Transformation of *Symphytum officinale* L. and *Panicum virgatum* L. Biomass to 5-Hydroxymethylfurfural for Biofuel Production

By

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Abstract

Lignocellulosic biomass has the potential to offer a cleaner alternative as a renewable source for fuel production. The present work aimed to use two plants, *Symphytum officinale* L. (common comfrey) and *Panicum virgatum* L. (switchgrass) to produce 5-hydroxymethylfurfural (HMF) using metal chloride catalysis in two ionic liquids, 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) or 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl). Pre-treatments were used to increase sugar availability, and two types of treatments were found to be suitable for HMF production. First, the 0.5 M sulfuric acid hydrolysis yielded 230±23 mg of sugars per g of hydrolysed comfrey, and 425±13 mg of sugars per g of hydrolysed switchgrass. Second, the methanol extraction yielded 300±60 mg of sugars per g of extracted comfrey, and 202±16 mg of sugars per g of extracted switchgrass. The yield of HMF produced was improved from <1% using untreated biomass, to 6.04% and 18.0% using methanol extracts of comfrey and switchgrass, respectively.

Keywords

5-hydroxymethylfurfural, biofuel, comfrey, ionic liquid, metal chloride catalysis, *Panicum virgatum* L., pre-treatment, switchgrass, *Symphytum officinale* L.

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List of Abbreviations

3,5-DNS: 3,5-dinitrosalicylic acid

µL: microliter

µm: micrometer

°C: degree Celsius

%: percent

Abs: absorbance

[AMIM]Cl: 1-allyl-3-methylimidazolium chloride

ADH: alcohol dehydrogenase

AFEX: ammonia fiber explosion

atm: atmosphere

[BDBU]Cl: 7-butyl-1,8-diazabicyclo[5,4,0]undec-7-ene chloride

[BMIM]: 1-butyl-3-methylimidazolium

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

[BMIM]Cl: 1-butyl-3-methylimidazolium chloride

[BMIM]HSO_4: 1-butyl-3-methylimidazolium hydrogen sulfate

$CHCl_3$: chloroform

BuOH: butanol

[C_3SO_3HMIM]HSO_4: 1-(4-sulfonic acid)-propyl-3-methylimidazolium hydrogen sulfate

DCM: dichloromethane

DF: dilution factor

dH_2O: distilled water

DMA: *N*,*N*-dimethylacetamine

DMF: 2,5-dimethylfuran

DMSO: dimethyl sulfoxide

DW: dry weight

[EMIM]: 1-ethyl-3-methylimidazolium

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

[EMIM]Cl: 1-ethyl-3-methylimidazolium chloride

EtOAc: ethyl acetate

EtOH: ethanol

g: gram

GC-MS: gas chromatography-mass spectrometry

GHG: greenhouse gas

h: hour

HCl: hydrochloric acid

HMF: 5-hydroxymethylfurfural

H_2O: water

L: liter

MBIK: methyl isobutyl ketone

MeOH: methanol

m: meter

M: molar

mg: milligram

MI: microwave irradiation

MJ: megajoule

min: minute

mL: milliliter

mm: millimeter

mM: millimolar

mol%: mol percent

nm: nanometer

[NMP]HSO_4: *N*-methyl-2-pyrrolidonium hydrogen sulfate

[OMIM]Cl: 1-octyl-3-methylimidazolium chloride

psi: pounds per square inch

TEACl: tetraethylammonium chloride

TLC: thin layer chromatography

TFA: trifluoroacetic acid

THF: tetrahydrofuran

v/v: volume/volume

wt/v: weight/volume

wt%: weight percent

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1. Introduction

1.1. Fossil Fuels

Nowadays, the use of fossil fuels is problematic due to the fast depletion and increased consumption of resources, as well as the high emission of CO_2 produced from those fuels [1]. From the feedstocks used to produce energy, 80% are fossil fuels [2]. This is problematic because energy production is currently responsible for up to 70% of the release of greenhouse gases (GHG), and is the principal contributor of CO_2 pollution [2, 3]. According to the statistical review of world energy, the oil production was up to 85 millions of barrels per day in 2010, which is equivalent to 3,900 millions of tons of oil equivalents each year, not including coal and natural gas which correspond to an additional 2,900 millions of tons of oil equivalent per year [4]. By 2014, the oil production had climbed to 89 millions of barrels per day [4]. Many scenarios have been hypothesized concerning the future of the oil industry and the associated impact on the environment, but regardless of the scenario, it is clear that CO_2 emissions associated with the oil industry are on the rise (Figure 1) [2, 3, 5]. The increased pollution caused by those CO_2 emissions leads to the need to find alternative methods for green and renewable energy production to be used as a replacement for fossil fuels.

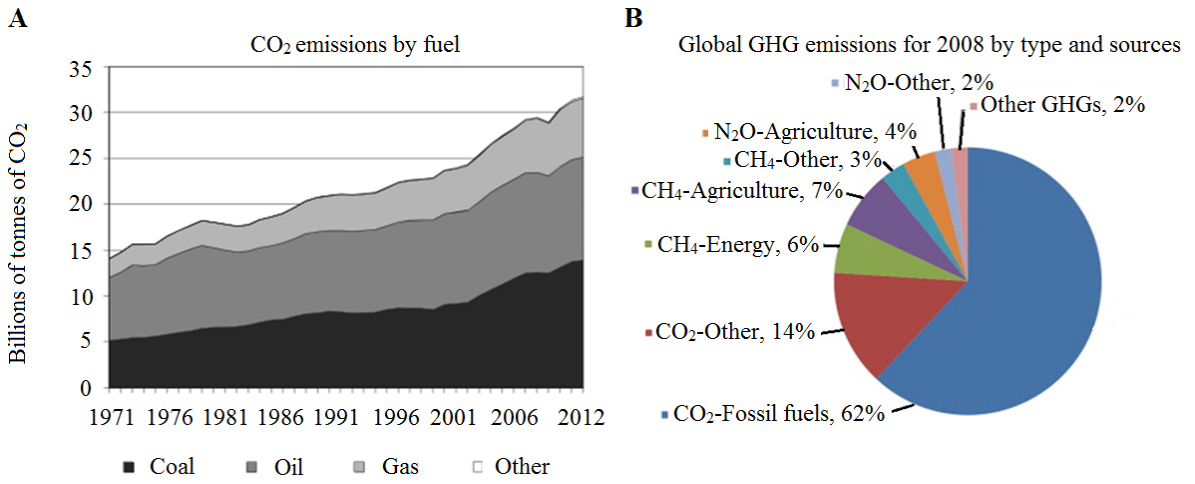


Figure 1. Emission of CO_2 from fossil fuel sources. Panel A. CO_2 emissions by fuel. Panel B. Greenhouse gases (GHG) emissions by sector.
Sources: [3, 5]

1.2. Biofuels

As a greener alternative, biofuels offer a potential replacement for fossil fuels. Biofuels are described as a fuel coming from biological sources [6]. Advantages of biofuels, including renewability and reduction of CO_2 emissions, have made this area of research popular in recent years [6]. The first studies concerning biofuels date back to the 1980's, where bioethanol was produced from corn [7, 8]. To this day, bioethanol is still the primary biofuel being produced and used. The process of EtOH production is done using yeasts which can utilize a metabolic process called alcoholic fermentation to metabolize carbohydrates to produce EtOH in aerobic or anaerobic conditions [9]. The reaction by which carbohydrates are transformed into EtOH is shown below.

$$C_6H_{12}O_6 \xrightarrow{\text{yeast}} 2C_2H_5OH + 2CO_2$$

One mole of any simple sugar (including hexoses such as glucose, galactose or mannose, as well as pentoses like xylose and arabinose) can be transformed into 2 moles of EtOH and 2

moles of CO_2 by the enzyme alcohol dehydrogenase (ADH) present in the yeasts [6, 9]. All fermentable sugars are monosaccharides, meaning that they are also considered reducing sugars as they can act as a reducing agent because they contain a free aldehyde group or ketone group. The theoretical maximal conversion for EtOH from sugars according to the stoichiometry is 51 wt%, but due to the utilization of glucose by the yeast for other metabolites and growth, the conversion is decreased to 40-48 wt%, which equates to 583 L of EtOH being produced for 1,000 kg of fermentable sugars [6]. Many different organisms can be used for fermentation, with two of the best producers of EtOH being *Saccharomyces cerevisiae* Meyen ex. E.C. Hansen, and *Saccharomyces uvarium* Nguyen & Gaillardin ex. Beijerinck [10, 11]. Fermentation can also be used as a tool to produce other fuel molecules such as BuOH and propanol, using different organism such as the bacteria *Clostridium acetobutylicum* McCoy *et al*. emend. Keis *et al*. [12].

Nowadays, production of bioethanol from corn and other crops is considered to be unsustainable due to many factors. These factors include a lower output of energy compared to the energy used to produce the bioethanol, as well as erosion of the land, wastewater production, and herbicide usage related to crop cultivation [13, 14]. The bioethanol produced from barley has even shown to generate more CO_2 compared to fossil fuels [15]. Additionally, there is an ethical dilemma associated with using a food resource to produce energy while world hunger is still occurring. At the moment, even with the known downsides mentioned above, bioethanol is in use as a replacement fuel for automobiles and machinery. It is therefore debatable whether or not production of bioethanol from crops is truly better on the environmental scale compared to fossil fuels. These concerns lead to the need to find a way to reduce the use of fossil fuel, but also to create a renewable source of energy that will be

economically viable, while protecting the environment from noxious byproduct. Currently, other types of biofuels are being studied for use at the pump, including hydrogen, methane, alcohols (EtOH, propanol, and BuOH), carboxylic acids, and other organic molecules [16]. Biodiesels are also utilized, but once again, CO_2 emissions have been reported as greater than the emissions produced from fossil fuels [15].

When dealing with biofuels, it is not only the type of molecules that must be studied, but also the different types of feedstocks that can be used to produce those molecules. Many different plants have already been studied as a biofuel source, including corn, grain, sugar cane, potato, aquatic plants, woods, grasses, and waste materials (eg: wood wastes and agricultural wastes) [6]. Plants can be separated in three categories: sugar-containing plants, starch-containing plants, and cellulosic biomass [6, 17]. The former two can be used to produce first generation biofuels, whereas cellulosic or lignocellulosic biomass can be used to produce second generation biofuels [6]. A third generation of biofuel has also recently been recognized in the literature as the production of fuels from algae [18].

Although simple sugars are required to produce biofuels, the simple sugar- and starch-containing plants are principally made of food sources such as corn, potato and grain, and therefore offer a poor choice for a biofuel feedstock due to the environmental implications (soil erosion, depletion of food resources, use of pesticides, and herbicides). Second generation fuels could offer a better alternative for a renewable source since lignocellulosic biomass is more readily available, and does not require the use of food sources and agricultural plants. Lignocellulosic materials such as hardwood, softwood, agricultural residues, and other plant materials also offer the most abundant renewable carbon source [17]. Figure 2 shows the different types of feedstocks that can be used for biofuel production.

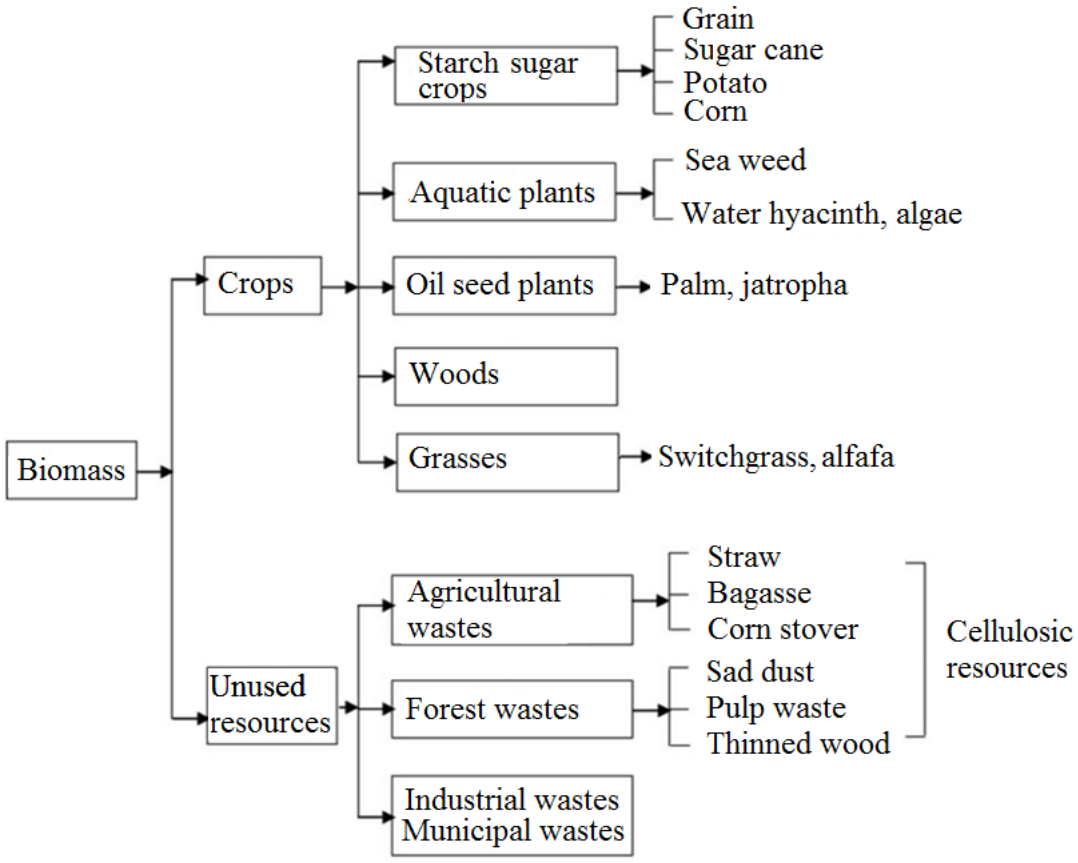
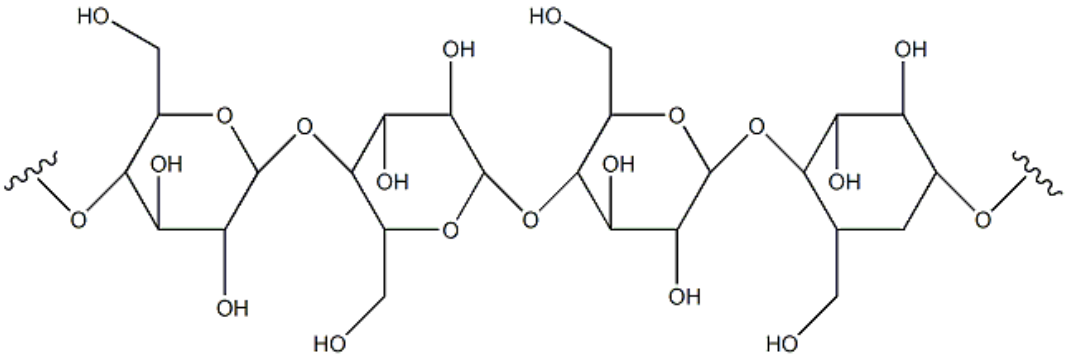


Figure 2. Biomass feedstock for biofuel conversion.
Source: Adapted from [17]

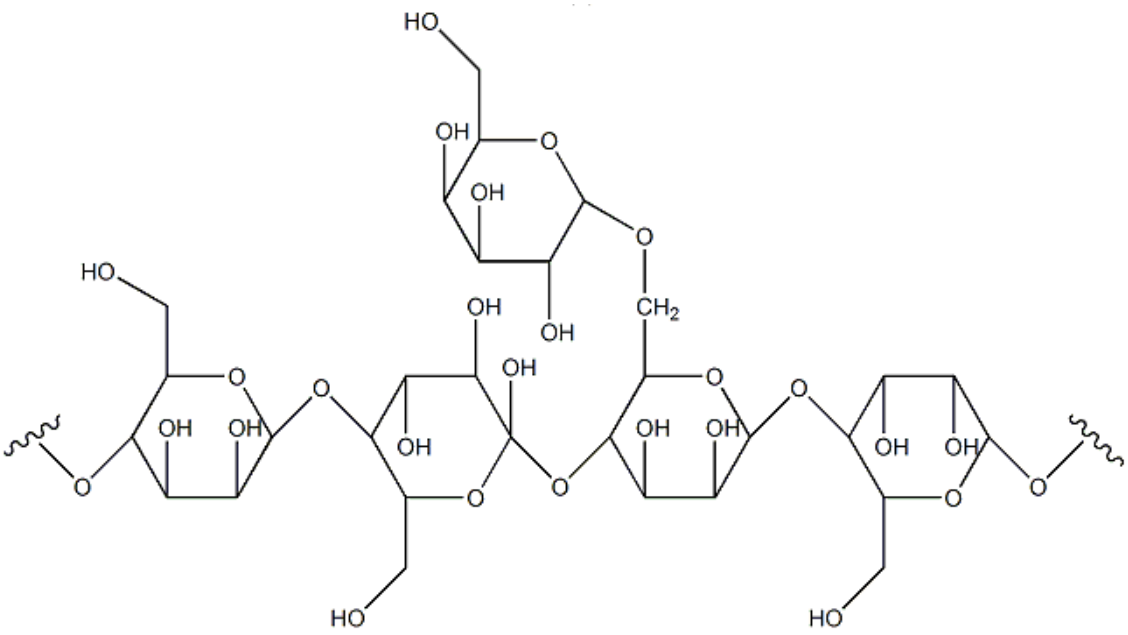
Lignocellulosic plants are composed of three main components: cellulose, hemicellulose, and lignin. Normally, the components need to be separated and hydrolysed into simple sugars to be used for biofuel production. Cellulose is the most abundant source of sugars in plants, and is constituted of a linear, semi-crystalline fibrous homopolysaccharide made of β(1,4) linked D-glucose (Figure 3) [19]. Strong hydrogen bonds between the glucose subunits create a lattice network in the cellulose, producing a stable and organized structure that interacts with hemicellulose [17]. Cellulose can be found either in a crystalline, or amorphous form [16]. The amorphous form is more easily degraded by enzymes, and is therefore easier to use for biofuel production, but the major portion of the cellulose in the plant is found in the crystalline form [16].



cellulose

Figure 3. Cellulose chain structure showing glucose molecules linked by β(1,4) glycosidic bonds.
Source: Adapted from [19]

Hemicellulose, the second most abundant source of sugars in the plant, is a branched heteropolysaccharide containing different sugars, including pentose (arabinose, xylose, and rhamnose) and hexose (glucose, mannose, and galactose) (Figure 4) [20]. Hemicellulose can also include a variety of uronic acids and can be acetylated [16, 20]. The linkage of hemicellulose is made of different α or β-glycosidic bonds, including (1,3), (1,4), or (1,6) linkage, which creates a branched polymer backbone [16]. Both cellulose and hemicellulose can be used for biofuel production, but hemicellulose is more easily hydrolysed, and the polymers do not aggregate, making the sugars in hemicellulose more accessible for biofuel production [16].



hemicellulose

Figure 4. Example of a section of hemicellulose. Galactoglucomannan can be typically found in conifer woods.
Source: Adapted from [21]

Lignin is an amorphous phenyl propanoid polymer, with the structural role of binding cellulose and hemicellulose by ester linkage and hydrogen bonds, respectively (Figure 5) [22]. The higher the content of lignin, the harder it is to hydrolyse the plant biomass [23]. Lignin therefore hinders the hydrolysis of the sugars, which makes biofuel transformation difficult due to the separation process [22, 24]. Three types of phenyl proprionic alcohols can be found as monomers of lignin, guaiacyl propanol, *p*-hydroxyphenyl propanol, and syringyl alcohol [16, 25]. The monomers are linked together by ether bonds (alkyl-aryl, alkyl-alkyl, or aryl-aryl) [16, 25].

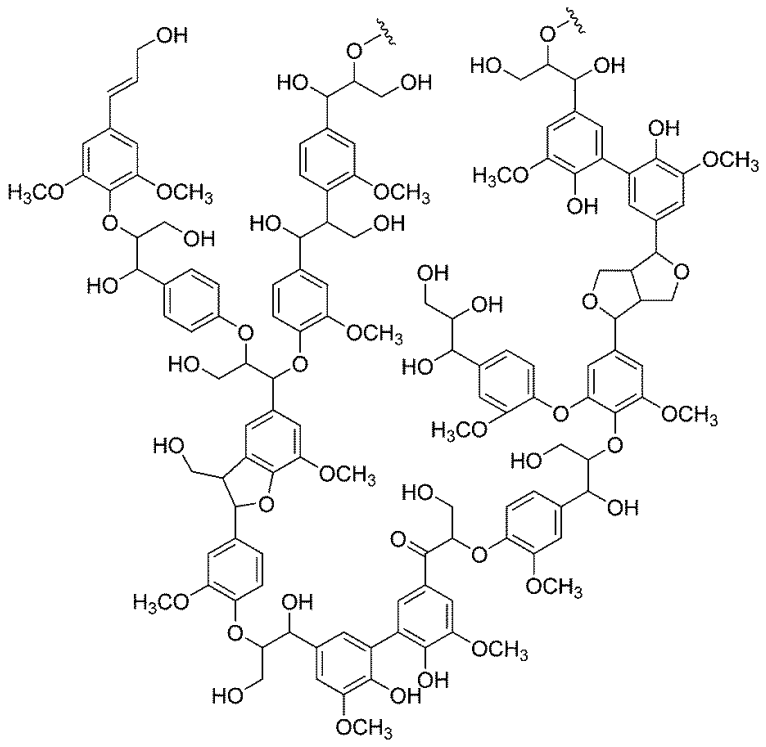


Figure 5. Example of a lignin structure.
Source: [25]

Our studies will focus on two lignocellulosic plants, *Symphytum officinale* L. (common comfrey, family Boraginaceae), and *Panicum virgatum* L. (switchgrass, family Gramineae). Switchgrass has previously been identified as a suitable feedstock for biofuel production, having been used for combustion, and as a feedstock to produce biodiesel and bioethanol [14, 26, 27]. Switchgrass has also proven to be a suitable substrate for acetone, BuOH and EtOH fermentation using yeasts [28]. Other biofuels such as fatty acid ethyl esters, BuOH and pinene have also been produced from switchgrass using bacterial enzymes [29]. On the other hand, although comfrey does not appear to have been used as a biofuel feedstock, the plant composition has been assessed by Godin e*t al*. (2010) and the sugar contents of

comfrey makes it a potential biofuel feedstock [30]. Both plants will be further discussed in section 1.6.

1.3. Biomass Treatment

Simple sugars are required for the conversion to biofuels. Exposing the carbohydrate fraction using thermochemical or physical pre-treatment is therefore an important aspect of biofuel production when using lignocellulosic materials [17, 31]. As previously discussed, once carbohydrates have been hydrolysed into simple sugars, transformation to bio-alcohols, bio-hydrogen, or methane, is possible through fermentation processes [14, 32, 33]. Pre-treating the biomass must remove hemicellulose and lignin, reduce cellulose crystallinity, and increase cellulose porosity [16]. The main goal of the pre-treatment is therefore to improve sugar availability, while also keeping sugar degradation to a minimum and avoiding the release of inhibitors which could influence the growth of yeasts and bacteria used for fermentation [16]. The efficiency of treatments will differ from plant to plant due to different content in cellulose, hemicellulose, and lignin in each plant, making achieving the goals of pre-treatments challenging [23]. Ideally, the method used to treat the plant biomass would also be environmentally friendly and cost efficient, but currently most methods are harsh and expensive [22]. Although pre-treatments require an investment in the cost of biomass processing, they are an important aspect of biofuel production. Treatments tremendously improve the yields of biofuel and are often necessary to achieve biofuel production [16].

