sulfonation of aldehyde is unfavourable in the presence of H₂SO₄; but, it is still possible if H₂SO₄ is heated at a high temperature, decomposing to SO₃ (as mentioned earlier, **Figure 5- 13**).

Each cellulose chain contains a reducing-end group, where both cyclic hemiacetal and aldehyde structures exist in equilibrium at C1 (chemical structure is shown as in **Figure 2-10, Chapter 2**). The aldehyde structure also contains an α -proton (**Figure 2-10, Chapter 2**). Thus, the sulfonation with a similar mechanism in **Figure 5- 17**, could be expected. However, it is implausible that the [HSO₄]⁻ anion decomposes to yield SO₃; thus, the reaction in **Figure 5- 17** appears to be unlikely to occur in the presence of the [C_nC_mim][HSO₄] ionic liquid (**Figure 5- 18**).

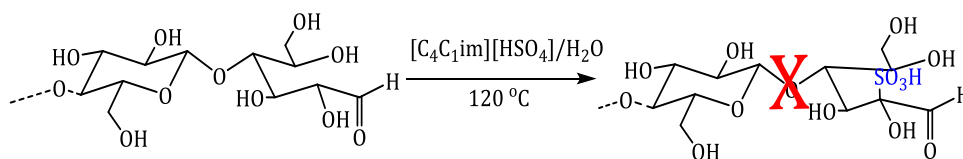


Figure 5- 18. Sulfation of an aldehyde on the reducing end group of a cellulose chain.

Summary

This study has demonstrated that the interplay of chemisorption and depolymerisation of cellulose during incubation process at 120 °C is significantly influenced by acid-to-water ([C₄C₁im][HSO₄]/H₂O) ratio. The chemisorption was enhanced with decreasing DP of MCC particles, observed when increasing the quantity of [C₄C₁im][HSO₄] in solvent systems with prolonging the incubation period. This trend was observed, particularly in **Mixture I** (MALDI-TOF, **Figure 5- 1**, and; viscosity measurement, **Figure 5- 2**) and **Mixture II** (MALDI-TOF, **Figure 5- 6**, and; viscosity measurement, **Figure 5- 9**). Using solvent mixtures containing more water enhanced depolymerisation of MCC particles (**Figure 5- 9**); however, the chemisorption was unfavourable (comparing MALDI-TOF spectra in **Figure 5- 1**, **Figure 5- 6**, **Figure 5- 7** and **Figure 5- 8**).

As overall, the impact of acid-to-water ratio to the chemisorption could be ranked as follows, [C₄C₁im][HSO₄]/H₂O (wt/wt): 96/4 (**Mixture I**) > 80/20 (**Mixture II**) > 50/50 (**Mixture III**) > 10/90 (**Mixture IV**). Regarding the depolymerisation, **Mixture III** was found to be the optimum solvent mixture for depolymerisation. Thus, the following order concludes the impact of acid-to-water ratio to the depolymerisation, [C₄C₁im][HSO₄]/H₂O (wt/wt): 50/50 (**Mixture III**) > 80/20 (**Mixture II**) > 96/4 (**Mixture I**) ≈ 10/90 (**Mixture IV**).

6 . Future Work

The following experiments can be performed in the future to have a better understanding on chemisorption interactions between cellulose and $[\text{C}_n\text{C}_m\text{im}][\text{HSO}_4]$ ionic liquids.

1. Characterisation of treated-cellulose samples using X-ray Photoelectron Spectroscopy (XPS)

XPS has been used previously to determine chemical state of sulfur in treated cellulose samples.^{56,65} For example, sulfonation, $-\text{C}-\text{S}-\text{O}-$ bond, was recorded at a binding energy of 2861 eV in a C 1s spectrum. Thus, this technique could potentially be used to determine whether chemisorption interactions between $[\text{HSO}_4]^-$ anion and cellulose occur through sulfonation or sulfation. However, it is highly advised to thoroughly purify the cellulose samples, eliminating physisorbed $[\text{C}_n\text{C}_m\text{im}][\text{HSO}_4]$ traces. This is because XPS is a highly surface sensitive technique; the presence of impurities can affect the analysis, such as the percentage atomic composition and the actual chemical state of the element of interest. It should also be noted that XPS does not work below 2.5 atom%.

2. Incubation of a carbonyl model compound in $[\text{C}_n\text{C}_m][\text{HSO}_4]/\text{H}_2\text{O}$ mixtures

As described previously, each cellulose chain has a reducing end group, which contains an aldehyde structure, with contains an α -proton attached to its adjacent carbon. (**Chapter 1, Section 1.7.3.2, Figure 1- 12**). A simple model compound, like phenyl acetaldehyde (comprising an aldehyde group and α -proton) could be used to examine the possibility of $[\text{HSO}_4]^-$ anions reacting with the aldehyde group.