Methods of treatment fall under many categories: physical, physiochemical, chemical, or biological [16]. Methods of treatment include steam explosion, pyrolysis, ammonia fiber explosion (AFEX), ozonolysis, enzymatic hydrolysis, acid hydrolysis, and alkaline hydrolysis [34]. From those methods, the most common ones are dilute acid hydrolysis, concentrated

acid hydrolysis, and enzymatic digestion by cellulases [35]. A list of different biofuel processing methods are presented in Figure 6. Four treatments will be used in this thesis research: mechanical breakdown, dilute acid hydrolysis, alkaline hydrolysis and solvent extraction.

1.3.1. Mechanical Breakdown

The simplest way to process biomass is to mechanically break it apart. Mechanical treatment includes chipping, grinding, and milling, and is most popular with woody materials and agricultural waste [16]. Mechanical breakdown works by increasing the surface area, and decreasing the crystallinity of cellulose, which increases sugar availability [16]. Unfortunately, the energy required to obtain particle sizes in the range of 3-6 mm is often beyond the energy potential of the feedstock, making mechanical breakage of the material often ineffective to produce a green energy [36]. For this reason, other treatments are often required for biofuel transformation.

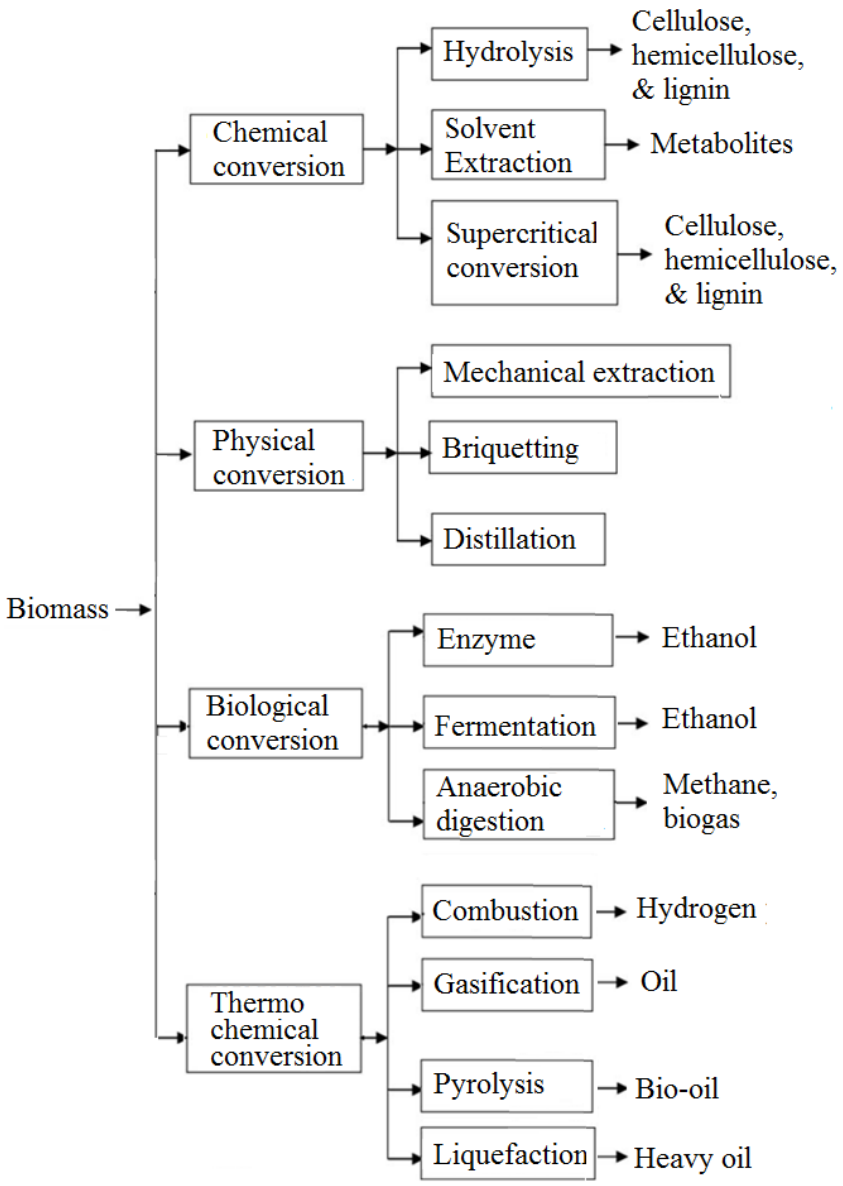


Figure 6. Biofuel treatments and biomass processing methods.
Source: Adapted from [6]

1.3.2. Acid Hydrolysis

Two types of acid hydrolysis are readily used to break down the carbohydrate chains in cellulose and hemicellulose: the concentrated acid hydrolysis, with concentrations ranging from 10-30% (wt/v), or the dilute acid hydrolysis, with concentrations ranging from 1-5% (wt/v) [35]. Many different acids can be used for treating biomass, including H_2SO_4, HNO_3, H_3PO_4 and HCl, with H_2SO_4 being the most commonly used [16]. Once again, depending on the types of biomass, different acids or different conditions might be necessary [35]. For example, hemicellulose is more easily hydrolysed, and therefore requires milder conditions, whereas cellulose usually requires stronger acids, with higher temperature and pressures in order to obtain simple glucose subunits [35]. Acid treatment is also known to degrade cellulose and hemicellulose in different yields [37].

For the concentrated acid hydrolysis, temperatures used are usually lower than 50°C while dilute acid hydrolysis requires high temperatures (normally ranging from 160°C to 230°C) and high pressures (usually around 10 atm) [35, 38]. Dilute acid hydrolysis has previously been shown to be more suitable for degrading high amounts of cellulose [37, 39]. Furthermore, dilute acid hydrolysis has been shown to offer better conditions for the conversion of xylan into xylose, which is important since xylan can make up to a third of the total sugars found in lignocellulosic materials [40]. Dilute acid hydrolysis therefore offers advantages over concentrated acid hydrolysis, including working in milder conditions, and obtaining better yields of cellulose and hemicellulose breakdown. Furthermore, dilute acid hydrolysis is appropriate for a large amount of feedstock, including agricultural waste (corn stover, bagasse), woods (aspen, balsam fir, red maple) and grasses (switchgrass) [16]. For

example, dilute acid treatment shows 96% of hydrolysis of hemicellulose into xylose, and 13.5% of hydrolysis of cellulose into glucose for switchgrass [41].

Although acid hydrolysis is extremely effective for producing simple sugars from cellulose and hemicellulose in a wide array of feedstocks, acids are also toxic and corrosive materials requiring special corrosive resistant reactors [34]. Thus this treatment is more expensive than physiochemical pre-treatments like AFEX [34]. The process requires recovery of the acids to reduce the costs and minimize the potential hazards for the environment [34, 42]. Another downfall of acid treatment is that the pH must be neutralized prior to using the recovered sugars for enzymatic treatments or fermentation processes, which adds to the cost of materials [16].

1.3.3. Alkaline Hydrolysis

Alkaline, or lime, pre-treatment involves the use of a diluted base, such as NaOH, KOH, $Ca(OH)_2$ or NH_4OH to break down the biomass [16]. The most commonly used base is NaOH, although less expensive lime solutions of KOH are also popular [16]. This type of pre-treatment does not require high temperature, and can be carried at room temperature, reducing the energy cost compared to acid hydrolysis [43]. However, treatment time varies from hours to days, instead of minutes in comparison to the acid treatment [43].

The efficiency of an alkaline treatment depends highly on the lignin content of the biomass being treated, with the treatment being inefficient on materials with lignin content above 26% [39, 44]. Alkaline hydrolysis is thought to work through saponification of the intermolecular ester links between hemicellulose and lignin [34]. Lime treatment has been shown to remove primarily lignin, and decreases acetylation present in hemicellulose, while cellulose remains mostly unbroken at temperature below 55°C [45]. However, the treatment

causes swelling, increases surface area, and decreases polymerization and crystallinity of cellulose [46].

Once again, lime treatment has been studied for a variety of feedstocks such as wood, straw, corn stover, and grasses [16]. For example, switchgrass digestion in $Ca(OH)_2$ resulted in 26% extraction of xylan, 10% extraction of glucans, and 29% extraction of lignin [47].

1.3.4. Plant Extracts

Solvent extraction offers a good tool to measure the soluble portion of the sugars in the plant biomass [48]. Methanol extraction has been shown to extract soluble sugars in plant [49]. Methanol solutions are also often used to extract medicinal compounds in plants to test for activity, or to quantify other compounds such as chlorophylls, carotenoids, phenols, proanthocyanidins, flavones, and flavonols [50-52]. Other methods of extraction include hot EtOH extraction and MeOH: $CHCl_3$: H_2O mixture extraction [48, 53, 54]. Samples extracted with warm EtOH have been shown to offer more accurate estimates of soluble sugars compared to the MeOH: $CHCl_3$: H_2O mixture with EtOH extracting 7-16% more soluble sugars [48]. However, the solvents mentioned above will not dissolve cellulose, hemicellulose, or lignin found in the plants, and are therefore mostly ineffective for the production of biofuels from lignocellulosic materials. The next section will explore the use of alternative solvents for lignocellulosic biomass dissolution.

1.4. Ionic Liquids

Due to the strong network of cellulose, sugars are presently extracted using harsh treatments. Dissolution of biomass would be more environmentally friendly if break down of cellulose used a milder and recyclable solvent. Furthermore, current methods of cellulose

breakdown usually also degrade a portion of the available sugars, thus making the processes wasteful [17]. Use of solvents to extract cellulose would therefore be a better alternative compared to those harsh acid and base conditions. Dissolution of cellulose in solvents may also open the door for organic transformations which cannot be performed in aqueous media [55]. Unfortunately, cellulose is difficult to dissolve due to the hydrogen-bond network which creates a chemically and thermally stable structure [56]. The lignin that "glues" cellulose and hemicellulose together further tends to decrease biomass solubility, making the discovery of a suitable solvent extremely difficult [24].

In recent years, some of these issues have been solved by using ionic liquids for biomass dissolution. Instead of attempting to break the biomass down into simple sugars like alkaline and acid treatments, this treatment serves to dissolve cellulose directly in the ionic liquid [17]. Cellulose can then be extracted and used with further treatment for fermentation or the dissolved biomass in the ionic liquid can be used directly for biofuel production by organic synthesis [17, 55]. In the following section ionic liquids will be discussed.

1.4.1. Definition of Ionic Liquids

Ionic liquids encompass any ionic salt with a melting point below 100°C [57]. Ionic liquids are known to display high thermal stability and are non-volatile, making them an environmentally friendly alternative for dissolving cellulosic materials [58]. The low vapor pressure reduces the risk of volatile toxic compound production, while thermal stability enables recycling of the solvent due to the low degradation rate under high temperatures [59, 60]. For example, only 1% of 1-butyl-3-methylimidazolium acetate ([BMIM]Ac) is decomposed within 10 h at 120°C [60].

A variety of ionic liquids have been studied, and some examples of cations and anions used in those solvents are shown in Figure 7. The most common cations used for the dissolution of biomass are 1-butyl-3-methylimidazolium [BMIM], 1-ethyl-3-methylimidazolium [EMIM], and 1-allyl-3-methylimidazolium [AMIM], and are often paired with a halide ion, or an acetate ion [17]. The structures of those cations are shown in Figure 8. Toxicity of the cations usually increases when the size of the alkyl chain increases [61].

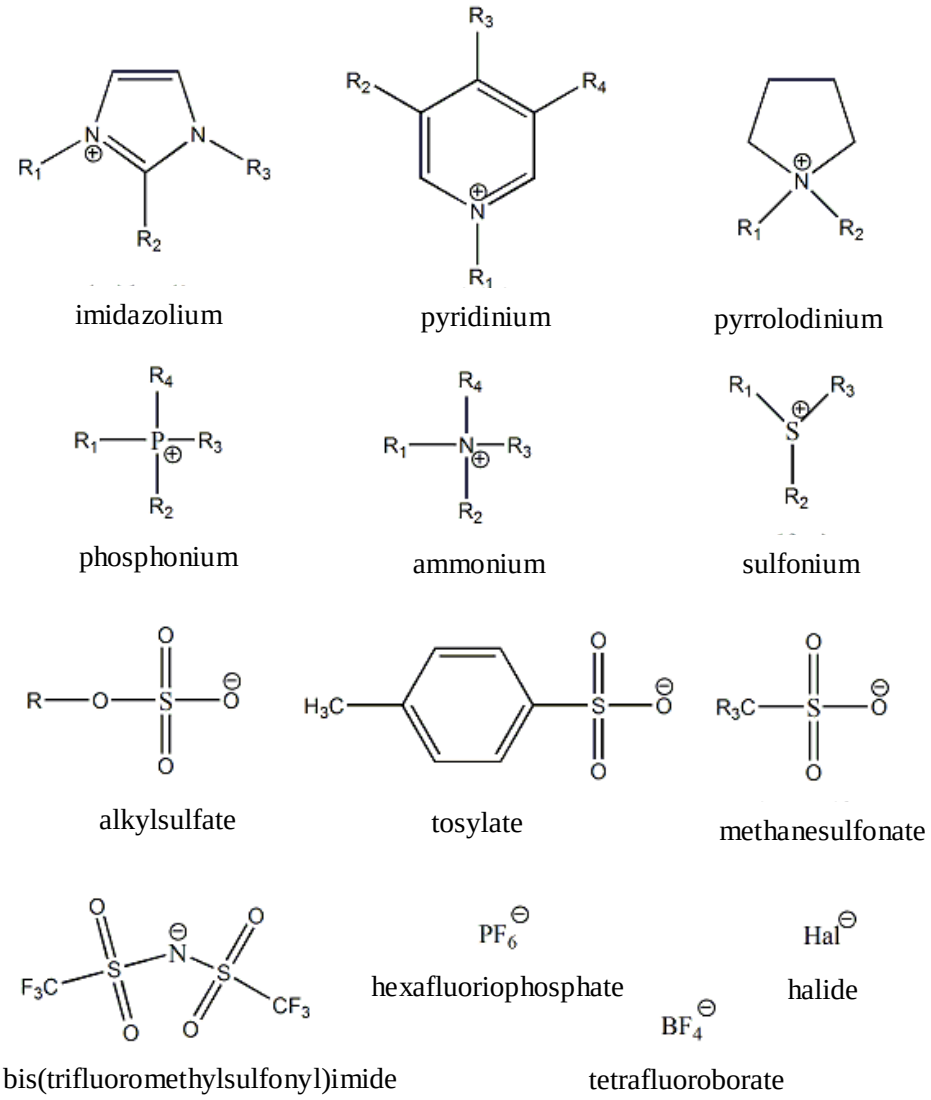


Figure 7. Example of cations and anions used in ionic liquids.
Source: [57]

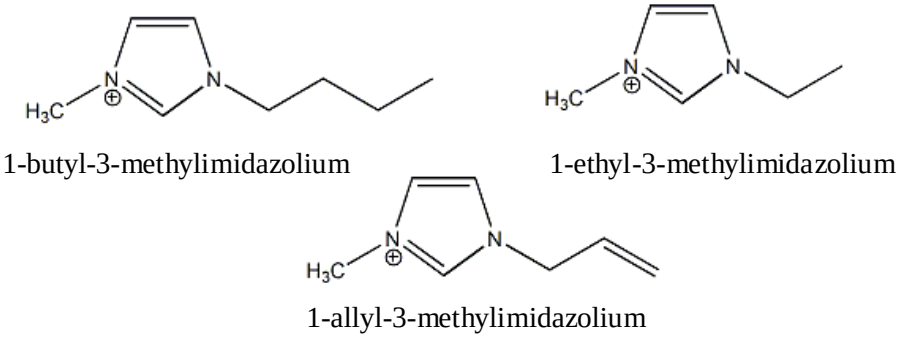


Figure 8. Common cations used in ionic liquids for dissolution of lignocellulosic biomass.
Source: [17]

1.4.2. Dissolution in Ionic Liquids

Many research groups have studied dissolution of biomass in ionic liquids [17, 62]. Even sawdust feedstocks from species such as Norway spruce and Southern yellow pine have been completely dissolved using ionic liquids [63]. The mechanism by which dissolution takes place is thought to involve the cation of the ionic liquid binding to the oxygen in the hydroxyl groups of the outside "wall" of cellulose, and the anions binding to the inside of the cellulose strands via H-bonds with the hydroxyl groups [64]. The electrostatic interactions between the outside cation, and the inserted anions, causes the intercalation of cellulose [64].

So far, alkylimidazolium cations with the acetate anion have displayed the highest rate of dissolution at low temperature (below 100°C), offering the advantage of being liquid at room temperature [65-68]. For example, switchgrass can be dissolved entirely in 1-ethyl-3-methylimidazolim acetate ([EMIM]Ac) after 3 h at 120°C [66]. Soft wood, such as Southern yellow pine, and hard wood, such as red oak, were also shown to dissolve in [EMIM]Ac (up to 90%) after 16 h at 110°C [69]. The acetate ion, CH_3COO^-, is thought to be the most efficient at dissolving biomass due to strong hydrogen bond acceptor properties [70]. Chloride anions are also often used, even though they demonstrate a reduced potential for separating cellulose chains due to the lower strength of the H-bonds being formed [64, 71]. For example, poplar wood can be dissolved to 96 wt% in [BMIM]Ac after 12 h at 130°C, but [BMIM]Cl only dissolved 23 wt% under the same conditions [72]. Therefore, the efficiency of the anion in the ionic liquid depends on the property by which they will accept hydrogen bonds, making strong anion basicity necessary in this type of dissolution. Ionic liquids with the anions $CH_3SO_4^-$, HSO_4^-, and $CH_3SO_3^-$ are therefore not suitable for dissolution of biomass due to the low basicity; the anion tetrafluoroacetate is also unsuitable due to the strong electron-withdrawing

property of F [73, 74]. Ionic liquids with such anions, for example [BMIM]HSO_4, might be used for the dissolution of simple sugars [75]. For the cation, the bigger the size of the chain, the smaller the solvating power, due to the bulkiness preventing interaction with cellulose, as well as decreasing the amount of anion available in solution [76].

A factor that also influences dissolution of plant derived-biomass in ionic liquids is the type of plant material being used. Different plant materials will dissolve with different efficiency, and although some ionic liquids are more commonly used than others for biomass dissolution such as [EMIM]Ac and [BMIM]Cl, predicting the efficiency of dissolution is difficult [17]. For example, although the acetate ion has previously been proven to be the best at dissolving cellulose, poor dissolution of wheat straw and pine wood biomass occurs after 24 h at 100°C [77]. This observation can be partially due to the fact that lignin decreases dissolution in ionic liquids [17]. Lignocellulosic materials containing a high percentage of lignin have been shown to create a brown viscous substance when mixed with the ionic liquid, an indication that the biomass material is being poorly dissolved due to the high lignin content [78].

Other variables can also affect biomass dissolution including the size of the particles, the ratio of biomass/ionic liquid, the temperature, and the time of dissolution [17]. Larger particle sizes tend to decrease the percentage of dissolution [69]. Since smaller particle sizes are more suitable for dissolution, a mechanical breakdown of the plant material may be necessary prior to dissolution [79]. Ideally, the size of the particles will be no smaller than necessary in order to save on the costs and the energy required at the grinding stage.

Lower temperatures will also generally increase reaction times necessary for dissolution of cellulose in ionic liquid. This is due to higher temperatures disrupting the

hydrogen network in cellulose more readily [78]. Once again, the appropriate temperature will be dependent on the type of ionic liquid, but usually temperatures above 100°C are used to reduce the volume of H_2O produced in the dehydration of cellulose [74]. Furthermore, higher temperatures will decrease the ionic liquid viscosity and increase mass transport, making the dissolution process more efficient [80]. Ionic liquids with lower viscosity, especially those which are liquid at room temperature (eg: imidazolium acetate), can be used at lower temperatures than more viscous ionic liquids with higher melting point (eg: imidazolium chloride). Consideration of the degradation of sugars and the degradation of the ionic liquids which can occur at higher temperatures is also important [81]. The reaction time is also directly linked to reaction temperature, with lower temperatures requiring longer dissolution times, and higher temperatures shorter reaction times. Longer reaction times have the advantage of normally increasing dissolution of lignin, but can also increase sugar degradation [69, 82]. For this reason, the study of correct temperature and time for a dissolution depending on the ionic liquid as well as the type of biomass used, attempting to keep both parameters as small as possible while maintaining maximal dissolution, is important.

Lower concentrations of substrates also increase dissolution by favouring the dispersion of the molecules in the solution and increasing the interaction between the ionic liquid and the available biomass [69]. The efficiency of the dissolution process for cellulose is dependent on the ratio between the glucose molecules, and the anion which can interact with the hydrogen of the hydroxyl group in a 1:1 ratio [64].

Although ionic liquids offer many interesting properties, they are not without challenges. Ionic liquids have a very high viscosity, and adding cellulose to the mixture

further increases this viscosity, which limits dissolution of the materials due to decreased mass transport [56, 83, 84]. Furthermore, negative solute-solvent interactions also play a role in the amount of cellulose that can be readily dissolved in ionic liquids. Addition of H_2O usually decreases dissolution in ionic liquids due to the interaction of the H_2O molecules with the glucose molecules at the hydroxyl positions in cellulose, preventing the interaction between the ionic liquid and the glucose molecules at those same positions [56, 64, 68, 85]. Normally, H_2O concentrations greater than 1% of the reaction mixture cause a decrease in dissolution [76]. Some studies have shown that use of a co-solvent or a gas alongside the ionic liquid may be beneficial, decreasing viscosity and increasing dissolution of cellulose [56, 84, 86]. Due to the issues associated with H_2O, the use of a protic solvent is therefore not suitable with the ionic liquid. Strongly polar and aprotic solvents, such as dimethyl sulfoxide (DMSO), work best at increasing dissolution because of the decrease in viscosity of the ionic liquid/solvent mixture, and associated low interaction of the aprotic solvent with glucose which accelerates mass transport and increases conductivity [56]. Another example of a co-solvent currently used is the LiCl/*N*,*N*-dimethylacetamide (DMA) mixtures [86]. Gas such as CO_2 have also proven to decrease viscosity, and improve mass transport for biomass dissolution in ionic liquids [84].

1.4.3. Chemical Reactions using Ionic Liquids

Currently, ionic liquids are used as a tool for cellulose extraction in plants. Regeneration of cellulose, but with altered structural properties and crystallinity, as well as carbohydrate fractioning is possible via the use of an anti-solvent such as H_2O, acetone, or acetonitrile which can be added to the ionic liquid after the completed dissolution [17]. The recovered materials have been shown to be more suitable for enzymatic hydrolysis followed

by bioethanol production [17]. The regeneration step can also be bypassed in favour of using direct organic synthesis to convert the dissolved biomass into a suitable biofuel. Ionic liquids are currently used as a solvent in the reaction transforming carbohydrates, or plant biomass, to the molecule 5-hydroxymethylfurfural (HMF), a promising molecule for the future of biofuel production [87].

1.5. 5-Hydroxymethylfurfural

5-Hydroxymethyfurfural (HMF), a derivative of furan containing an aldehyde and an alcohol functional group, can be synthesized from glucose and subsequently transformed to 2,5-dimethylfuran (DMF) by hydrogenation (Figure 9) [86]. The energy content of DMF (31.5 MJ/L) is closer to the energy content of gasoline (35 MJ/L) and better than the energy content of EtOH (23 MJ/L); DMF also has a higher boiling point (92-94°C) than EtOH (78°C), making it an important molecule for usage as a biofuel [86].

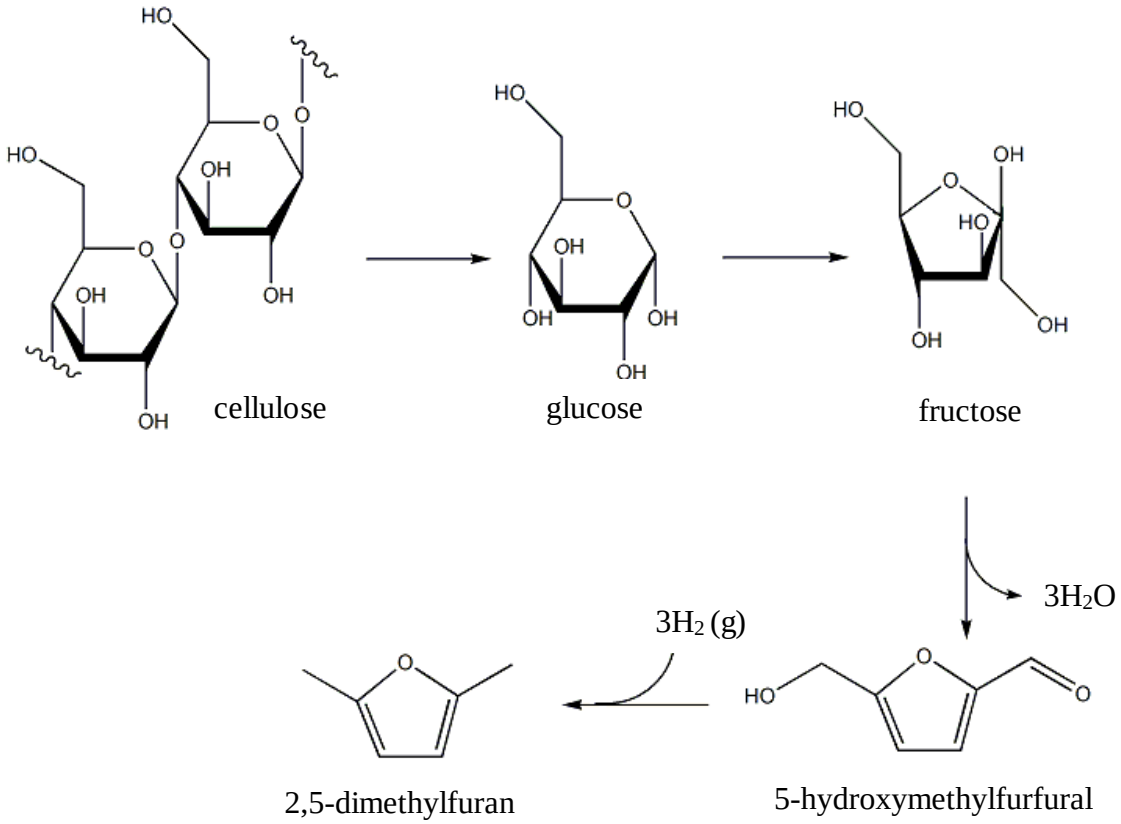


Figure 9. Synthesis of HMF and DMF from cellulose.
Source: Adapted from [86]

HMF is naturally found in sugar-containing plants and foods such as milk, honey, fruits and bread, and can be produced during cooking of sugary foods due to the dehydration of sugars, but the quantity of HMF in those sources remains very low [88]. HMF is not considered a harmful substance, but it is recognized as a molecule involved in food spoilage [88].

1.5.1. Reaction Mechanism from Glucose to HMF Using Ionic Liquids and Metal Halide Catalysis

Transforming fructose into HMF is a simple process of dehydration (Figure 9), while using glucose as a feedstock requires an isomeration of glucose into fructose prior to the

transformation into HMF. Other hexoses can also be used as a feedstock to produce HMF, such as mannose, xylose and arabinose [89]. Methods for transforming carbohydrates, cellulose, as well as cellulosic materials (such as corn stover) are currently being studied [86, 90-94]. An important step which allows the use of biomass to produce HMF is the dissolution of cellulose, making ionic liquids interesting for the organic synthesis of HMF.

The mechanism of transformation of simple sugars to HMF has previously been hypothesized by Guan *et al*. (2011) for the catalysis in [BMIM]Cl with a MCl_3 (where M is a transition metal cation with an oxidation number of 3) [87]. The first step of the reaction is the transformation of glucose to fructose. The proposed mechanism by Guan *et al*. (2011) is reported in Figure 10 [87]. First, the glucose-MCl_3 complex **1** is generated via interactions between the H in the hydroxyl groups of the glucose at position C1 and C2, and the Cl atoms of [BMIM]/MCl_3, and interactions between the O in the hydroxyl groups and the metal atom. The ring from **1** is opened to form the open-glucose-MCl_3 complex **2** and the open –CHO group binds to MCl_3. The subsequent formation of a five membered ring chelate structure occurs between the two neighboring OH groups in glucose and the metal atom. This structure forms the 1,2-enediol-MCl_3 intermediate (**3**). Intermediate **3** then takes the open form of the fructose-metal complex (**4**). Finally, [BMIM]Cl and MCl_3 are released and the open-fructose structure (**5**) is closed via a ring closure to form fructofuranose.

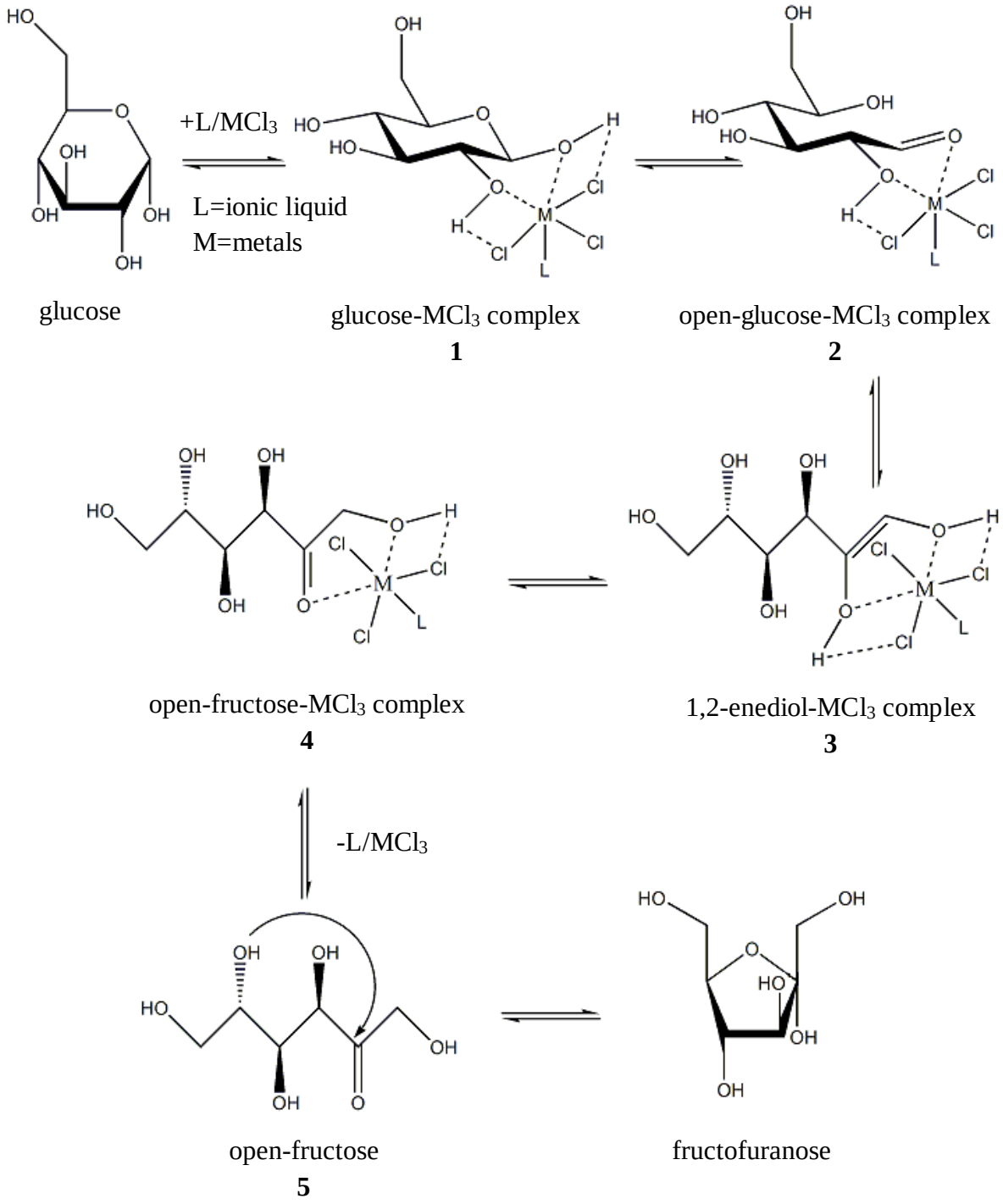


Figure 10. Formation of fructose from glucose in [BMIM]Cl (L) with a metal chloride (MCl_3). Source: [87]

Fructose, once formed, can be transformed to HMF by dehydration where three molecules of H_2O are lost [87]. The proposed mechanism to form HMF from fructose using [BMIM]Cl and MCl_3 is described in Figure 11 [87]. First, two O atoms at position C1 and C5 interact with the metal atom and form a fructose-MCl_3 complex **6**. A H_2O molecule, fragmented from fructose, interacts with the L/MCl_3 complex to form intermediate **7**. The L/MCl_3 complex alongside the H_2O molecule are then lost forming **8** which is rapidly isomerized into its aldehyde form **9**. During the second step, the metal atom interacts with the O atom at the C2 position and the aldehyde group at the C5 position to form **10**. A second H_2O molecule is released from the 5-membered ring and interacts with L/MCl_3 to form **11.** The L/MCl_3 complex alongside the H_2O molecule are then lost, and a double bond is formed between C1 and C2 yielding **12**. Finally, the O atom at C3 is coordinated to the metal site forming **13**. A third H_2O molecule is lost from the 5-membered ring, and the H_2O molecule interacts with the L/MCl_3 forming **14**. Lastly, the L/MCl_3 complex and the H_2O molecule are lost, with a double bond being formed between C3 and C4 to produce HMF.

Different ionic liquids, with or without co-solvents, can be used for the transformation of sugars to HMF. As mentioned before, co-solvents can include LiCl/DMA mixtures or DMSO [86, 91, 94]. Addition of acid could also favor the transformation of biomass into HMF, as acid can be used as a replacement for the metal halides in reactions for the transformation of fructose to HMF [86]. A variety of metal halides can also be used to produce HMF from biomass, including $CrCl_3$, $CrCl_2$, $CuCl_2$, $ZnCl_2$, etc. [90].

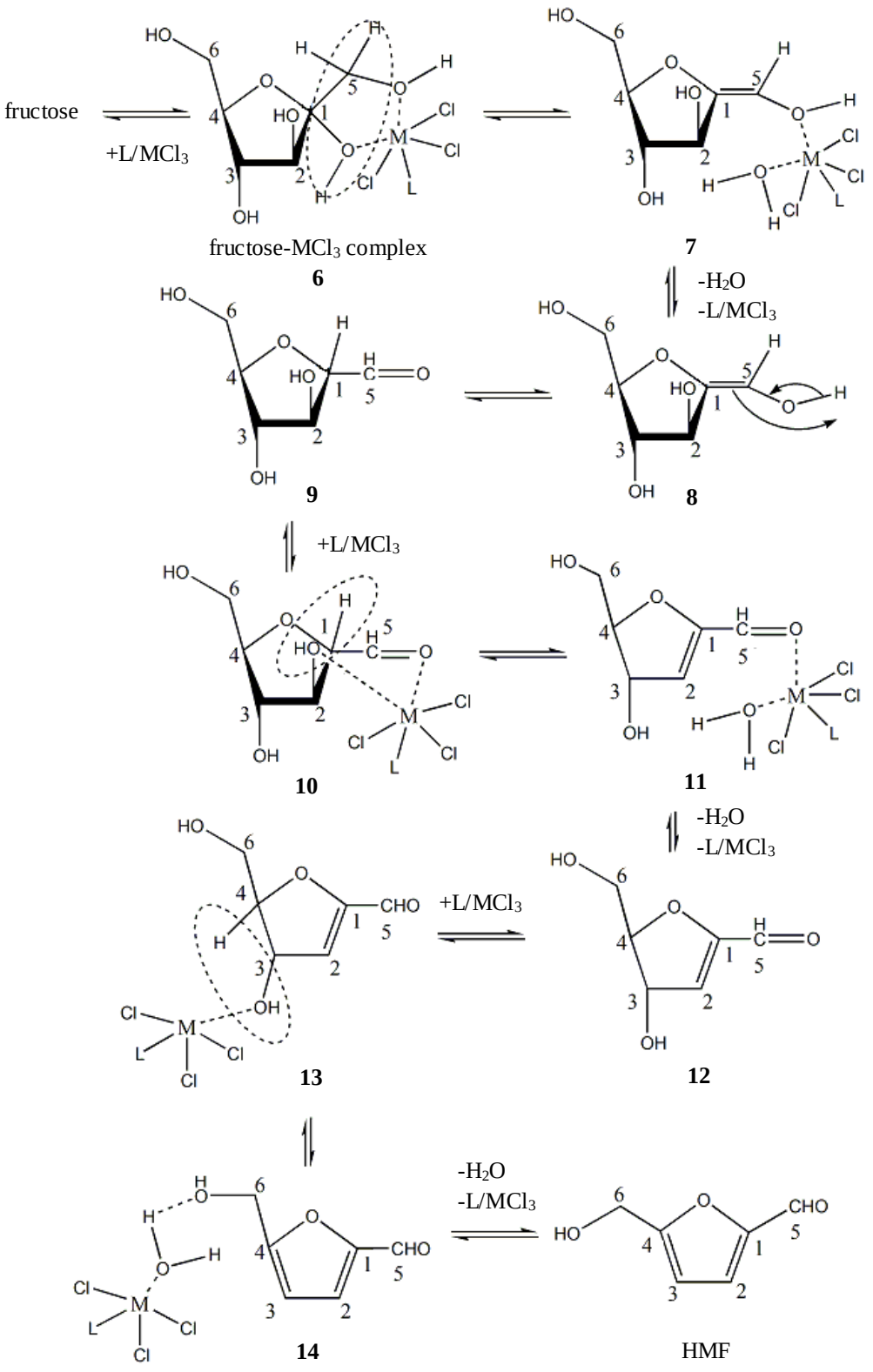


Figure 11. Formation of HMF from fructose in [BMIM]Cl (L) with a metal chloride (MCl_3). Source: [87]

1.5.2. Organic Synthesis of HMF from Mono- and Disaccharide Feedstocks Using Ionic Liquids and Metal Halide Catalysis

As explained in the previous section, the simplest way to produce HMF is through the conversion of glucose or fructose, but starch and disaccharides (such as maltose, lactose, and sucrose) can also be utilized as a feedstock for easy conversion to HMF due to the high solubility and simplicity of those sugar feedstocks [95]. Starch, a polymer of sugar made of amylose (glucose units linked by α(1,4)-glycosidic bonds) and amylopectin branches (glucose units linked by *α*(1,6)-glycosidic bonds), can be found in corn, wheat, rice, potato tuber, sweet potato, tapioca, acorn, and kudzu starch [95]. On the other hand, galactose has been found to be a poor feedstock for production of HMF using metal halide catalysis in ionic liquid [89].

With high temperatures and long reaction times, HMF has been shown to degrade to side products by rehydration to levulinic acid or formic acid [91]. Harsh conditions also result in the generation of humins which can be visualized by their brown colour [89]. Therefore, time and temperature must be optimized to find the most suitable conditions for production of HMF for a variety of feedstocks. In the case of simple sugars and disaccharides, time varies from minutes to 24 h, while temperatures are usually maintained between 80°C to 140°C (Table 1).

Ionic liquids are primarily used in the dissolution of sugars for HMF production. Other solvents have also been studied for the conversion of sugars to HMF, including H_2O and DMSO [96]. However, H_2O is not appropriate for the dissolution of cellulose or biomass and DMSO poses problems related to poor extractability and purification of HMF. Those solvents also offer lower yields of HMF compared to ionic liquids [97].

Table 1. Literature overview for the transformation of sugars to HMF using ionic liquids and other solvents in the presence of metal halide and acid catalysts. List of ionic liquids: [AMIM]Cl, 1-allyl-3-methylimidazolium chloride; [BDBU]Cl, 7-butyl-1,8-diazabicyclo[5,4,0]undec-7-ene chloride; [BMIM]Cl, 1-butyl-3-methylimidazolium chloride; [BMIM]HSO_4, 1-butyl-3-methylimidazolium hydrogen sulfate; [C_3SO_3HMIM]HSO_4, 1-(4-sulfonic acid)-propyl-3-methylimidazolium hydrogen sulfate; [NMP]HSO_4, *N*-methyl-2-pyrrolidonium hydrogen sulfate; [OMIM]Cl, 1-octyl-3-methylimidazolium chloride. DMA, *N*,*N*-dimethylacetamine; MI, microwave. Catalyst concentration reported per mol or per weight of substrate loading.

Substrate	**Solvent**	**Catalyst**	**t (h or min), T (°C)**	**HMF yield (%)**	**Reference**
Fructose	[BMIM]Cl	$CrCl_2$ (6 mol%)	3 h, 120	70	[55]
Fructose	[BMIM]HSO_4	$CrCl_3$•$6H_2O$ (7 mol%)	24 h, 80	80	[75]
Fructose	[BMIM]HSO_4	$CrCl_2$ (7 mol%)	24 h, 80	74	[75]
Fructose	[BMIM]HSO_4	WCl_4 (7 mol%)	24 h, 80	65	[75]
Fructose	[BMIM]HSO_4	$ZnCl_2$ (7 mol%)	24 h, 80	38	[75]
Fructose	[AMIM]Cl	-	30 min, 100	91.1	[98]
Fructose	[AMIM]Cl	[C_3SO_3HMIM]HSO_4 (9 mol%)	10 min, 100	91.1	[98]
Fructose	[BDBU]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	1.5 h, 100	69	[92]
Fructose	DMA	$CrCl_3$ (9.5 wt%), NH_4Br (0.16M)	1 h, 100	92	[97]
Fructose	Isopropanol	NH_4Cl (5 mol%)	12 h, 120	97.2	[99]
Fructose	DMSO	-	5 h, 150	90	[100]
Glucose	[BMIM]Cl	$CrCl_3$ (5 wt%)	10 min, 140	68.8	[89]
Glucose	[BDBU]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	3 h, 100	64	[92]
Glucose	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3.6 mol%)	1 min, MI	91	[101]
Glucose	[AMIM]Cl	[C_3SO_3HMIM]HSO_4 (9 mol%), $CoCl_2$ (4 mol%)	2 h, 120	62.2	[98]

Glucose	DMA	AlI_3 (20 mol%)	15 min, 120	52	[91]
Glucose	DMA	$CrCl_3$ (9.5 wt%), NH_4Br (0.16 M)	1 h, 100	74	[97]
Mannose	[BMIM]Cl	$CrCl_3$ (5 wt%)	2 h, 120	62.4	[89]
Galactose	[AMIM]Cl	$[C_3SO_3HMIM]HSO_4$ (9 mol%), $CoCl_2$ (4 mol%)	1 h, 120	19.7	[98]
Sucrose	[AMIM]Cl	$[NMP]HSO_4$ (9 mol%)	1 h, 120	82.3	[98]
Sucrose	[AMIM]Cl	H_2SO_4 (9 mol%)	1 h, 120	50.2	[98]
Sucrose	[BDBU]Cl	$CrCl_3 \cdot 6H_2O$ (10 wt%)	2 h, 100	63	[92]
Sucrose	DMA	$CrCl_3$ (9.5 wt%), NH_4Br (0.16 M)	1 h, 100	87	[97]
Maltose	[AMIM]Cl	$[C_3SO_3HMIM]HSO_4$ (9 mol%), $CoCl_2$ (4 mol%)	30 min, 140	55.7	[98]
Lactose	[AMIM]Cl	$[C_3SO_3HMIM]HSO_4$ (9 mol%), $CoCl_2$ (4 mol%)	30 min, 140	36.1	[98]
Tapioca starch	[OMIM]Cl	HCl (0.5 M), $CrCl_2$ (5 wt%)	1 h, 120	73.0	[95]

The solvent *N*,*N*-dimethylacetamide (DMA) has shown to offer good dissolution of sugars and produce good yields of HMF [91, 97]. In the case of DMA, the solvent can also be used for cellulose dissolution, with use of an ionic liquid in conjunction with DMA potentially increasing dissolution and conversion of HMF [86].

A summary of the literature concerning the conversion of mono- and disaccharide, as well as starch, to HMF utilising ionic liquids, and other solvents, with metal halides catalysis, or acid catalysis, are reported in Table 1. Yields of HMF produced from glucose or fructose vary from 38%, to 97.2% [55, 75, 89, 91, 92, 95, 97-101]. Disaccharide such as sucrose and maltose can also produce good yields of HMF which are comparable to fructose (above 50%), while lactose can be converted with the slightly lower yields of 36.1%, and galactose can be converted at the low yield of 19.7% of HMF [92, 97, 98]. Starch can be converted in yields comparable to fructose with a yield of HMF of 73.0% [95].

1.5.3. Organic Synthesis of HMF from Cellulose and Biomass Using Ionic Liquids and Metal Halide Catalysis

Although simple sugars offer easy feedstocks to produce HMF, most of the sugars on this planet are locked away in lignocellulosic plants. The ability to directly use plants would remove the need for the expensive and harsh pre-treatments currently necessary to produce simple sugars for biofuel conversion. As previously discussed, ionic liquids are known to dissolve lignocellulosic biomass, which makes HMF production directly from plant materials possible. In this section, a summary of the literature concerning the transformation of cellulose or lignocellulosic biomass to HMF using ionic liquids, with or without co-solvent, will be explored. A literature overview of the reactions for production of HMF is reported in Table 2, with a focus put on metal halide catalysis in ionic liquids.