3. Use of unhydrolysed cotton linters as a cellulose model compound

Microcrystalline sigmacell-cellulose (MCC) is generally obtained via hydrolysis of cotton linters, which contain amorphous and crystalline regions, with hydrochloric acid (HCl). This reaction degrades the amorphous regions, leaving mostly crystalline cellulose.¹³⁸ Thus, MCC is not a very suitable model compound for plant cellulose, which consists of both amorphous and crystalline regions (**Section 1.3.4**). By replicating the treatment using unhydrolysed cotton linters, it would be interesting to

examine whether the presence of amorphous regions has an impact on the chemisorption of $[\text{HSO}_4]^-$ anions on cellulose particles.

4. Determination of average degree of polymerisation (DP_v) and number of chain scissions (S)

From determination of kinematic viscosity (ϵ) of CUEN-MCC mixtures using an Ostwald viscometer, degree of polymerisation (DP_v) and number of chain scissions (S) of treated-MCC samples following the treatment could also be calculated. The following steps lead to attaining these values:

(a) Relative viscosity (η_{rel})

Relative viscosity (η_{rel}) can be calculated using **Equation 6-1** (since the densities of CUEN solvent and CUEN-MCC mixture are practically the same, they cancel in determining η_{rel}).⁸³

$$\eta_{\text{rel}} = \epsilon/\epsilon_0$$

Equation 6-1

ϵ : CUEN-MCC mixture, and; ϵ_0 : CUEN solution

(b) Intrinsic Viscosity $[\eta]$

The Martin equation can then be applied to determine the intrinsic viscosity $[\eta]$ (**Equation 6-2**).⁸³

$$\log [(\eta_{\text{rel}} - 1)/c] = \log [\eta] + k[\eta]c$$

Equation 6-2

k : 0.13, and; c : concentration of CUEN-MCC mixture.

Values of $[\eta]$ can be obtained by finding η_{rel} for three or more concentrations, plotting $\log [(\eta_{\text{rel}} - 1)/c]$ against c and extrapolating the straight line through the points to $c = 0$. The intercept gives $\log [\eta]$.

(c) Average degree of polymerisation (DP_v)

DP_v can be calculated using Mark-Houwink equation as described in **Equation 6-3**.⁸⁵

$$\text{DP}_v = 190 [\eta]$$

Equation 6-3

(d) Number of chain scissions (S)

Equation 6-4 provides a formula to determine number of chain scissions (S).

$$S = \text{DP}_{\text{av0}}/\text{DP}_{\text{av}} - 1$$

Equation 6-4

DP_{av0} : CUEN-Untreated MCC mixture, and; DP_{av} : CUEN-Treated MCC mixture.

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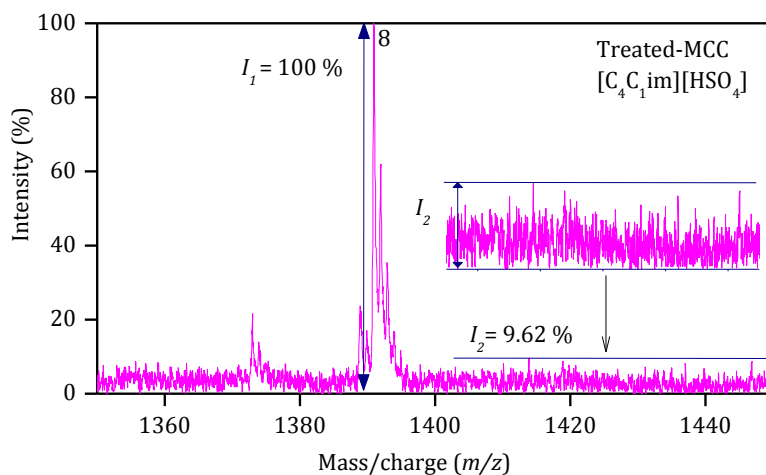
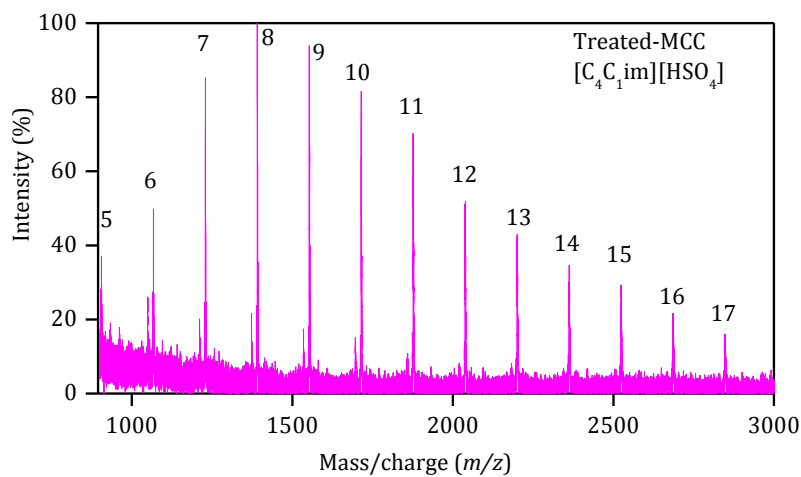
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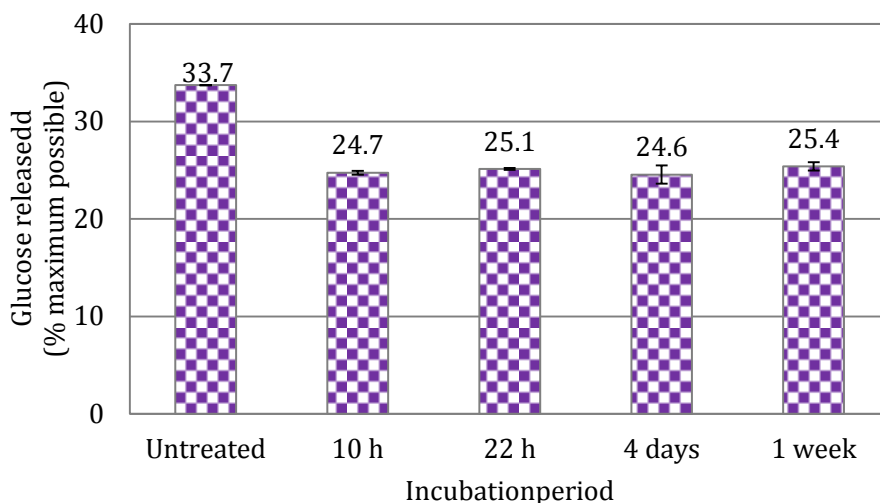
Appendix

A- 1. Determination of S/N ratio for a MALDI-TOF spectrum.

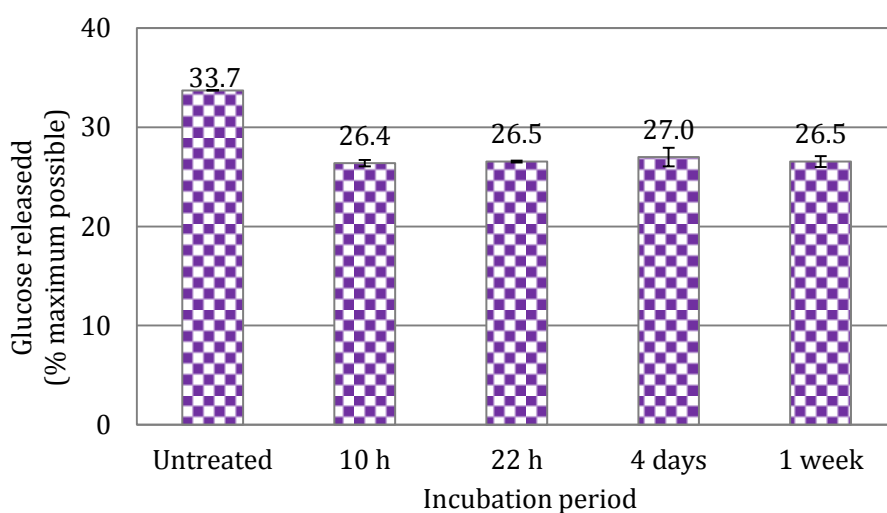


DP	I_1 (%)	I_2 (%)	I_1/I_2
5	33.73	16.23	2.078250154
6	48.68	12.44	3.91318328
7	84.87	11.791	7.197862777
8	100	9.62	10.3950104
9	92.905	8.544	10.87371255
10	80.866	7.3786	10.95953162
11	69.916	6.637	10.53427754
12	51.196	6.877	7.444525229
13	41.802	6.481	6.449930566
14	34.417	6.678	5.153788559
15	29.024	6.06466	4.785758806
16	21.411	5.316	4.02765237
17	15.938	5.7219	2.785438403
SUM			86.59892224

$$\text{S/N ratio} = 86.5 / 13 = 6.66$$



A- 2. Impact of incubation period on the glucose released (2 h of assay) from the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC in a **Mixture III** ([C₄C₁im][HSO₄]/H₂O (50/50 wt/wt)) at 120°C. Error bars ($\pm$) are standard deviations of triplicate samples.



A- 3. Impact of incubation period on the glucose released (2 h of assay) from the Treated-MCC samples. The samples were obtained following incubation of the Untreated-MCC in a **Mixture IV** ([C₄C₁im][HSO₄]/H₂O (10/90 wt/wt)) at 120 °C. Error bars ($\pm$) are standard deviations of triplicate samples.