Table 2. Literature overview for the transformation of cellulose and lignocellulosic biomass to HMF using ionic liquids and other solvents in the presence of metal halide and acid catalysts. List of ionic liquids: [AMIM]Cl, 1-allyl-3-methylimidazolium chloride; [BDBU]Cl, 7-butyl-1,8-diazabicyclo[5,4,0]undec-7-ene chloride; [BMIM]Cl, 1-butyl-3-methylimidazolium chloride; [EMIM]Cl, 1-ethyl-3-methylimidazolium chloride; [OMIM]Cl, 1-octyl-3-methylimidazolium chloride. AFEX, ammonia fiber explosion; DMA-LiCl, *N*,*N*-dimethylacetamide containing 10 wt% LiCl; MI, microwave; TEACl, tetraethylammonium chloride; THF, tetrahydrofuran. Catalyst concentration reported per mol or per weight of substrate loading.

Biomass	**Solvent**	**Catalyst**	**t (min or h), T (°C)**	**HMF yield (%)**	**Reference**
Inulin	[BDBU]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	2 h, 100	46	[92]
Inulin	H_2O	H_2SO_4 (6 mM)	20 min, 170	50.6	[102]
Cellobiose	[BDBU]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	3 h, 100	39	[92]
Cellulose	[BDBU]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	2 h, 130	41	[92]
Cellulose	[EMIM]Cl	$FeCl_3$ (6 wt%)	10 min, 140	23.6	[90]
Cellulose	[EMIM]Cl	$CrCl_2$ (6 wt%)	10 min, 140	31.8	[90]
Cellulose	[EMIM]Cl	$CuCl_2$ (6 wt%)	10 min, 140	11.3	[90]
Cellulose	[EMIM]Cl	$CuCl_2$ (3 wt%), $CrCl_3$ (3 wt%)	10 min, 140	37.7	[90]
Cellulose	[BMIM]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	2 min, MI	62	[101]
Cellulose	[BMIM]Cl	LiCl (50 mol%), $CrCl_3$ (50 mol%)	10 min, 160	62.3	[93]
Cellulose	DMSO, [BMIM]Cl	$AlCl_3$ (10 wt%)	9 h, 150	54.9	[94]
Cellulose	DMSO, TEACl	$AlCl_3$ (10 mol%)	7 h, 150	32	[100]
Cellulose	DMSO, TEACl	$CrCl_3$ (10 mol%)	7 h, 150	26	[100]
Cellulose	DMA-LiCl, [EMIM]Cl	$CrCl_2$ (25 mol%), HCl (6 mol%)	2 h, 140	54	[86]
AFEX treated corn stover	DMA-LiCl, [EMIM]Cl	$CrCl_2$ (38 mol%)	6 h, 140	16	[86]

Corn stover	DMA-LiCl, [EMIM]Cl	$CrCl_2$ (38 mol%)	6 h, 140	16	[86]
Corn stover	DMA-LiCl, [EMIM]Cl	$CrCl_3$ (10 mol%), HCl (10 mol%)	2 h, 140	48	[86]
Corn stalk	[BMIM]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	3 min, 100, MI	45	[103]
Rice Straw	[BMIM]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	3 min, 100, MI	47	[103]
Wheat Straw	[BMIM]Cl	LiCl (50 mol%), $CrCl_3$ (50 mol%)	15 min, 160	61.4	[93]
Wheat Straw	DMSO, TEACl	$AlCl_3$ (20 mol%)	4 h, 150	13	[100]
Wheat Straw	DMSO	H_2SO_4 (20 mol%)	4 h, 150	7	[100]
Pine Wood	[BMIM]Cl	$CrCl_3$•$6H_2O$ (10 wt%)	3 min, 100, MI	52	[103]
Poplar Wood	H_2O, THF	$AlCl_3$•$6H_2O$	30 min, 180, MI	26	[104]
Poplar Wood	DMSO, TEACl	$AlCl_3$ (20 mol%)	4 h, 150	24	[100]
Poplar Wood	DMSO	H_2SO_4 (20 mol%)	4 h, 150	7	[100]
Switchgrass	H_2O, THF	$AlCl_3$•$6H_2O$	30 min, 180, MI	21	[104]
Switchgrass	1% H_2SO_4	-	2 min, 220	4.5	[105]
Bamboo fiber	H_2O, THF, NaCl	NH_2SO_3H (40 mol%)	40 min, 180, MI	52.2	[106]
Dioscorea composite (yam)	DMA-LiCl	$CrCl_3$•$6H_2O$, $LaCl_3$•$6H_2O$	4 h, 120	33.2	[107]
0.3 M HCl extract of girasol tuber	[OMIM]Cl, EtOAc	-	1 h, 120	58.3	[108]
0.5 M HCl extract of potato tuber	[OMIM]Cl, EtOAc	$CrCl_2$	1 h, 120	54.4	[108]
0.3 M HCl extract of acorn	[OMIM]Cl, EtOAc	$CrBr_3$, CrF_3	1.5 h, 120	58.7	[109]
0.3 M HCl extract of chicory root	[OMIM]Cl, EtOAc	-	1 h, 120	50.9	[110]

Once again, temperatures varying from 100°C to 140°C are recommended based on the literature for the conversion of HMF in ionic liquids, with Gaikwad and Chakraborty (2014) suggesting an ideal temperature of 120°C when the reaction is performed in [BMIM]Cl with $CuCl_2$ [111]. However, use of different solvents and different catalysts requires an adjustment of the temperature [90]. Reaction times vary from minutes to hours depending on the feedstock, and the type of ionic liquid used (Table 2). When using cellulose or biomass, yields are generally lower compared to the ones obtained from glucose or fructose (Table 1). The highest reported yield of HMF from cellulose found in the chemical literature was about 62% using [BMIM]Cl (with or without LiCl) and using $CrCl_3$ as the catalyst [93, 101]. The highest yield of 61.4% of HMF was found using a biomass feedstock, wheat straw, dissolved in [BMIM]Cl with LiCl and $CrCl_3$ [93]. However, in most cases, conversion of cellulose or biomass to HMF offer yields near, or below 50% [86, 90, 92, 94, 100, 102-110].

Different types of biomass, most of which are materials high in starch and simple sugars, have been studied which explains why yields of HMF are nearly as high as observed for simpler feedstocks such as glucose with feedstocks including corn stover, corn stalk, rice straw, wheat straw, bamboo fiber, yams, girasol tubers, potato tubers, acorns, and chicory roots [86, 93, 100, 103, 106-110]. The production of HMF from biomass in combination with another pre-treatment has only been examined in a few studies. Binder and Raines (2009), for example, found the transformation of AFEX treated corn stover did not produce more HMF compared to untreated corn stover under the same conditions [86]. The transformations of diluted HCl treated girasol tubers, potato tubers, acorns, and chicory roots were also studied with yields of HMF between 50-59% [108-110]. Utilizing feedstocks, such as grasses and wood, containing complex lignocellulosic materials remains mostly unexplored in the

literature. Two studies have found that wood can be used to produce HMF, with pine wood yielding 52% of HMF, and poplar wood yielding 26% of HMF [103, 104]. In another study, yields of HMF of 24% and 7% were also obtained from poplar wood [100]. Lignin content in both types of wood is similar, with pine wood containing 26.7±0.7% of lignin and poplar wood containing 25.7±1.1% of lignin [112]. Therefore, the lower yield of HMF produced from poplar is not likely due to the lignin content, but some other differences found in the composition of the two woods.

To our knowledge, only two studies reported HMF yields from switchgrass, neither of which used metal chloride catalysis in the presence of an ionic liquid [104, 105]. In one study, HMF was produced from switchgrass in a H_2O/THF mixture with $AlCl_3 \cdot 6H_2O$ as the catalyst which produced a yield of 21% of HMF [104]. In a mechanistic study, HMF was also quantified after switchgrass was placed in a solution of 1% H_2SO_4 and a yield of 4.5% of HMF was obtained after 2 min [105]. The HMF produced during a diluted acid treatment will rapidly degrade to other by-products within a few minutes, and this method may therefore not be suitable for production of HMF on the industrial scale [105]. As previously mentioned, no studies are known to have been conducted for the conversion of comfrey to HMF, and observation which makes switchgrass and comfrey two interesting feedstocks since both plants have already been identified for potential biofuel production based on their high sugar contents [30]. However, neither plant has been studied for production of HMF using ionic liquids and metal chloride catalysis. Furthermore, both plants address the urgent need to move to non-edible plants to provide added benefits for the environment compared to conventional biomass sources like edible corn stover (for animals), which requires the use of agricultural plants.

1.6. *Symphytum officinale* L. and *Panicum virgatum* L.

For this research, not only plants high in sugar are necessary, but plants which can be grown locally in Sudbury are also required. Most of the land surrounding Sudbury contains varying level of contaminants coming from smelters, including SO_2 emissions, as well as metals (such as arsenic, cadmium, copper, nickel, etc.), which may impact plant cultivation [113, 114]. Furthermore, reports have shown that factors related to soil pollution from the mining activity, such as soil erosion, low nutrient levels, lack of soil organic matter, and low soil pH can also have an impact on plant growth and cultivation [113, 114]. We selected two plants for the production of HMF which can be grown in Sudbury's soil and climate, *Symphytum officinale* L. (common comfrey, family Boraginaceae), and *Panicum virgatum* L. (switchgrass, family Gramineae). Plants are displayed in Figure 12.

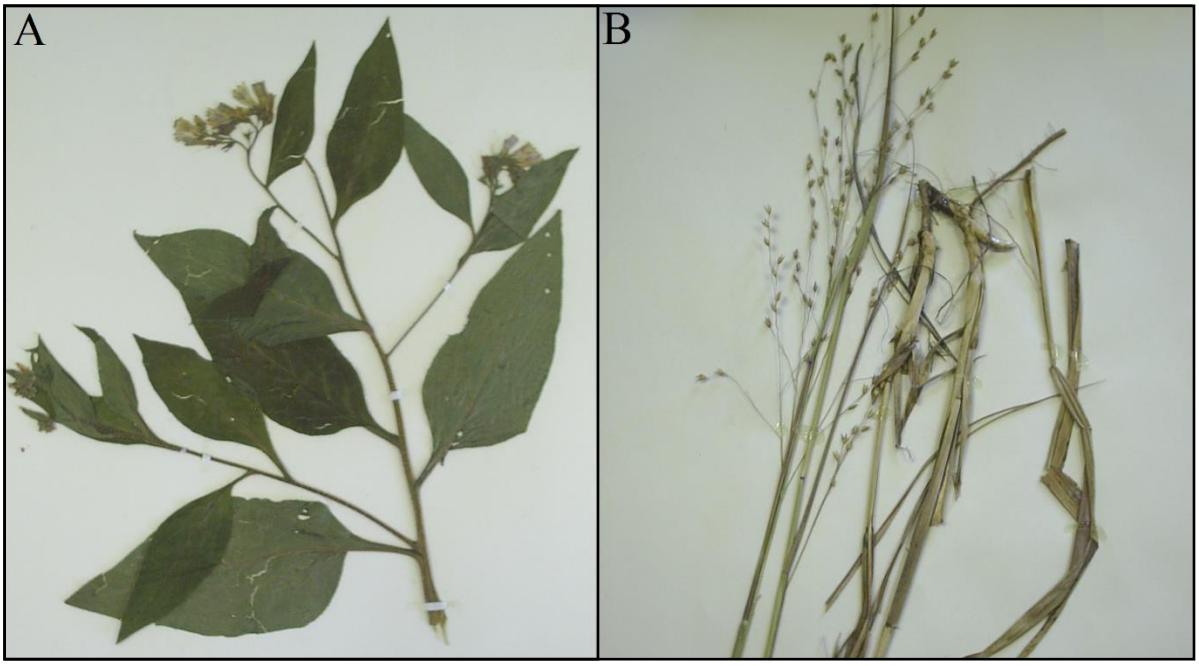
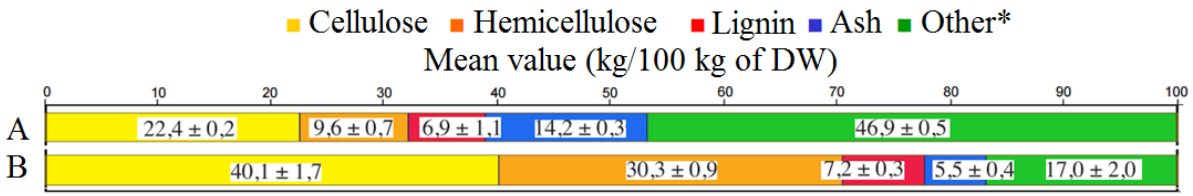


Figure 12. Comfrey (panel A) and Switchgrass (panel B).
Photo credit: Northern Ontario Herbarium Database

Although it is not the aim of this study, both plants were also chosen on the basis that they can accumulate metals and could potentially be used for the phytoremediation of the mine landfills [115-119]. The wasted plant material after collection could then be transformed into HMF, and subsequently DMF. Both plants were chosen on the basis that they produce a very large amount of biomass (up to 20 tonnes/hectare/year for switchgrass), and also have high contents of sugars [30, 120]. Therefore, not only would the plants be used for remediation purposes, but transforming that biomass into a green source of energy would reduce the overall carbon footprint of the mining industry by bringing biofuel production locally and decreasing the amount of fossil fuel needed. Comfrey is also a known medicinal plant, which offers the potential of using the biomass as a source for medicinal compounds [121, 122].

The most important aspect for biofuel production from plants is the amount of sugars found in those plants in the form of cellulose, hemicellulose, and simple sugars. The composition of *S. officinale* and *P. virgatum* are shown in Figure 13 [30].



*Other includes soluble polysaccharides (such as starch and fructans), soluble sugars, organic acids, proteins, and lipids.

Figure 13. Plant composition of comfrey (A) and switchgrass (B). DW, dry weight. Source: Adapted from [30]

Objectives

Producing green energy is becoming one of the growing areas of interest for environmental chemists. The first goal of this thesis is to develop and validate a method for sugar extraction for two plants, *Symphytum officinale* L. (common comfrey) and *Panicum virgatum* L. (switchgrass) using methods such as acid hydrolysis, alkaline hydrolysis, and solvent extraction.

The second goal of this thesis is to use chemical transformation of the untreated and pre-treated plant biomass to produce HMF, a platform chemical which can then be used to produce the biofuel DMF. The use of ionic liquids, with or without co-solvents, will be used to dissolve the biomass. Different metal halide catalysts will be studied for the production of HMF. A quantification method will be developed using gas chromatography coupled with a mass spectrometer detector (GC-MS).

2. Methodology

2.1. List of Chemicals

The following chemicals were purchased from Sigma-Aldrich (St-Louis, Missouri): 1-butyl-3-methylimidazolium chloride (≥95%), 1-ethyl-3-methylimidazolium chloride (≥95%), 2-methylpyridine *N*-oxide (≥96%), 3-picoline *N*-oxide (≥98%), 3,5-dinitrosalicylic acid, activated charcoal (untreated powder, 100-400 mesh), $Ca(OH)_2$ (≥96%), $CuCl_2$ (≥97%), $CrCl_3 \bullet 6H_2O$ (≥98%), H_2SO_4 (95-98%), *N*,*N*-dimethylacetamide (98.8%), NaOH (≥98%), sodium potassium tartrate (≥99%), and trifluoroacetic acid (≥99.9%). The following chemicals were purchased from Fisher Scientific (Unionville, Ontario): 5-hydroxymethylfurfural (≥98%), acetone (≥99.5%) DCM (≥99.9%), diethyl ether (≥99.9%), EtOH (≥95%), EtOAc (99.9%), glacial acetic acid (≥99%), glucose (anhydrous), MeOH (≥99.9%), and phenol (≥99%). $AlCl_3$ (anhydrous, ≥98%), DMSO (99%), and LiCl (≥99%) were purchased from BDH Chemicals (Toronto, Ontario). $CrCl_2$ (anhydrous, 99.9%) was purchased from Strem Chemicals (Newburyport, Massachussetts). HCl (36-38%) was purchased from Stanchem (East Berlin, Pennsylvania).

2.2. List of Materials

For the spectrophotometric analysis, 1.5 mL plastic cuvettes, and an Ultrospec 3100 pro UV-Vis spectrophotometer from Fisher Scientific (Unionville, Ontario) were used.

For drying of the samples, a Heidolph Collegiate rotary evaporator from Fisher Scientific was used.

A pH meter (model AB15) from Fisher Scientific was used to monitor the pH during neutralisation.

Thin layer chromatography (TLC) was performed using aluminum backed silica TLC plates (20×20 cm, 200 µm thickness) from Silicycle Ultra Pure Silica Gels (Quebec, Quebec) and a mineralight UV lamp (model UVS-54) (San Gabriel, California) at 254 nm was used to detect the products.

For the GC-MS analysis, a Finnigan TRACE GC Ultra connected to a Finnigan Polaris Q MS detector using electron ionization (EI) and a TriPlus AS auto-injector were used (Thermo Scientific). The column used was a TR-5MS SQC column (L 30 m, ID 0.25 mm, film 0.25 µm) (Thermo Scientific). The following materials used in chromatographic analysis were purchased from Canadian Life Sciences (Peterborough, Ontario): 0.45 µm syringe filters (13 mm nylon), 1 mL syringes, 2 mL clear Robo vials (12×32 mm, 9 mm thread), 9 mm blue screw caps, and 350 µL glass flat bottom insert (6×30 mm). He gas for the GC-MS analysis were obtained at a purity of 5.0 from Praxair Canada (Sudbury, Ontario).

2.3. Plant Material

Symphytum officinale L. (common comfrey) was obtained from Ritchers Herbs (Goodwood, ON). The plants were ordered in spring 2014, and upon arrival, the plants were kept in small pots for approximately 2 weeks, and were watered bi-weekly, or when the soil was dry. After approximately 2 weeks of growth, the plants were transferred in bigger pots in Home Gardener top soil. The plants were kept in a partially shaded area outside, until maturity was reached (plant height reaching approximately 60 cm, after 3 to 4 months of growth).

Panicum virgatum L. (switchgrass) seeds of the "Sunburst" cultivar were obtained from Ernst Seeds (Meadville, Pennsylvania). The switchgrass was cultivated as part of a study by Smith (2012) to determine the feasibility of growing the plant on low sulphur mine tailing

with an approximate one meter compost cover (manufactured by GroBark) [123]. The switchgrass seeds were planted in Xstrata Nickel's Strathcona tailings facility in Onaping, Ontario, in the summer of 2009 and 2010. A municipal compost cover (provided by GroBark) over a fine ground woody construction material was applied over the tailing as growth media for the plants, and fertilizers and urea were added to the soil. Plants were grown in full sun, and seasonal precipitation was sufficient to cultivate the plants without further irrigation being necessary. Switchgrass was collected in July 2015 for use in our studies.

After harvest, the leaves and stems of the green plants were immediately frozen. Before use, the plants were air dried at room temperature, avoiding direct sunlight for approximately 72 h, or until weight was constant. Plant material was ground into to a fine powder using a Magic Bullet® blender, and the biomass was stored at room temperature in dry and airtight containers, away from direct sunlight.

2.4. Plant Pre-treatment for Sugar Extraction

2.4.1. Methanol Extraction

The soluble sugars in the plants were quantified by extracting plant biomass with MeOH. Up to 5% (wt/v) of comfrey or switchgrass were incubated in MeOH at 40°C for 24 h under a condenser. Solutions were filtered by gravity filtration, and recovered biomass was thoroughly washed with MeOH and filtered under vacuum. Recovered biomass was dried in the oven at 50°C until weight no longer fluctuated (approximately 72 h). Masses of the biomass and volumes of solution were recorded before and after the extraction. The percentage of dissolved biomass in the treatment was calculated using equation 1 where $M_{recovered}$ is the mass of the plant after extraction (g) and M_{DW} is the initial plant dry weight (DW) before extraction (g):

$$\% \text{ dissolved biomass} = \frac{M_{DW} - M_{recovered}}{M_{DW}} \times 100 \quad [1]$$

The amounts of total and reducing sugars found in the recovered MeOH fraction were quantified (section 2.5). Total sugar analysis of the MeOH comfrey extract was repeated 4 times. Reducing sugar analysis of the MeOH comfrey extract, as well as the total sugar and reducing sugar analysis of the MeOH switchgrass extract were performed in triplicates. The extract for quantification could be prepared using two methods. In method 1, a fraction of the MeOH solution was evaporated using a rotary evaporator, and the solid was weighed, and re-dissolved in a known, minimal, volume of MeOH. In method 2, the MeOH was not evaporated and the MeOH solution was directly used for sugar determination. In method 2, the weight of the plant material in the extract was measured by subtracting the recovered dried biomass ($M_{recovered}$) after extraction from the initial biomass weight (M_{DW}), and the known volume of MeOH used for the extraction was used in the calculations (section 2.5.3). In order to confirm whether or not pigments interfered with sugar quantification, the total and reducing sugar assays were performed on both a MeOH extract with pigment, and on a MeOH extract filtered through activated charcoal to remove the pigments.

2.4.2. Acid Pre-treatment

A concentration of 5% (wt/v) of comfrey was placed in either a 0.5 M or a 0.1 M H_2SO_4 solution (n=1 for 0.1 M treatment and n=4 for the 0.5 M treatment). The weights of the plant material and the volumes of the H_2SO_4 solutions were recorded. The samples were then either autoclaved immediately, or were incubated for 24 h at room temperature before being autoclaved (n=2 for incubated samples and n=4 for samples without incubation). Autoclave was conducted for 30 min at 121°C and 17-20 psi, in a covered Erlenmeyer flask. The flasks were subsequently cooled to room temperature. The samples were vacuum filtered

using a fritted Buchner funnel, with biomass being recovered and dried in an oven at 50°C to constant mass. The weight of the plants was recorded. The experiments using the optimal conditions of 0.5 M H_2SO_4, no incubation, autoclaved for 30 min were repeated in triplicates using switchgrass biomass. Percentage of dissolved biomass in the acid for each sample was calculated using equation 1.

All filtered solutions following hydrolysis were neutralized to a pH of 7 using either $Ca(OH)_2$ or a 2 M solution of NaOH. In the case of $Ca(OH)_2$, the solution was filtered using a Buchner funnel, and the $CaSO_4$ precipitate was discarded. Solutions were kept refrigerated and the total and reducing sugars were quantified within 24 h of preparing the solutions (section 2.5).

In order to assess the efficiency of the 0.5 M H_2SO_4 hydrolysis, recovered dried comfrey material obtained from the 0.5 M H_2SO_4 treatment with no incubation prior to a 30 min autoclave (optimal conditions) was further hydrolyzed using the same conditions. Biomass was again recovered and dried, and the recovered material was hydrolyzed a third time using the optimal conditions. Solutions were filtered and neutralized as mentioned above, and total sugars were quantified (section 2.5). This experiment was performed in duplicate.

2.4.3. Base Pre-treatment

Base hydrolysis of comfrey was done in duplicate. Comfrey was incubated in a solution of 2 M NaOH at a concentration of 10% (wt/v). Solutions of comfrey were heated at 50°C for 24 h, and subsequently vacuum filtered using a fritted Buchner funnel. Recovered biomass was dried in the oven at 50°C to constant mass, and the recorded weights were used to calculate the percentage of hydrolysed biomass in the NaOH solution (equation 1). The

recovered liquid was neutralized to a pH of 7 with 2 M HCl, and the total sugars in the solution were quantified (section 2.5).

2.4.4. Combination of Pre-treatments

A combination of pre-treatments was used to study the amounts of sugars extracted from comfrey using MeOH, acid, and base treatment, consecutively. The MeOH treatment was used first in order to extract the soluble portion of the biomass. The preparation of the samples is outlined in section 2.4.1. Five percent (wt/v) of comfrey was incubated in MeOH at 40°C for 24 h, and placed under a condenser to prevent evaporation of the solutions. The recovered plant material was dried in the oven at 50°C to constant mass, and the dried recovered plant was further hydrolysed using the optimal conditions for acid treatment (0.5 M H_2SO_4, no incubation, 30 min autoclave). The recovered biomass from the acid treatment was again dried at 50°C to constant mass, and the dried plant material underwent a base treatment of 2 M NaOH at 50°C, for 24 h. The recovered material was dried at 50°C in the oven. Liquid samples were filtered and neutralized to pH 7 with $Ca(OH)_2$ or a 2 M solution of HCl. All solutions were assayed for total sugars (section 2.5.). All masses recorded were used to calculate the percentage of hydrolysed biomass after each treatment using equation 1.

2.5. Sugar Quantification

2.5.1. Total Sugar Assay

The phenol/H_2SO_4 spectrophotometric assay, outlined by DuBois *et al*. (1956), was used to quantify the amount of total sugars in the solutions [124]. The phenol/H_2SO_4 method works by converting carbohydrates into furfural and furfural derivatives (such as HMF) in the presence of acid and heat [125]. Those complexes are then polymerized and/or condensed

with phenol to produce complexes which absorb light at 490 nm, and the absorbance offers a direct correlation to the amount of total sugars found in the media [125]. Figure 14 shows the mechanism by which the total sugar assay works.

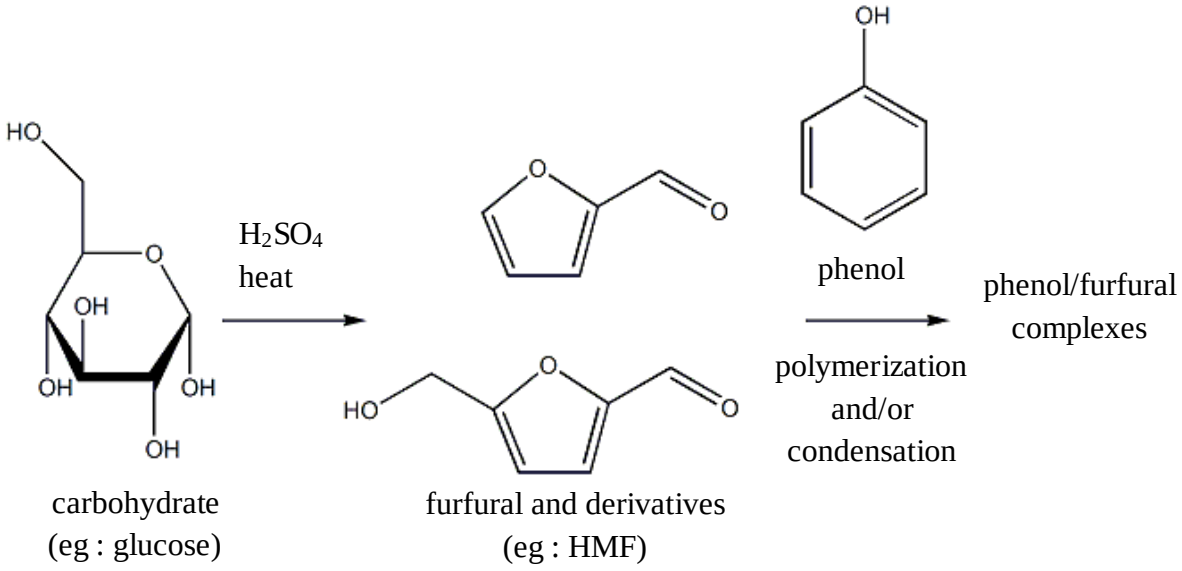


Figure 14. Mechanism of the phenol/H_2SO_4 assay for total sugars.
Source: Adapted from [125]

Volumes ranging from 5 µL to 200 µL of plant samples were diluted to 2000 µL in dH_2O in test tubes. The standard curve was built using glucose, and was repeated 5 times. Standards were made using a 0.1 g/L solution of glucose, with volumes ranging from 200 µL to 2000 µL (in increments of 200 µL) diluted to 2000 µL in dH_2O, to give concentrations ranging from 10 mg/L to 100 mg/L. The blank contained 2000 µL of dH_2O (or a mixture of MeOH and dH_2O when MeOH extracts were tested). To each sample 1 mL of 5% (wt/v) phenol solution was added, followed by 5 mL of concentrated H_2SO_4. Samples were incubated at room temperature for 10 min, and then incubated at 30°C for 20 min. Test tubes were cooled to room temperature in an ice bath and absorbance was measured at 490 nm using a UV/Vis spectrophotometer.

2.5.2. Reducing Sugar Assay

The 3,5-dinitrosalicylic acid (3,5-DNS) spectrophotometric assay was used to quantify the reducing sugars using the protocol explained by Wood *et al*. (2012) [126]. In this assay, the 3,5-DNS is reduced to 3-amino-nitrosalicylic acid in the presence of reducing sugars, and absorbs the light at a wavelength of 540 nm (Figure 15) [126]. Non-reducing sugars will not react with the 3,5-DNS due to the lack of hydroxyl group at the anomeric carbon.

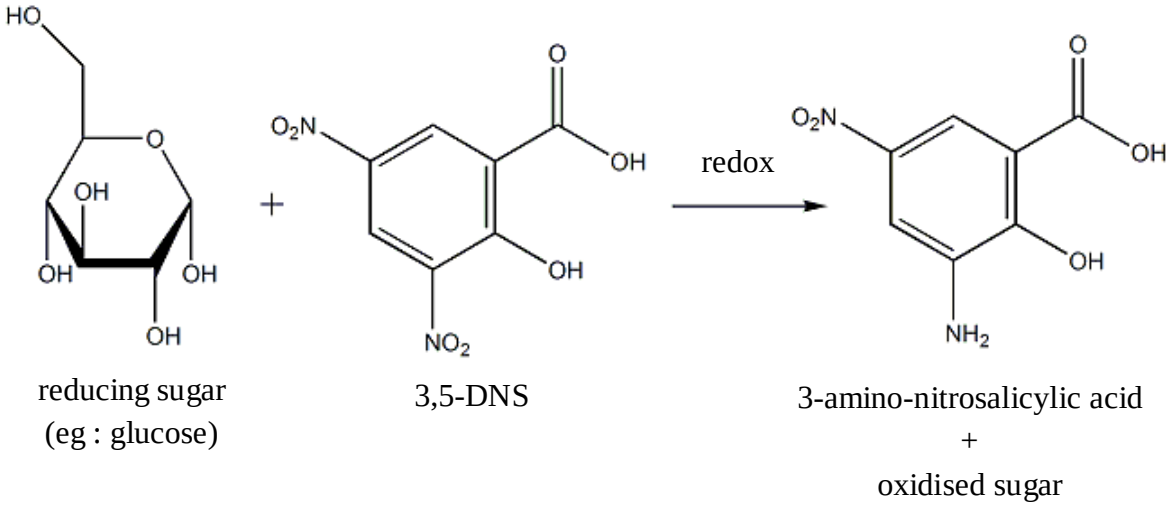


Figure 15. Mechanism of the 3,5-DNS assay for reducing sugar.
Source: Adapted from [126]

The 3,5-DNS reagent was prepared by dissolving 8 g of NaOH in 200 mL of dH_2O. The solution was heated to 70°C and 150 g of sodium-potassium tartrate was added while mixing with a magnetic stirrer. The volume was brought to 400 mL of dH_2O, heated to 70°C, and 5 g of 3,5-DNS was mixed into the solution. The solution was cooled to room temperature and volume was brought to 500 mL with dH_2O. The solution was autoclaved and kept in a capped container at 4°C.

Measurement of reducing sugars was done by diluting 10 µL to 150 µL of plant extract to 500 µL (in dH_2O). The standard curve was performed in triplicates using glucose standards

which were prepared using a 2 g/L solution, with volumes ranging from 50 µL to 500 µL (in increments of 50 µL) diluted to 500 µL in dH_2O, to give glucose concentrations ranging from 0.2 g/L to 2 g/L. The blank contained 500 µL of dH_2O (or a mixture of MeOH and dH_2O when MeOH extracts were tested). To each sample 0.5 mL of 3,5-DNS reagent was added and test tubes were lightly capped with aluminum foil to prevent evaporation. Test tubes were incubated at 100°C for 5 min, and were subsequently cooled to room temperature. Samples were diluted by adding 5 mL of dH_2O. Absorbance was read at 540 nm using a UV-Vis spectrophotometer.

2.5.3. Calculations for Sugar Quantification

A series of parameters were calculated for both the total, and the reducing sugars assay (Table 3). The concentration of the extracts in mg of sugars/L of the extracts ($C_{solution}$) was calculated using the equation obtained from the standard calibration curve of glucose and using the dilution factor (DF) of the samples. The amount of sugars extracted in mg ($M_{extract}$) was then calculated by multiplying $C_{solution}$ (mg of sugars/L) by the total volume of the extract in L (V). The concentrations were then reported in mg of sugars/g of extracted biomass ($C_{extract}$) for the MeOH extracts, or in mg of sugars/g of hydrolysed biomass (C_{hydro}) for the H_2SO_4 and the NaOH hydrolysis, by dividing $M_{extract}$ with M_{DW}-$M_{recovered}$ (initial dry weight of the plant-recovered weight). Sugar concentrations were also reported in mg of sugars/g of dry weight (DW) (C_{DW}), where $M_{extract}$ was divided by M_{DW} (initial weight of dry plant used).

Table 3. Calculation for the amount of sugars obtained after a pre-treatment of the biomass. Abs, absorbance; C, concentration; DF, dilution factor; M, mass; V, volume. Abs=m(C)+b is the linear equation calculated from the standard calibration curve of glucose, and is used to find $C_{cuvette}$.

Parameter	$C_{cuvette}$ (mg of sugars/L)	$C_{solution}$ (mg of sugars/L)	$M_{extract}$ (mg of sugars)	$C_{extract}$ or C_{hydro} (mg of sugars/g of extracted or hydrolysed biomass)	C_{DW} (mg of sugars/g of DW)
Calculation	Abs=m(C)+b	$C_{cuvette}\times DF$	$C_{solution}\times V$	$\frac{M_{extract}}{(M_{DW}-M_{recovered})}$	$\frac{M_{extract}}{M_{DW}}$

2.6. HMF Production from Glucose, Comfrey, and Switchgrass

2.6.1. HMF Production from Glucose

As a control, transformation of HMF from glucose was performed prior to using plants as the substrate (Table 4). A 10 wt% substrate loading of glucose was put in either [BMIM]Cl (entries 1, 3, and 5) or [EMIM]Cl (entries 2, 4, and 6). There was no additional dissolution step in the ionic liquids because glucose readily dissolves in the solvents. The catalytic step was performed at 140°C for 30 min, with a catalyst loading of 3 mol% of $CuCl_2$ and 3 mol% of $CrCl_3\bullet 6H_2O$ (entries 1 and 2), or 3 wt% of $CuCl_2$ and 3 wt% of $CrCl_3\bullet 6H_2O$ (entries 3 and 4) based on the amount of glucose used. The experiment was also repeated switching $CrCl_3\bullet 6H_2O$ for 3 mol% of $CrCl_2$ (entries 5 and 6).

Table 4. Summary of the reactions using glucose as a substrate (10 wt% substrate loading).

Entry	**Solvent**	**Catalyst 1**	**Catalyst 2**	**Catalytic step**
1	[BMIM]Cl	$CrCl_3\bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	30 min, 140°C
2	[EMIM]Cl	$CrCl_3\bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	30 min, 140°C
3	[BMIM]Cl	$CrCl_3\bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 140°C
4	[EMIM]Cl	$CrCl_3\bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 140°C
5	[BMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	30 min, 140°C
6	[EMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	30 min, 140°C

2.6.2. Preparation of Untreated and Treated Comfrey and Switchgrass for HMF Production

Both untreated and treated comfrey and switchgrass were used as a feedstock for conversion to HMF. The ground dry plant material is considered to be the untreated material, even though the material underwent mechanical breakage prior to the reaction. The treated biomass for both plants includes the MeOH extracts, and the 0.5 M H_2SO_4 hydrolysis that was autoclaved for 30 min, with no incubation prior to the autoclave. Before being used for production of HMF, both types of extracts were dried using a rotary evaporator. The solid obtained from drying those extracts contained the sugars which could be transformed to HMF. The base extract was not used to produce HMF due to the low amount of sugars found in the extract.

2.6.3. HMF Production from Untreated Comfrey and Switchgrass

Two steps were involved in each reaction for HMF production, the dissolution step and the catalytic step. The dissolution step involved dissolving the biomass in one, or more solvents (Table 5). Four ionic liquids were used to dissolve the biomass, [BMIM]Cl (entries 1 to 4, 10 and 11), [EMIM]Cl (entries 5 and 12), 2-methylpyridine *N*-oxide (entry 6), and 3-picoline *N*-oxide (entry 7). Two more solvents were also studied for the dissolution of the biomass, DMSO, and DMA-LiCl (10% LiCl in DMA) which were added to [BMIM]Cl as a co-solvent (entries 8, 9, 13, and 14).

Table 5. List of solvents studied for comfrey and switchgrass dissolution using different reaction times and substrate loading.

Entry	Substrate	Solvent	Dissolution step	Catalytic Step
1	Comfrey (2.5 wt%)	[BMIM]Cl	30 min, 120°C	2 h, 140°C
2	Comfrey (5 wt%)	[BMIM]Cl	30 min, 120°C	15 min, 140°C
3	Comfrey (10 wt%)	[BMIM]Cl	30 min, 120°C	30 min, 140°C
4	Comfrey (10 wt%)	[BMIM]Cl	24 h, 80°C	30 min, 140°C
5	Comfrey (10 wt%)	[EMIM]Cl	30 min, 120°C	30 min, 140°C
6	Comfrey (5 wt%)	2-methylpyridine *N*-oxide	30 min, 100°C	15 min, 120°C
7	Comfrey (5 wt%)	3-picoline *N*-oxide	30 min, 100°C	15 min, 120°C
8	Comfrey (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	24 h, 75°C	2 h, 140°C
9	Comfrey (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	-	9 h, 150°C
10	Switchgrass (10 wt%)	[BMIM]Cl	30 min, 120°C	30 min, 140°C
11	Switchgrass (10 wt%)	[BMIM]Cl	24 h, 80°C	30 min, 140°C
12	Switchgrass (10 wt%)	[EMIM]Cl	30 min, 120°C	30 min, 140°C
13	Switchgrass (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	24 h, 75°C	2 h, 140°C
14	Switchgrass (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	-	9 h, 150°C

Substrate loading for untreated comfrey in [BMIM]Cl was tested at 2.5, 5 and 10 wt% of the reaction mixture (entries 1 to 3). In [EMIM]Cl, comfrey was loaded at 10 wt% of the reaction mixture (entry 4), while in 2-methylpyridine *N*-oxide (entry 6) and 3-picoline *N*-oxide (entry 7), substrate loading was 5 wt%. Switchgrass was always loaded at 10 wt% of the reaction mixture when using [BMIM]Cl and [EMIM]Cl as solvents (entries 10 to 12). Dissolution time was normally maintained at 120°C for 30 min when using [BMIM]Cl or [EMIM]Cl, however dissolution time of 24 h in [BMIM]Cl were also studied using a 10 wt% substrate loading for comfrey (entry 4) and switchgrass (entry 8). When using 2-methylpyridine *N*-oxide (entry 6) and 3-picoline *N*-oxide (entry 7) as the solvents, temperature was brought down to 100°C due to the lower melting point of those ionic liquids. When using co-solvents, a certain quantity of a specific solvent was added to [BMIM]Cl. DMA-LiCl was tested as a co-solvent and was added to [BMIM]Cl. The [BMIM]Cl made up 60 wt% of the reaction mixture (entries 8 and 13). The rest of the reaction mixture was composed of the

DMA-LiCl and the 10 wt% substrate loading (entries 8 and 13). The dissolution step was performed at 75°C for 24 h following the procedure described by Binder and Raines (2009) (entries 8 and 13) [86]. When using DMSO as a co-solvent with [BMIM]Cl, [BMIM]Cl was made up 10 wt% of the reaction mixture, and a 2 wt% catalyst loading was used (entries 9 and 14). The dissolution step was omitted in favour of a longer reaction time, which followed the procedure outlined by Xiao *et al*. (2014) (entries 9 and 14) [94].

Following the dissolution step, a catalytic step was used to produce HMF. The catalytic step involved adding one, or more, metal halide catalyst(s) to the reaction mixture containing the solvent, and the dissolved biomass (Table 6). Three different metal chloride were used as catalyst for conversion of untreated biomass to HMF: $CuCl_2$, $CrCl_3 \cdot 6H_2O$, and $AlCl_3$. Catalyst loading for $CuCl_2$ and $CrCl_3 \cdot 6H_2O$ in [BMIM]Cl was either 3 wt% or 3 mol% based on the amount of substrate loading (entries 1 to 13). Acids were also tested as co-catalysts in the reactions with comfrey and switchgrass as the substrate, including H_2SO_4, HCl, TFA, and CH_3COOH in concentrations of 10 or 20 mol% based on the substrate loading (entries 6 to 13). For the untreated biomass, the concentration of sugars (mg of sugars/g of DW, C_{DW}, Table 3) hydrolysed using the 0.5 M H_2SO_4 treatment was used as an estimate to find the mol% for catalyst loading as it represented our highest estimate of extractable sugars found in comfrey and switchgrass.

Table 6. Summary of the reactions for the conversion of untreated comfrey and switchgrass to HMF.

Entry	Substrate	Solvent	Catalyst 1	Catalyst 2	Catalyst 3	Dissolution step (min, °C)	Catalytic step (min, °C)
1	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	10, 140
2	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	20, 140
3	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	30 , 140
4	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	180, 140
5	Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	30, 140
6	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (10 mol%)	30, 120	30, 140
7	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (20 mol%)	30, 120	30, 140
8	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	CH_3COOH (10 mol%)	30, 120	30, 140
9	Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	TFA (20 mol%)	30, 120	30, 140
10	Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (10 mol%)	30, 120	30, 140
11	Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (20 mol%)	30, 120	30, 140
12	Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	CH_3COOH (10 mol%)	30, 120	30, 140
13	Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	TFA (20 mol%)	30, 120	30, 140
14	Comfrey (5 wt%)	2-methylpyridine *N*-oxide	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	15, 120
15	Comfrey (5 wt%)	3-picoline *N*-oxide	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	15, 120
16	Comfrey (10 wt%)	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	30, 140
17	Switchgrass (10 wt%)	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	30, 140

18	Comfrey (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	$CrCl_3 \bullet 6H_2O$ (10 mol%)	HCl (10 mol%)	-	24[a], 75	120, 140
19	Switchgrass (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	$CrCl_3 \bullet 6H_2O$ (10 mol%)	HCl (10 mol%)	-	24[a], 75	120, 140
20	Comfrey (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	$AlCl_3$ (10 mol%)	-	-	-	540, 150
21	Switchgrass (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	$AlCl_3$ (10 mol%)	-	-	-	540, 150

[a] Time is reported in hours

Using the untreated comfrey, a series of reaction times for the production of HMF were tested at 140°C with a catalyst loading of 3 wt% of $CuCl_2$ and $CrCl_3 \cdot 6H_2O$ with [BMIM]Cl as the solvent (entries 1 to 4). The times were either 10 min (entry 1), 20 min (entry 2), 30 min (entry 3), or 180 min (entry 4). Using untreated switchgrass, only the reaction time of 30 min was tested (entry 5). Using the reaction time of 30 min at 140°C with a catalyst loading of 3 mol% of $CuCl_2$ and 3 mol% $CrCl_3 \cdot 6H_2O$ in [BMIM]Cl, the influence of the addition of acids was tested on both comfrey and switchgrass by adding 10 mol% of H_2SO_4 (entries 6 and 10), 20 mol% of H_2SO_4 (entries 7 and 11), 10 mol% of CH_3COOH (entries 8 and 12), or 20 mol% of TFA (entries 9 and 13).

When using 2-methylpyridine *N*-oxide (entry 14) and 3-picoline *N*-oxide (entry 15) as the solvents, and untreated comfrey as the substrate, temperature of the catalytic step was brought down to 120°C due to the lower melting point of those ionic liquids, and times of 15 min were used with a 3 wt% catalyst loading of $CuCl_2$ and $CrCl_3 \cdot 6H_2O$. When using [EMIM]Cl as the solvent, for both comfrey and switchgrass, the catalytic step was performed at 140°C for 30 min with a 3 wt% catalyst loading of $CuCl_2$ and $CrCl_3 \cdot 6H_2O$ (entries 16 and 17). When using DMA-LiCl as the co-solvent with [BMIM]Cl (60 wt% of the reaction mixture), the catalytic step was performed at 140°C for 120 min with 10 mol% catalyst loading of $CrCl_3 \cdot 6H_2O$ and 10 mol% catalyst loading of HCl (37%), which followed the procedure described by Binder and Raines (2009) (entries 18 and 19) [86]. When using DMSO as a co-solvent with [BMIM]Cl (10 wt% of the reaction mixture), the catalytic step had a duration of 540 min at 150°C with 10 mol% $AlCl_3$ catalyst loading, which followed the procedures of Xiao *et al*. (2014) (entries 20 and 21) [94].

2.6.4. HMF Production from Treated Comfrey and Switchgrass

For the treated comfrey and switchgrass, including the MeOH and the 0.5 M H_2SO_4 dried extract, substrate loading was maintained at 10 wt% of the reaction mixture and only the 30 min dissolution at 120°C in [BMIM]Cl or [EMIM]Cl was studied (Tables 7 and 8).

For the MeOH extracts (Table 7), the catalytic step was performed at 140°C for 15 min (entries 1, 2, and 5) or 30 min (entries 3, 4, and 6), in [BMIM]Cl (entries 1, 2, 3, and 5) or [EMIM]Cl (entries 4 and 6), and each reaction had a catalyst loading of 3 mol% or 3 wt% of $CuCl_2$ and $CrCl_3 \bullet 6H_2O$. The mol% was calculated for the MeOH extract for both plants using the amount of total sugars calculated in each extract (mg of sugars/g of extract, $C_{extract}$, Table 3). Using the highest HMF yielding reactions (15 min catalytic step at 140°C in [BMIM]Cl for comfrey, entry 1, and 30 min catalytic step at 140°C in [EMIM]Cl for switchgrass, entry 4), the reactions were also repeated switching the 3 wt% $CrCl_3 \bullet 6H_2O$ catalyst loading for 3 wt% of $CrCl_2$ loading (entries 5 and 6).

For the dry 0.5 M H_2SO_4 extracts of comfrey and switchgrass (Table 8), the dissolution step was done either in [BMIM]Cl (entry 1) or [EMIM]Cl (entry 2) for 30 min at 120°C, while the catalytic step had a duration of 30 min at 140°C with 3 wt% of $CuCl_2$ and 3 wt% of $CrCl_3 \bullet 6H_2O$.

Table 7. Summary of the reactions for the conversion of the dry MeOH extracts for comfrey and switchgrass with a substrate loading of 10 wt%.

Entry	Substrate	Solvent	Catalyst 1	Catalyst 2	Dissolution step	Catalytic step
1	Comfrey or Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 120°C	15 min, 140°C
2	Comfrey or Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	30 min, 120°C	15 min, 140°C
3	Comfrey or Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 120°C	30 min, 140°C
4	Comfrey or Switchgrass	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 120°C	30 min, 140°C
5	Comfrey	[BMIM]Cl	$CrCl_2$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 120°C	15 min, 140°C
6	Switchgrass	[EMIM]Cl	$CrCl_2$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 120°C	30 min, 140°C

Table 8. Summary of the reactions for the conversion of the dry 0.5 M H_2SO_4 extracts for comfrey and switchgrass with a substrate loading of 10 wt%.

Entry	Substrate	Solvent	Catalyst 1	Catalyst 2	Dissolution step	Catalytic step
1	Comfrey or Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 120°C	30 min, 140°C
2	Comfrey or Switchgrass	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30 min, 120°C	30 min, 140°C

2.6.5. Work-up of the Reaction Mixture and Sample Preparation

After completion of the reaction, each solution was diluted with dH_2O to reduce viscosity, and hot solutions were filtered in a fritted Buchner funnel by air filtration to prevent clogging of the pores. Undissolved biomass was then thoroughly washed with dH_2O and the solution was filtered under vacuum to remove any remnant of the ionic liquid. The biomass was subsequently dried at 100°C for 24 h to constant weight. The recovered dry biomass was weighed and the percentage of dissolution was calculated from the initial weight using equation 1.

Liquid/liquid extraction was used to extract HMF from the reaction mixtures. For all reaction mixtures containing only ionic liquids as the solvent, and for the reaction mixtures containing DMA-LiCl, fractions of 10 mL of HPLC grade EtOAc were used to extract the mixture. For the reaction mixture containing DMSO, the DMSO was first diluted with icy cold dH_2O, and HPLC grade diethyl ether was used for extraction. The extracts obtained were washed thoroughly with cold dH_2O.

All extracts were analysed by TLC. The solutions were applied against an HMF standard (14.2 mM HMF in EtOAC) on an aluminum backed silica TLC plate. Compounds were migrated with a 10% MeOH/DCM mixture and presence of the compound was detected by UV light at a wavelength of 254 nm. The reaction mixtures were extracted until the EtOAc phase no longer showed HMF on the TLC.

In the case of the MeOH extracts samples, chlorophylls were present in the extracts and therefore the samples were filtered over activated charcoal, and washed with EtOAc (3×10 mL), to remove the pigments. Untreated biomass and the acid treated biomass tended to yield a slightly yellow/brown sample which did not require filtration on activated charcoal prior to

injection in the GC-MS for quantification of HMF. All EtOAc extracts were subsequently concentrated by drying the extract using a rotary evaporator, and re-dissolving in minimal EtOAc for GC-MS injection (500 µL for untreated biomass samples and H_2SO_4 treated samples, and 2000 µL for MeOH treated biomass samples). The EtOAc extracts of the glucose standards did not require concentration. All samples were filtered in a 0.45 µm filter, and were subsequently ready for injection in the GC-MS.

2.6.6. Quantification of HMF by GC-MS

To quantify HMF in reactions, the EtOAc extracts were injected in the GC-MS onto a TR-5MS SQC column (L 30 m, ID 0.25 mm, film 0.25 µm). Samples were injected using the TriPlus As auto injector at a volume of 1.0 µL. Scans were started at 3 min to prevent overload of the solvent on the detector. The ion source was set at 200°C. Damping gas flow was set at 0.3 mL/min. Carrier gas used was He at a constant flow rate of 1.5 mL/min. The initial oven temperature was set at 50°C and held for 0.50 min, followed by an increased temperature slope of 15.0°C/min up to a final temperature of 250°C which was held for 1.00 min. The injector was set at 200°C with a split ratio of 40.

The standard curve was prepared using a stock solution of 100 mM HMF in EtOAc, and concentration ranging from 5 mM to 50 mM, in increments of 5 mM, were prepared for injection. The XCaliber program was used to plot the standard curve and for quantification of HMF in the samples. Presence of HMF in the samples was identified both by retention time, and by mass spectra with the NIST Mass Spectral Library (version 2.0).

To calculate the experimental yields of HMF, the standard curve obtained using the GC-MS is used. Equation 2 is used to find the concentration in mmol of HMF/g of substrate, where C is the concentration in mM found using the standard curve, V is the volume in L of

EtOAc used to re-dissolve the dried samples, and $M_{substrate}$ is the initial weight of the substrate (untreated biomass or dry extract) in g. Units are shown in brackets.

$$C_{experimental}\left(\frac{\text{mmol HMF}}{\text{g of substrate}}\right) = \frac{C\ (\text{mM}) \times V\ (\text{L})}{M_{substrate}(\text{g})} \quad [2]$$

The theoretical yield for untreated biomass ($C_{HMF\ untreated}$) was calculated using equation 3 where C_{DW} is calculated using the amount of total sugars in the 0.5 M H_2SO_4 pre-treatment (Table 3) and $MW_{glucose}$ is the molecular weight of glucose. Units are shown in brackets.

$$C_{HMF\ untreated}\left(\frac{\text{mmol HMF}}{\text{g of biomass}}\right) = C_{DW}\left(\frac{\text{mg sugars}}{\text{g of DW}}\right) \times \frac{1}{MW_{glucose}} \times \frac{1\ \text{mol}_{HMF}}{1\ \text{mol}_{glucose}} \quad [3]$$

The theoretical yield for the dry MeOH extracts ($C_{HMF\ MeOH}$) was calculated using equation 4 where $C_{extract}$ is calculated using the total sugars found in the MeOH extract (Table 3). Units are shown in brackets.

$$C_{HMF\ MeOH}\left(\frac{\text{mmol HMF}}{\text{g of extract}}\right) = C_{\ extract}\left(\frac{\text{mg sugars}}{\text{g of extract}}\right) \times \frac{1}{MW_{glucose}} \times \frac{1\ \text{mol}_{HMF}}{1\ \text{mol}_{glucose}} \quad [4]$$

For the 0.5 M H_2SO_4 treatment, the solution was neutralized using NaOH, and the production of the salt Na_2SO_4 is not negligible when weighting the dry extracts. Following the stoichiometry of the reaction, we can determine the amount of Na_2SO_4 produced in the reaction, and therefore calculate the mass of Na_2SO_4 in g ($M_{Na_2SO_4}$) found in a specific volume of extract in L (V) (equation 5). Here, the $C_{H_2SO_4}$ is the concentration of the H_2SO_4 solution in mol/L (0.5 M H_2SO_4 is used), V is the amount of extract used in L, and $MW_{Na_2SO_4}$ is the molecular weight of Na_2SO_4.

$$H_2SO_4 + 2NaOH \rightarrow\ 2H_2O + Na_2SO_4$$

$$M_{Na_2SO_4} = C_{H_2SO_4} \times \frac{1\ \text{mol}_{\ Na_2SO_4}}{1\ \text{mol}_{H_2SO_4}} \times MW_{Na_2SO_4} \times V \quad [5]$$

The mass in the dry extract attributed to the hydrolysed biomass ($M_{hydrolysed\ biomass}$) can then be found for a specific volume by subtracting the total weight of the dried material ($M_{DW\ of\ extract}$), with the known weight of Na_2SO_4 ($M_{Na_2SO_4}$) (equation 6).

$$M_{hydrolysed\ biomass} = M_{DW\ of\ extract} - M_{Na_2SO_4} \quad [6]$$

The theoretical yield for the dry 0.5 M H_2SO_4 extract ($C_{HMF\ H_2SO_4}$) can then be calculated using equation 7, where the concentration of sugars (C_{hydro}) is calculated using the total sugars found in the H_2SO_4 extract (Table 3). Units are shown in brackets.

$$C_{HMF\ H_2SO_4}\left(\frac{\text{mmol HMF}}{\text{g of extract}}\right)$$

$$= C_{\ hydro}\left(\frac{\text{mg sugars}}{\text{g of hydrolysed biomass}}\right) \times \frac{1}{MW_{glucose}} \times \frac{1\ mol_{HMF}}{1\ mol_{sugar}} \times \frac{M_{hydrolysed\ biomass}}{M_{DW\ of\ extract}} \quad [7]$$

For all types of biomass, the percentage yield (mol%) was then calculated by dividing the experimental yield by the theoretical yield and multiplying by 100.

3. Results

3.1. Pre-Treatments

3.1.1. Glucose Standard Calibration Curves

Standard curves were prepared before each analysis with a coefficient of determination above 0.99. The standard curves for total sugars using the phenol/H_2SO_4 assay, and for reducing sugars using the 3,5-DNS assay are presented in Figures 16 and 17.

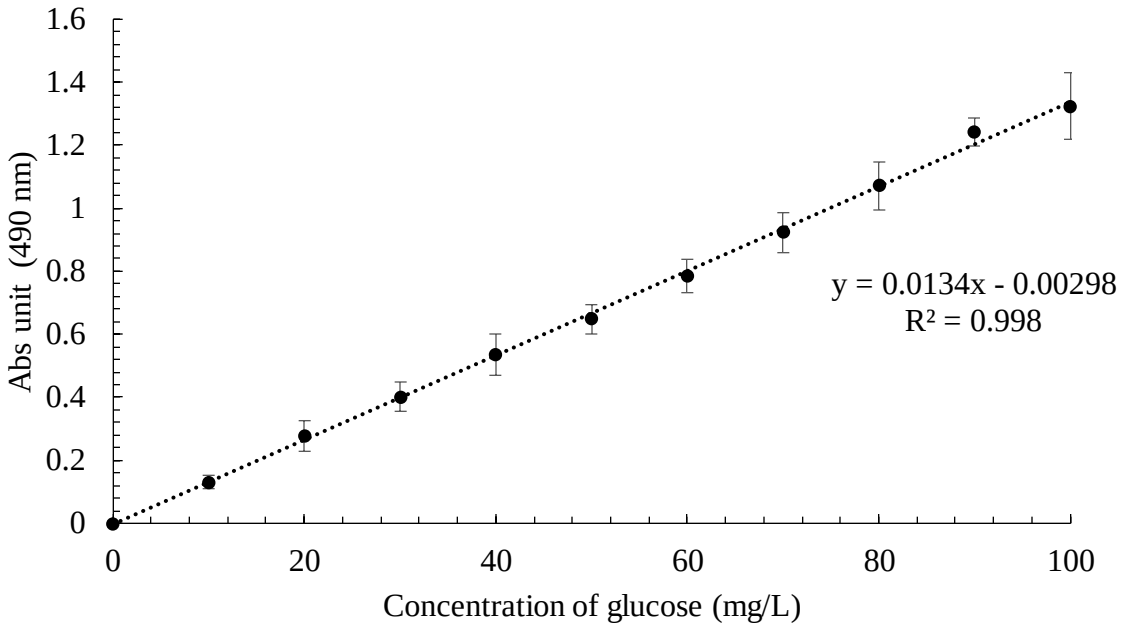


Figure 16. Total sugar standard calibration curve. Concentrations of glucose measured using the phenol/H_2SO_4 assay at a wavelength of 490 nm (n=5).

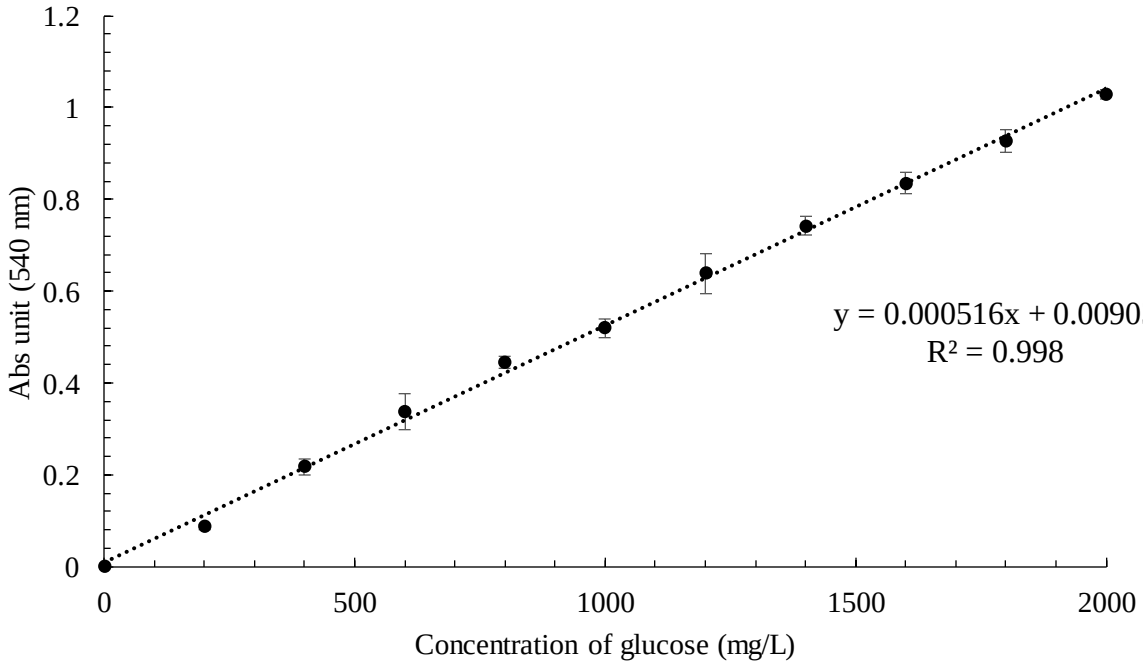


Figure 17. Reducing sugar standard calibration curve. Concentrations of glucose measured using the 3,5-DNS assay at a wavelength of 540 nm (n=3).

3.1.2. Total Sugars and Reducing Sugars in Methanol Extracts of Comfrey and Switchgrass

The concentration of total sugars and reducing sugars in the MeOH extract of comfrey are reported in Table 9. Total sugars were found at a concentration of 300±60 mg of sugars/g of extracted biomass or 47.0±13.1 mg of sugars/g of DW (total biomass). For reducing sugars, 264±106 mg of sugars/g of extracted biomass or 38.8±2.8 mg of sugars/g of DW were obtained. Therefore, all sugars found in the MeOH extract of comfrey were reducing. The percentage of biomass extracted in the MeOH was 19.6±11.1% for comfrey.

Table 9. Total and reducing sugar concentrations in the MeOH extract of comfrey (n=4 for total sugars, n=3 for reducing sugars).

	C_{sample} (mg/L)	$C_{extract}$ (mg of sugars/g of extracted biomass)	C_{DW} (mg of sugars/g of DW)	% of sugars in extracted biomass	% of sugars in DW
Total sugars	2587±408	300±60	47.0±13.1	30.0±6.0	4.70±1.31
Reducing sugars	2552±212	264±106	38.8±2.8	26.4±10.6	3.88±0.28

Table 10 reports the concentration of total and reducing sugars in the MeOH extract of switchgrass. Unlike for comfrey, reducing sugar concentration was lower than total sugar concentration. Total sugars in the extract were found at a concentration of 202±16 mg of sugars/g of extracted biomass or 34.7±4.8 mg of sugars/g of DW. Reducing sugars were found in concentrations of 91.9±1.6 mg of sugars/g of extracted biomass or 11.9±0.3 mg of sugars/g of DW. For switchgrass, 17.7±2.3% of the biomass was dissolved in warm MeOH.

Table 10. Total and reducing sugar concentrations in the MeOH extract of switchgrass (n=3 for total and reducing sugars).

	C_{sample} (mg/L)	$C_{extract}$ (mg of sugars/g of extracted biomass)	C_{DW} (mg of sugars/g of DW)	% of sugars in extracted biomass	% of sugars in DW
Total sugars	2214±229	202±16	34.7±4.8	20.2±1.6	3.47±0.48
Reducing sugars	778±21	91.9±1.6	11.9±0.3	9.19±0.16	1.19±0.03

Pigments did not interfere with the quantification of the sugars. The amount of total sugars in the comfrey MeOH extract filtered through activated charcoal to remove pigments was found to be 30.2% in the extracted biomass, which is within the range of the 30.0±6.0% of sugars found in the extracted biomass when the pigments were present in the extract (Table 9). Similar results were found for the switchgrass MeOH extract, where the extract without pigments yielded 20.3% of sugars in the extracted biomass, which is within the range of 20.2±1.6% of sugars found when the pigments were left in the extract (Table 10).

Overall, total sugars in the MeOH extracts were lower for switchgrass compared to comfrey, indicating a lower amount of soluble sugars in switchgrass. However, the amount of dissolved biomass is essentially similar (19.6±11.1% for comfrey and 17.7±2.3% for switchgrass). Figure 18 shows the differences in sugars extracted with MeOH for comfrey and switchgrass.

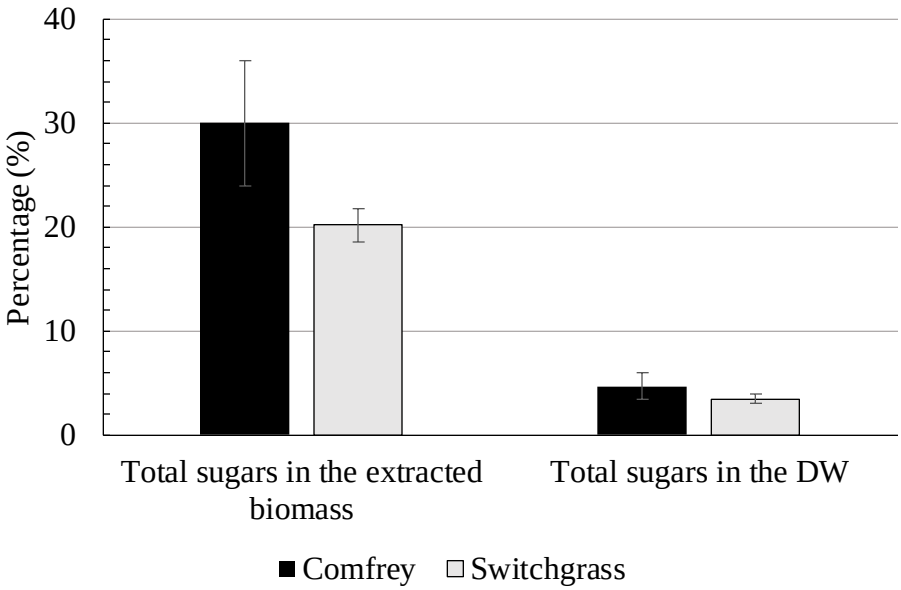


Figure 18. Comparison of total sugars obtained after a MeOH extraction of comfrey and switchgrass (n=4 for comfrey, n=3 for switchgrass).

3.1.3. Total Sugars and Reducing Sugars in Acid Pre-treated Comfrey and Switchgrass

The optimal conditions for hydrolysis were chosen based on a series of experiments done with comfrey. Both 0.1 M and 0.5 M H_2SO_4 solutions were tested. An incubation period of 24 h in the 0.5 M H_2SO_4 solution prior to autoclave was also tested. Autoclave parameters were set at a constant temperature and time of 121°C at 17-20 psi for 30 min. The 0.5 M H_2SO_4 with no incubation (n=4) yielded the best results with 230±29 mg of sugars/g of hydrolysed biomass, and was chosen as the optimal condition for subsequent hydrolysis (Table 11). There was no important difference found with the samples that were incubated for 24 h prior to autoclave, with results showing 247±23 mg of sugars/g of hydrolysed biomass (n=2). The 0.1 M H_2SO_4 hydrolysis yielded poor results with concentrations of sugars of 117 mg of sugars/g of hydrolysed biomass (n=1). Percentage of biomass hydrolysed was also superior using the 0.5 M H_2SO_4 which hydrolysed 57.2±3.2% of the biomass (no incubation)

or 53.4±1.1% of the biomass (incubated) compared to a 46.4% dissolution of the biomass for the 0.1 M treatment.

Choosing the optimal conditions of 0.5 M H_2SO_4 treatment under autoclave for 30 min with no incubation, both total and reducing sugars were quantified in the extracts for comfrey and switchgrass (Tables 11 and 12). Once again, for comfrey, total sugars and reducing sugars were similar (Table 11). Concentration of total sugars were 230±29 mg of sugars/g of hydrolysed biomass or 130±18 mg of sugars/g of DW. Reducing sugars concentrations were 256±13 mg of sugars/g of hydrolysed biomass or 147±11 mg of sugars/g of DW. Dissolution of the comfrey biomass using the 0.5 M H_2SO_4 treatment was 57.2±3.2%.

Table 11. Total and reducing sugar concentrations in the 0.5 M H_2SO_4 hydrolysis of 5% (wt/v) of comfrey, no incubation, after a 30 min autoclave (n=4 for total sugars, n=3 for reducing sugars).

	C_{sample} (mg/L)	**$C_{extract}$ (mg of sugars/g of hydrolysed biomass)**	**C_{DW} (mg of sugars/g of DW)**	**% of sugars in hydrolysed biomass**	**% of sugars in DW**
Total sugars	8596±1268	230±29	130±18	23.0±2.9	13.0±1.8
Reducing sugars	7957±126	256±13	147±11	25.6±1.3	14.7±1.1

For the switchgrass treated with 0.5 M H_2SO_4 at a concentration of 5% (wt/v) substrate loading and autoclaved for 30 min without prior incubation, reducing sugars were also equal to total sugars, indicating that most, if not all sugars found in solution were reducing (Table 12). This is to be expected since plants are primarily composed of reducing sugar subunits (mainly glucose, but also xylose, mannose, galactose, etc.) found in the cellulose and hemicellulose fractions, and the H_2SO_4 treatment will break down those components, making those reducing sugars available for quantification. Concentration of total sugars were 425±13

mg of sugars/g of hydrolysed biomass or 189±3.7 mg of sugars/g of DW. Reducing sugars concentrations were 474±120 mg of sugars/g of hydrolysed biomass or 204±3.2 mg of sugars/g of DW. Dissolution of the switchgrass biomass using the 0.5 M H_2SO_4 treatment was 44.2±7.2%.

Table 12. Total and reducing sugar concentrations in the 0.5 M H_2SO_4 hydrolysis of 5% (wt/v) of switchgrass, no incubation, after a 30 min autoclave (n=3 for total and reducing sugars).

	C_{sample} (mg/L)	**$C_{extract}$ (mg of sugars/g of hydrolysed biomass)**	**C_{DW} (mg of sugars/g of DW)**	**% of sugars in hydrolysed biomass**	**% of sugars in DW**
Total sugars	8957±1972	425±13	189±3.7	42.5±1.3	18.9±0.37
Reducing sugars	10037±1160	474±120	204±3.2	47.4±12.0	20.4±0.32

Using the optimal conditions of 0.5 M H_2SO_4 treatment autoclaved for 30 min without prior incubation, switchgrass yielded nearly double the amount of sugars per weight of hydrolysed biomass compared to comfrey under the same conditions (Figure 19). However, dissolution of switchgrass in the acid was slightly less (44.2±7.2%) compared to the dissolution of comfrey (57.2±3.2%). The concentration of sugars in mg/g of DW remained higher for switchgrass.

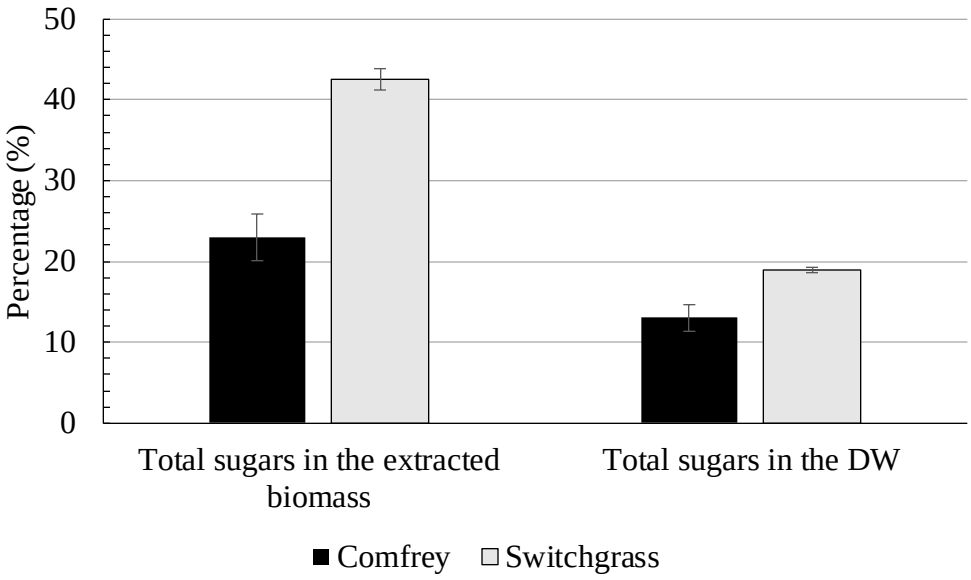


Figure 19. Comparison of total sugars obtained after a 0.5 M H_2SO_4 treatment after a 30 min autoclave for comfrey and switchgrass (n=4 for comfrey, n=3 for switchgrass).

Multiple consecutive hydrolyses were completed on comfrey with the optimal hydrolysis condition in order to assess the efficiency of the hydrolysis on the feedstock. A first hydrolysis was performed with 0.5 M H_2SO_4 and recovered biomass was dried, and used for a subsequent hydrolysis. This step was repeated a third time. As the results in Table 13 show, the concentration of total sugars extracted from the hydrolysed biomass decreased after each hydrolysis, starting from 235±40 mg of sugars/g of hydrolysed biomass to 164±18 mg of sugars/g of hydrolysed biomass to 71.6±11.5 mg of sugars/g of hydrolysed biomass by the third hydrolysis. However, the percentage of hydrolysed biomass decreased significantly after each hydrolysis, starting with a 55.0±1.2% dissolution to a 16.3±0.9% dissolution to a 14.6±0.1% dissolution by the third hydrolysis in acid (Table 13). This decrease in dissolution is reflected in the concentration of total sugars in the initial DW (C_{DW}), starting at 129±19 mg of sugars/g of DW to 13.5±0.02 mg of sugars/g of DW to 3.96±0.71 mg of sugars/g of DW by the third hydrolysis (Table 13). Compared to a single hydrolysis, yield of total sugars was

increased from 129±19 mg of sugars/g of DW to 147±19 mg of sugars/g of DW after three hydrolyses. Multiple treatments did not improve the yield of extracted sugars significantly, with approximately 88% of sugars being extracted in the initial hydrolysis.

Table 13. Total sugars concentrations obtained after three consecutive 0.5 M H_2SO_4 hydrolyses of 5% (wt/v) of comfrey, after a 30 min autoclave (n=2).

	C_{sample} (mg/L)	$C_{extract}$ (mg of sugars/g of hydrolysed biomass)	C_{DW} (mg of sugars/g of DW)	% of sugars in hydrolysed biomass	% of sugars in DW	% of hydrolysed biomass
Hydro 1	8596±1268	235±40	129±19	23.5±4.0	12.9±1.9	55.0±1.2
Hydro 2	1196±222	164±18	13.5±0.02	16.4±1.8	1.35±0.002	16.3±0.9
Hydro 3	396±71	71.6±11.5	3.96±0.71	7.16±1.15	0.396±0.071	14.6±0.1

The Student's t-test was performed using Excel, and we confirmed that the difference in base used for neutralization of acidic extracts did not significantly affect the quantification of the sugars in solution ($p>0.05$). Total sugars were found to be 114±14 mg of sugars/g of DW when NaOH was used to neutralize the solution, and 135±19 mg of sugars/g of DW when using $Ca(OH)_2$ to neutralize the solution.

3.1.4. Total Sugars Extracted from Comfrey with Other Treatments and Comparison of Treatments

The base pre-treatment of 2 M NaOH, at 50°C for 24 h, yielded very low amounts of sugars when using 10% (wt/v) loading of comfrey. The amount of total sugars obtained after this treatment for comfrey was 59.5±4.0 mg of sugars/g of hydrolysed biomass or 20.8±12 mg of sugars/g of DW (n=2). The percentage of sugars obtained using this method was 5.95±0.4% of the hydrolysed biomass or 2.08±1.2% of the DW (Figure 20). Only 19.4±1.0% of the comfrey biomass was hydrolysed using this treatment (Figure 20). The treatment was

not repeated using switchgrass under the assumption that, for comfrey, the NaOH treatment caused heavy degradation of the plant material, and offered poor yields of sugars. Furthermore, reports in the literature showed poor results for cellulose breakdown using base treatment [45, 47]. The recovered comfrey biomass from the 2 M NaOH treatment formed a thick brown sludge, and was therefore not suitable for further treatment.

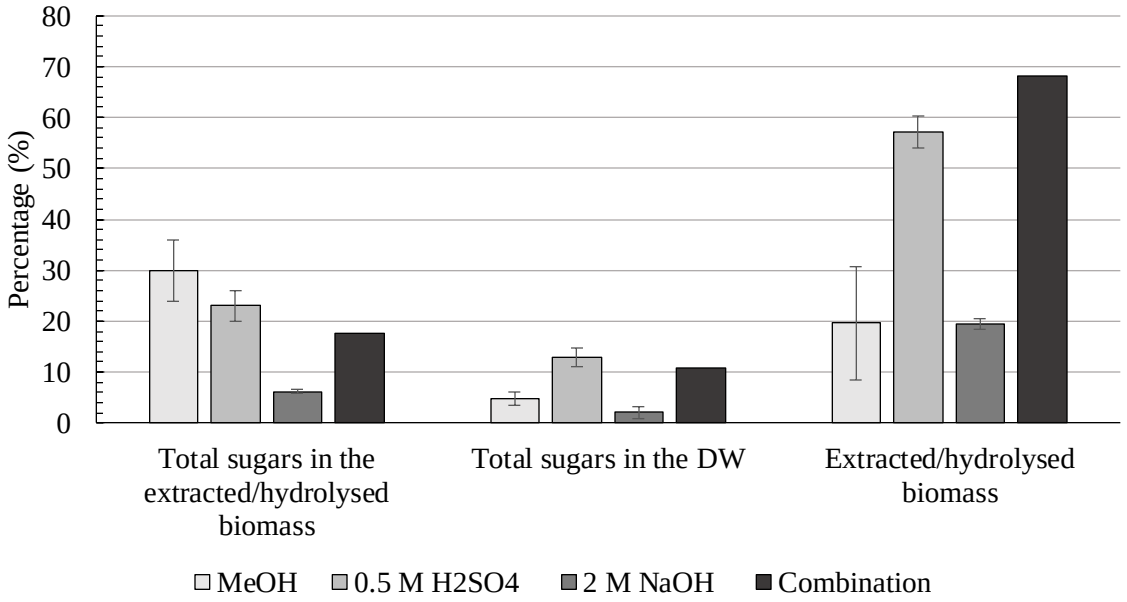


Figure 20. Comparison of the treatments for comfrey (n=4 for MeOH and 0.5 M H_2SO_4, n=2 for 2 M NaOH, and n=1 for the combination of treatments). DW, dry weight.

A combination of treatments was used on comfrey to determine if the yield of extractable sugars from the biomass could be improved (Table 14). In this case, the MeOH treatment was used first in order to remove the soluble fraction. The recovered biomass then underwent a 0.5 M H_2SO_4 treatment at optimal conditions. Finally, the recovered biomass from the acid treatment underwent a 2 M NaOH treatment. Overall, 68.1% of the biomass was dissolved following the three treatments compared to our highest dissolution of 57.2±3.2% using a single 0.5 M H_2SO_4. However, the total sugars after all three treatments (108 mg of sugars/g of DW) were not higher than what we obtained after a single 0.5 M H_2SO_4

treatment (130±18 mg of sugars/g of DW) (Table 11). This could be due to a fraction of the sugars being degraded, or lost, when using multiple treatments.

Table 14. Combination of treatments on comfrey biomass.

	C_{sample} (mg/L)	$C_{extract}$ (mg of sugars/g of extracted biomass)	C_{DW} (mg of sugars/g of DW)	% of sugars in hydrolysed biomass	% of sugars in DW	% of hydrolysed biomass
MeOH extract	2095	231	61.2	23.1	6.12	26.5
H_2SO_4 treatment	4537	234	35.1	23.4	3.51	21.3
NaOH treatment	5789	62.3	12.1	6.23	1.21	19.4
Total	-	175	108	17.5	10.8	68.1

Figure 20 shows the comparison of the combination of treatments to the single treatment for comfrey (including the MeOH treatment, the 0.5 M H_2SO_4 treatment, and the 2 M NaOH treatment). The MeOH extract was found to be best at producing an extract rich in sugars, however dissolution of the biomass in MeOH was low. The 0.5 M H_2SO_4 treatment on the other hand dissolved a high amount of biomass and produced the highest amount of extractable sugars per DW used.

3.2. HMF Production

3.2.1. Catalyst Loading and Theoretical Yield of HMF

In some reactions, the catalyst loading is based on the mol% of catalyst in relation to the mol of sugars in the substrate. For untreated plant, the estimated amount of sugars is based on the concentration of sugars in comfrey and switchgrass per initial dry weight according to our best treatment (0.5 M H_2SO_4, no incubation, 30 min autoclave) (C_{DW}, Table 3). Although those numbers likely do not represent all hydrolysable sugars from lignocellulose, it is a reliable estimate as to the amount of sugars that can be expected in the reaction. For the

MeOH extract and the 0.5 M H_2SO_4 extract, the exact amount of sugars per dry amount of the extract was calculated using the total sugar assay ($C_{extract}$ or C_{hydro}, Table 3). The concentration of sugars in each type of substrate is summarized in Table 15. Furthermore, the expected theoretical yield of HMF was calculated for each type of biomass using the equations 2 to 7 presented in section 2.6.6. The expected yields are also reported in Table 15. The yields presented for the 0.5 M H_2SO_4 extract are only valid when the solution is neutralized with NaOH, as the amount of Na_2SO_4 is accounted for in the calculation.

Table 15. Average total sugar concentrations for untreated biomass, MeOH extracted biomass, and 0.5 M H_2SO_4 hydrolysed biomass, and theoretical HMF yielded from conversion of the biomass.

Biomass	Concentration	HMF yield
Untreated comfrey	0.130[a]	0.722[b]
Untreated switchgrass	0.189[a]	1.049[b]
MeOH extract comfrey	0.300[c]	1.665[d]
MeOH extract switchgrass	0.202[c]	1.121[d]
0.5 M H_2SO_4 extract comfrey[e]	0.230[f]	0.243[d]
0.5 M H_2SO_4 extract switchgrass[e]	0.425[f]	0.411[d]

[a] In g of sugars/g of dry weight of untreated plant material.
[b] In mmol of HMF/g of dry weight of untreated plant material.
[c] In g of sugars/g of extracted biomass.
[d] In mmol of HMF/g of dry extract.
[e] Includes the Na_2SO_4 obtained from the neutralisation with NaOH and the hydrolysed biomass.
[f] In g of sugars/g of hydrolysed biomass.

The theoretical yield for the untreated biomass is based on our estimate of hydrolysable sugars in the biomass using the 0.5 M H_2SO_4 treatment. An example for comfrey biomass is shown in equation 8. Here, although total sugar concentrations are used in the equation, we can assume that each unit of sugars is equivalent to one unit of glucose in the transformation to HMF, since the majority of the sugars found in switchgrass and comfrey are present in the form of glucose found in the cellulose fraction.

$$C_{\text{mmol HMF/g of DW}} = \frac{0.130\ g_{\text{sugar}}}{\text{g of DW}} \times \frac{1\ \text{mol}_{\text{glucose}}}{180.16\ g_{\text{glucose}}} \times \frac{1\ \text{mol}_{\text{HMF}}}{1\ \text{mol}_{\text{sugar}}} \times \frac{1000\ \text{mmol}}{1\ \text{mol}}$$

$$= \frac{0.722\ \text{mmol HMF}}{\text{g of DW}} \quad [8]$$

The concentration can also be calculated using the literature cellulose content (22.4% for comfrey) [30]. In this case, our theoretical yield is close to the literature based yield. An example of the calculation is shown for comfrey in equation 9.

$$C_{\text{mmol HMF/g of DW}} = \frac{0.224\ g_{\text{sugar}}}{\text{g of DW}} \times \frac{1\ \text{mol}_{\text{glucose}}}{180.16\ g_{\text{glucose}}} \times \frac{1\ \text{mol}_{\text{HMF}}}{1\ \text{mol}_{\text{sugar}}} \times \frac{1000\ \text{mmol}}{1\ \text{mol}}$$

$$= \frac{1.24\ \text{mmol HMF}}{\text{g of DW}} \quad [9]$$

For switchgrass, we expect a theoretical yield of 1.05 mmol HMF/g of extract according to our estimate, which is lower than the estimate of 2.23 mmol HMF/g of extract based on the cellulose content found in the literature [30].

Using the concentrations of sugars in the untreated biomass or the dried extracts, quantities of catalyst can be calculated in mol%. For example, the volume of H_2SO_4 at a concentration of 3 mol% can be calculated using the concentration of sugars in the untreated comfrey (Table 16) using equation 10.

$$V_{H_2SO_4} = \frac{0.130\ g_{\text{sugar}}}{\text{g DW}} \times \frac{1\ \text{mol}_{\text{sugar}}}{180.1559 g_{\text{sugar}}} \times \frac{5\ \text{mol}_{H_2SO_4}}{100\ \text{mol}_{\text{sugar}}} \times \frac{98.079\ g_{H_2SO_4}}{\text{mol}_{H_2SO_4}} \times \frac{1\ \text{ml}_{H_2SO_4}}{1.84\ g_{H_2SO_4}}$$

$$V_{H_2SO_4} = 1.92\ \text{mL}_{H_2SO_4} \quad [10]$$

Quantities of catalyst used in the reactions are reported in Table 16 for different mol%.

Table 16. List of catalyst loading in mol% used for HMF production from untreated comfrey and switchgrass, and from the MeOH extracts of comfrey and switchgrass.

Compound	mol%	Quantity per g of DW			
		Untreated comfrey	Untreated switchgrass	Dry MeOH extract of comfrey	Dry MeOH extract of switchgrass
$CrCl_3 \cdot 6H_2O$	3	0.0058 g	0.0102 g	0.0133 g	0.0096 g
$CuCl_2$	3	0.0029 g	0.0052 g	0.0067 g	0.0048 g
$AlCl_3$	3	0.0096 g	0.0170 g	-	-
H_2SO_4	5	1.92 µL	3.40 µL	-	-
TFA	10	5.52 µL	9.77 µL	-	-
CH_3COOH	10	4.12 µL	7.29 µL	-	-

3.2.2. Standard Calibration Curve of HMF

Figure 21 shows the standard calibration curve for HMF obtained by GC-MS, and a GC chromatogram for an HMF standard is shown in Figure 22. The retention time of HMF was around 6.25 min. A head-to-tail comparison of the mass spectra of our HMF standard with the MS library mass spectra is shown in Figure 23. The main signals in the MS spectrum of HMF are seen at a *m/z* of 127 (M+1 peak), 126 (M^+ peak), 109 (lost of the OH group), 97 (lost of the CHO group), 95 (lost of the CH_2OH group), 81 (lost of formic acid) and 69 (lost of glyoxal).

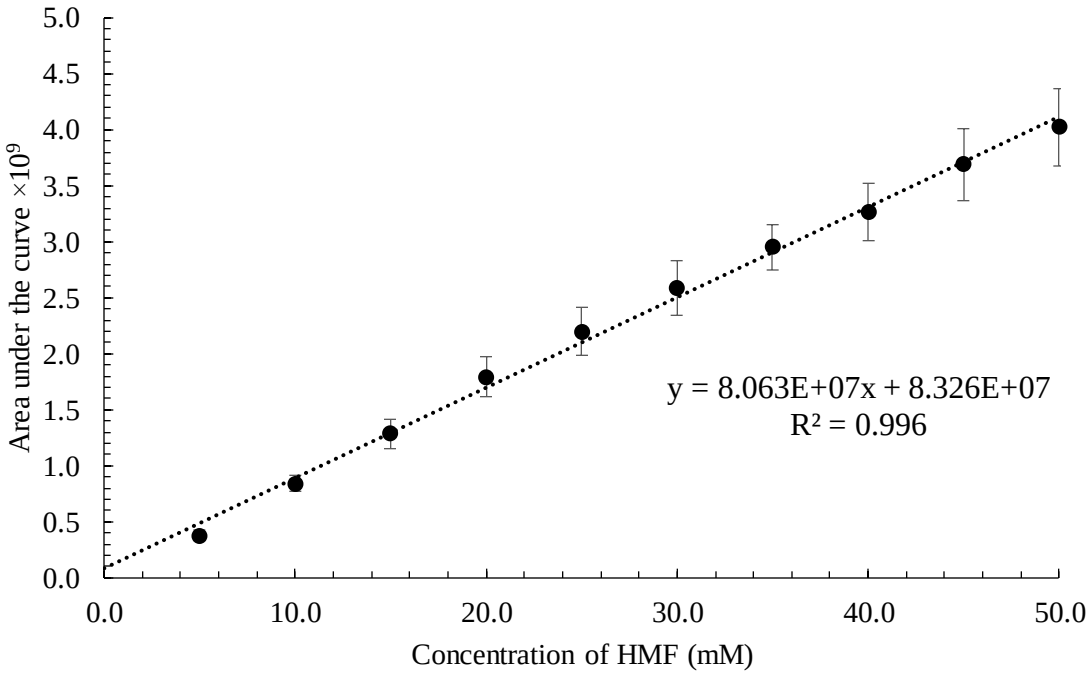


Figure 21. Standard calibration curve for HMF (n=3).

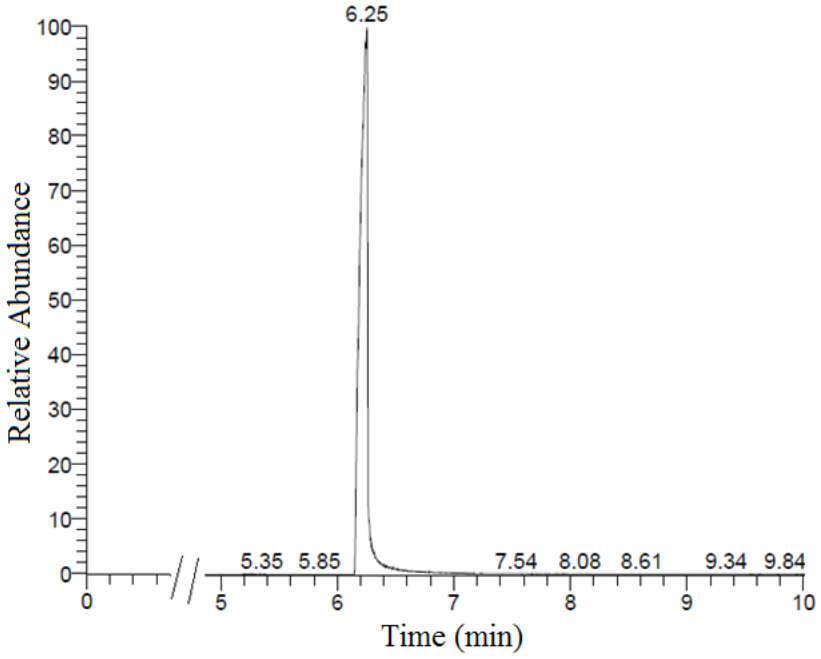


Figure 22. GC chromatogram for an HMF standard at a concentration of 40 mM.

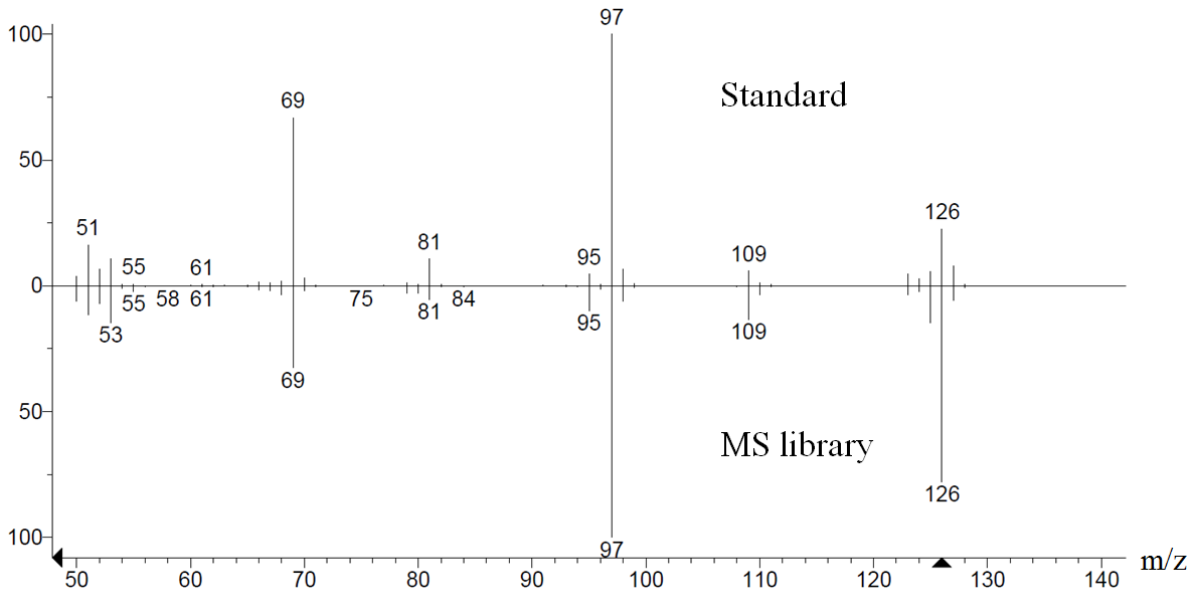


Figure 23. Head-to-tail comparison of the mass spectra of our HMF standard with the MS library mass spectra. Standard matches to 89% with the MS library spectrum.

3.2.3. HMF Production from Glucose

Controls were performed using our best reaction conditions in order to ensure the reaction system's suitability for production of HMF using a simple feedstock. Glucose loaded at 10 wt% of the reaction mixture was used as the substrate. The reaction time was kept constant at 30 min at 140°C, and no dissolution step was used since glucose dissolved readily in the ionic liquids. Results are reported in Table 17. The highest yield of HMF (based on the amount of glucose used) of 50.0% was obtained using [BMIM]Cl with 3 mol% catalyst loading of $CrCl_3 \bullet 6H_2O$ and $CuCl_2$ (entry 1). Using [EMIM]Cl under the same conditions, the yield of HMF decreased to 31.0% (entry 2). A catalyst loading of 3 wt% instead of 3 mol% also decreased the yield of HMF to 24.5% in [BMIM]Cl (entry 3) and 24.4% in [EMIM]Cl (entry 4). The control using wt% for the catalyst loading were performed because wt% is sometimes a more accurate measurement when using lignocellulosic biomass due to the difficulty in estimating the amount of glucose available for transformation in the plant. The

use of $CrCl_2$ instead of $CrCl_3 \bullet 6H_2O$ did not change the yield of HMF produced from the reaction (entries 5 and 6 compared to entries 1 and 2).

Table 17. HMF production from glucose with a reaction time of 30 min at 140°C with a 10 wt% substrate loading.

Entry	Solvent	Catalyst 1	Catalyst 2	HMF Yield (%)
1	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	50.0
2	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	31.0
3	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	24.5
4	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	24.4
5	[BMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	48.7
6	[EMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	31.0

3.2.4. HMF Production from Untreated Comfrey and Switchgrass

The dissolution of untreated biomass using different solvents was studied (Table 18). The best dissolution of the biomass was obtained with 68% dissolution of comfrey and 62% dissolution of switchgrass using a DMSO/[BMIM]Cl (10 wt%) mixture (entries 9 and 14). For comfrey biomass, dissolution varied from 27 to 53% in [BMIM]Cl with lower substrate loading showing better dissolution (entries 1 to 3). A higher dissolution time (24 h compared to 30 min) slightly improved the dissolution of comfrey in [BMIM]Cl from 27% to 39% when substrate loading was 10 wt% (entries 3 and 4). Switchgrass was harder to dissolve in [BMIM]Cl with 12 to 30% of the biomass dissolving (entries 10 and 11). Longer dissolution time (24 h compared to 30 min) did not improve the dissolution of switchgrass, with only 12% of the initial biomass dissolving after 24 h compared to 30% after 30 min (entries 10 and 11). However, temperature for the 24 h reaction was lower (80°C compared to 120°C), which could explain the lower dissolution of the biomass.

Table 18. Dissolution of comfrey and switchgrass under different conditions.

Entry	Biomass	Solvent	Dissolution step	Catalytic Step	% dissolved biomass
1	Comfrey (2.5 wt%)	[BMIM]Cl	30 min, 120°C	2 h, 140°C	53
2	Comfrey (5 wt%)	[BMIM]Cl	30 min, 120°C	15 min, 140°C	39
3	Comfrey (10 wt%)	[BMIM]Cl	30 min, 120°C	30 min, 140°C	27
4	Comfrey (10 wt%)	[BMIM]Cl	24 h, 80°C	30 min, 140°C	39
5	Comfrey (10 wt%)	[EMIM]Cl	30 min, 120°C	30 min, 140°C	49
6	Comfrey (5 wt%)	2-methylpyridine *N*-oxide	30 min, 100°C	15 min, 120°C	38
7	Comfrey (5 wt%)	3-picoline *N*-oxide	30 min, 100°C	15 min, 120°C	36
8	Comfrey (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	24 h, 75°C	2 h, 140°C	35
9	Comfrey (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	-	9 h, 150°C	68
10	Switchgrass (10 wt%)	[BMIM]Cl	30 min, 120°C	30 min, 140°C	30
11	Switchgrass (10 wt%)	[BMIM]Cl	24 h, 80°C	30 min, 140°C	12
12	Switchgrass (10 wt%)	[EMIM]Cl	30 min, 120°C	30 min, 140°C	54
13	Switchgrass (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	24 h, 75°C	2 h, 140°C	14
14	Switchgrass (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	-	9 h, 150°C	62

Dissolution of comfrey in 2-methylpyridine *N*-oxide and 3-picoline *N*-oxide was 38% and 36%, respectively (entries 6 and 7). Using a DMA-LiCl/BMIM]Cl (60 wt%) mixture did not improve the dissolution of comfrey nor switchgrass, with dissolutions of 35% and 14%, respectively (entries 8 and 13).

Dissolution in [EMIM]Cl for comfrey and switchgrass was better than dissolution in [BMIM]Cl under the same condition, with dissolutions of 49% for comfrey and 54% for switchgrass in [EMIM]Cl (entries 5 and 12) compared to dissolutions of 27% for comfrey and 30% for switchgrass in [BMIM]Cl (entries 3 and 10).

The yield of HMF for all reactions tested using untreated biomass have been found to be under 1% (Table 19). In most cases, exact concentrations could not be calculated because the detected HMF was below quantification limit (the concentration of the samples was inferior to 5 mM), and samples could not be further concentrated. The detection of HMF for the different reactions conditions is reported in Table 19.

Table 19. HMF production for untreated plants.

Biomass	Solvent	Catalyst 1	Catalyst 2	Catalyst 3	Dissolution step (min, °C)	Catalytic step (min, °C)	HMF
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	10, 140	ND[a]
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	20, 140	D[b]
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	30, 140	D
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	180, 140	D
Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 120	30, 140	D
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (10 mol%)	30, 120	30, 140	D
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (20 mol%)	30, 120	30, 140	D
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	CH_3COOH (10 mol%)	30, 120	30, 140	D
Comfrey (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	TFA (20 mol%)	30, 120	30, 140	ND
Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (10 mol%)	30, 120	30, 140	D
Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	H_2SO_4 (20 mol%)	30, 120	30, 140	D
Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	CH_3COOH (10 mol%)	30, 120	30, 140	D
Switchgrass (10 wt%)	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	TFA (20 mol%)	30, 120	30, 140	ND

Comfrey (5wt%)	2-methylpyridine *N*-oxide	$CrCl_3 \cdot 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 100	15, 120	ND
Comfrey (5 wt%)	3-picoline *N*-oxide	$CrCl_3 \cdot 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	-	30, 100	15, 120	ND
Comfrey (10 wt%)	[EMIM]Cl	$CrCl_3 \cdot 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)		30, 120	30, 140	D
Switchgrass (10 wt%)	[EMIM]Cl	$CrCl_3 \cdot 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)		30, 120	30, 140	D
Comfrey (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	$CrCl_3 \cdot 6H_2O$ (10 mol%)	HCl (10 mol%)	-	24[c], 75	120, 140	D
Switchgrass (10 wt%)	DMA-LiCl : [BMIM]Cl (60 wt%)	$CrCl_3 \cdot 6H_2O$ (10 mol%)	HCl (10 mol%)	-	24[c], 75	120, 140	D
Comfrey (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	$AlCl_3$ (10 mol%)	-	-	-	540, 150	ND
Switchgrass (2 wt%)	DMSO : [BMIM]Cl (10 wt%)	$AlCl_3$ (10 mol%)	-	-	-	540, 150	ND

[a] ND: not detected.
[b] D: detected (<1% yield of HMF).
[c] Time reported in hours.

Figure 24 shows an example of chromatographic identification of HMF using GC-MS for a reaction of comfrey (10 wt% loading) in DMA-LiCl and [BMIM]Cl (60 wt%) with 10 mol% of $CrCl_3 \cdot 6H_2O$ and 10 mol% of HCl. Dissolution time was 24 h at 75°C, followed with a reaction time of 2 h at 140°C. Sample was extracted with EtOAc (4×10 mL), dried, and re-dissolved in 500 µL of EtOAc. The peak was identified using the mass spectrum and the retention time of HMF using a standard to compare. In this case, HMF is below detection limit. Figure 25 shows an example of chromatographic detection of HMF for untreated switchgrass conversion (10 wt%) in [BMIM]Cl with 20 mol% of CH_3COOH, 3 mol% of $CrCl_3 \cdot 6H_2O$ and 3 mol% of $CuCl_2$. The dissolution step was 24 h at 100°C and the catalytic step was 30 min at 140°C. The yield of HMF found was below 1%.

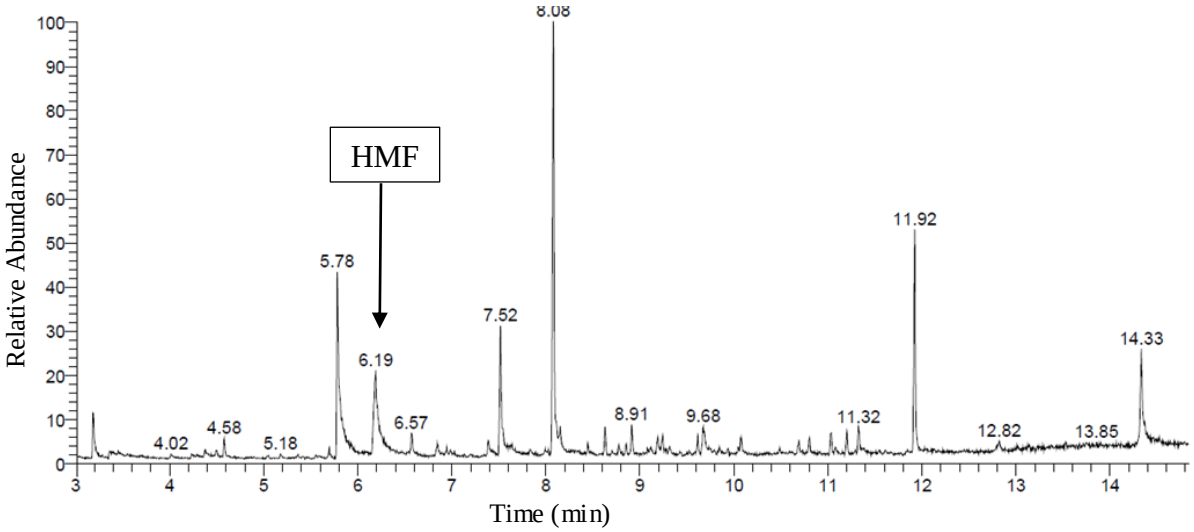


Figure 24. GC chromatogram of untreated comfrey converted into HMF.

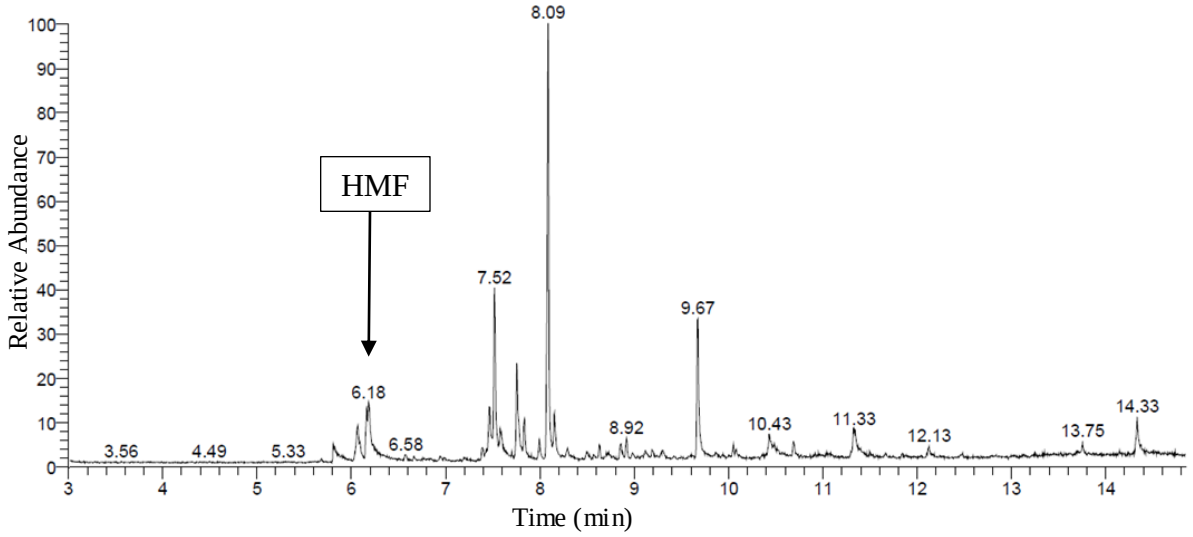


Figure 25. GC chromatogram of untreated switchgrass converted into HMF.

3.2.5. HMF Production from Treated Comfrey and Switchgrass

The MeOH extracts (filtered through activated charcoal) were directly injected in the GC-MS as a control. No HMF was present in the extracts prior to the reactions. Figure 26 and Figure 27 show the chromatograms of the MeOH extracts prior to reaction for comfrey and switchgrass, respectively. The high amount of noise is due to the lack of detectable compounds in the extracts.

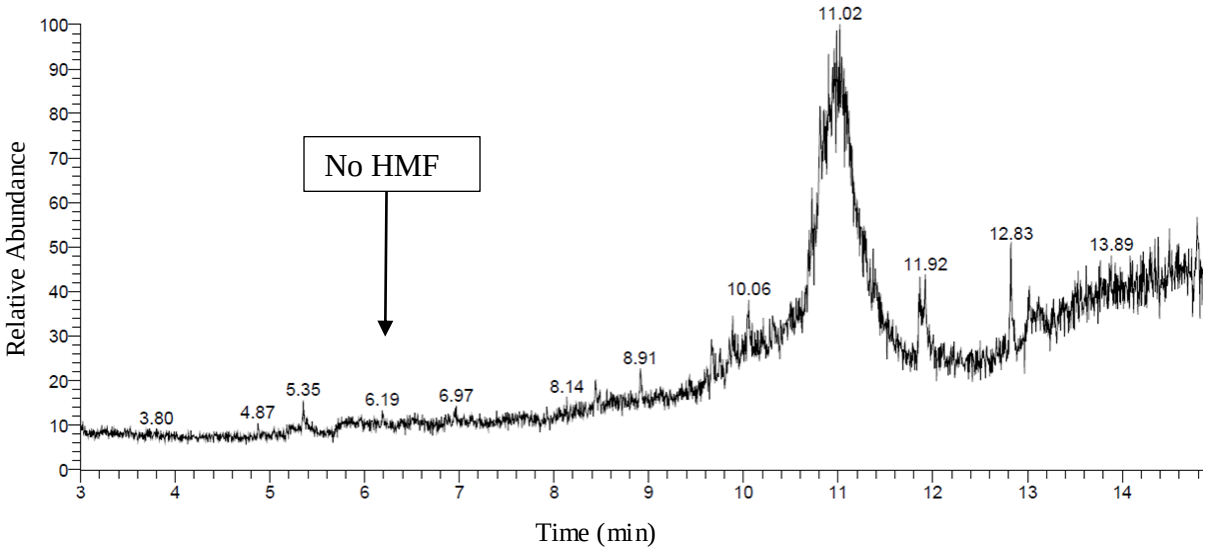


Figure 26. GC chromatogram of the MeOH comfrey extract prior to reaction. No HMF is detected.

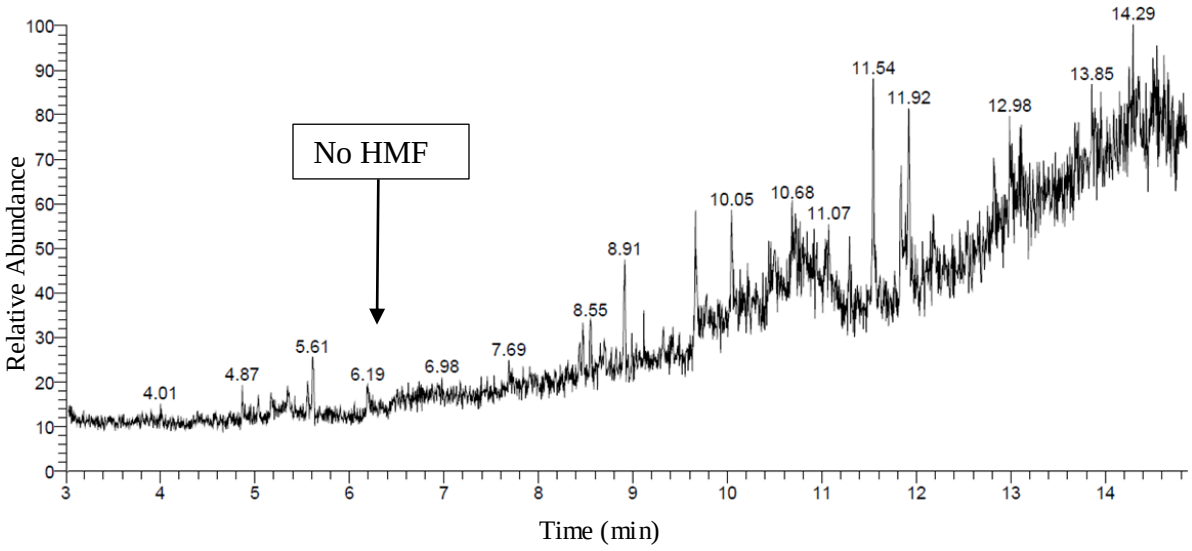


Figure 27. GC chromatogram of the MeOH switchgrass extract prior to reaction. No HMF is detected.

Different reaction conditions were tested for conversion to HMF from the MeOH extract of switchgrass and comfrey (Table 20). Substrate loading was always 10 wt% of the reaction mixture, and the dissolution step was always 30 min at 120°C.

Table 20. HMF production from the dry MeOH extract of comfrey and switchgrass. Substrate loading was 10 wt% of the reaction mixture.

Entry	Dry MeOH extract	Solvent	Catalyst 1	Catalyst 2	Dissolution step (min, °C)	Catalytic step (min, °C)	% dissolved biomass	C_{HMF} (mg/g of extract)	Yield of HMF (%)
1	Comfrey	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	15, 140	57	14.7	6.04
2	Comfrey	[BMIM]Cl	$CrCl_2$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	15, 140	71	10.4	4.93
3	Comfrey	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	30, 120	15, 140	81	D[a]	D[a]
4	Comfrey	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	61	6.66	3.17
5	Comfrey	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	66	5.74	2.73
6	Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	15, 140	43	D	D
7	Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	30, 120	15, 140	-	D	D
8	Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	60	3.90	2.00
9	Switchgrass	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	52	25.4	18.0
10	Switchgrass	[EMIM]Cl	$CrCl_2$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	48	19.9	14.1

[a] D: detected (<1% yield of HMF).

Catalyst loading using 3 wt% of $CrCl_3 \cdot 6H_2O$ and 3 wt% of $CuCl_2$ in [BMIM]Cl (entry 1) was compared with a catalyst loading of 3 mol% of $CrCl_3 \cdot 6H_2O$ and 3 mol% of $CuCl_2$ in [BMIM]Cl (entry 3). The transformation of the MeOH comfrey extract at a reaction time of 15 min with a 3 wt% catalyst loading yielded a higher amount of HMF (6.04%, entry 1) compared to the 3 mol% catalyst loading (<1%, entry 3). For the transformation of the MeOH switchgrass extract at a reaction time of 15 min, yields were <1% for the both the 3 wt% catalyst loading and the 3 mol% catalyst loading (entries 6 and 7).

The effect of reaction time was also studied, with a reaction time of 15 min at 140°C yielding the best result for the production of HMF from the dry MeOH comfrey extract (6.04%, entry 1), while an increased reaction time of 30 min decreased the yield to 3.17% for this extract (entry 4). It is unclear as to why this decrease occurs, since the opposite trend is seen using switchgrass where an increase in reaction time results in an increased yield. Using the MeOH extract of switchgrass, the reaction time of 15 min (entry 6) showed lower production of HMF (<1%) compared to the 30 min reaction time (2.00%) under the same conditions (entry 8). Using [EMIM]Cl instead of [BMIM]Cl was also studied using a 3 wt% catalyst loading, with a 30 min reaction time (entries 5 and 9). For the comfrey extract, the yield of HMF produced was slightly decreased from 3.17% (entry 4) to 2.73% (entry 5). For the switchgrass extract, the yield was sharply increased from 2.00% (entry 8) to 18.0% (entry 9). Optimum reaction conditions seem to be plant specific. Using the best conditions for each plant extract (entries 1 and 9), the $CrCl_3 \cdot 6H_2O$ was swapped for $CrCl_2$ (entries 2 and 10). Yields of HMF decreased slightly from 6.04% (entry 1) to 4.93% (entry 2) for the comfrey extract. A similar trend of yield reduction from 18.0% (entry 9) to 14.1% (entry 10) was found for the switchgrass extract. Dissolution of the MeOH comfrey extract ranged from 57 to 81%

in [BMIM]Cl (entries 1 to 4) and was 66% in [EMIM]Cl (entry 5). Dissolution of switchgrass MeOH extract ranged from 43 to 60% in [BMIM]Cl (entries 6 to 8) and 48 to 52% in [EMIM]Cl (entries 9 and 10), therefore dissolution was not substantially different depending on the ionic liquid used. Figures 28 and 29 show an example of GC chromatograms for the production of HMF with the best reaction conditions obtained from the comfrey extract (entry 1) and the switchgrass extract (entry 9), respectively.

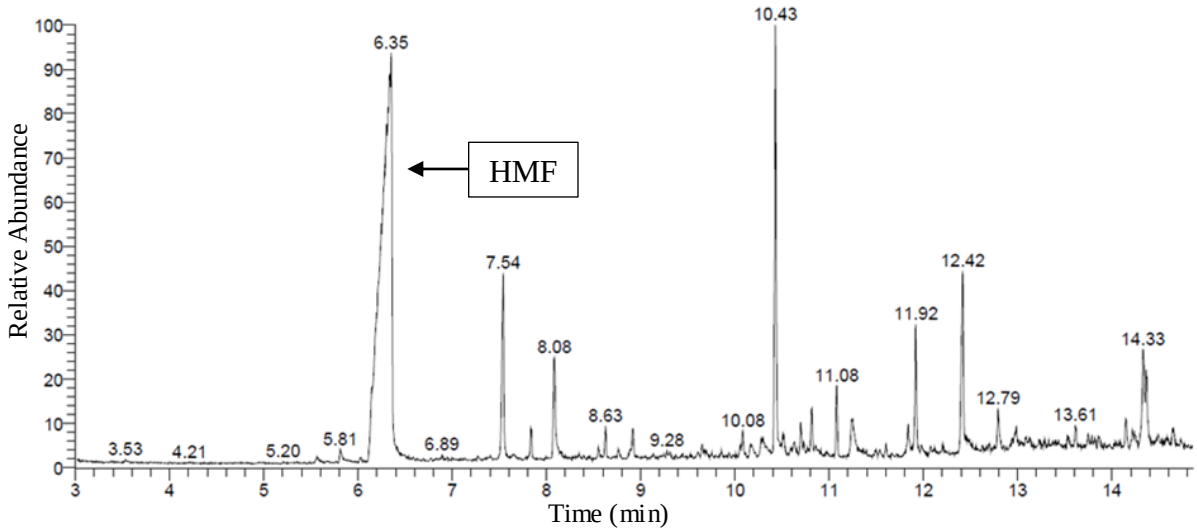


Figure 28. GC chromatogram of the reaction mixture obtained from the transformation of the dry MeOH extract of comfrey to HMF using a 3 wt% catalyst loading in [BMIM]Cl after a 15 min reaction time.

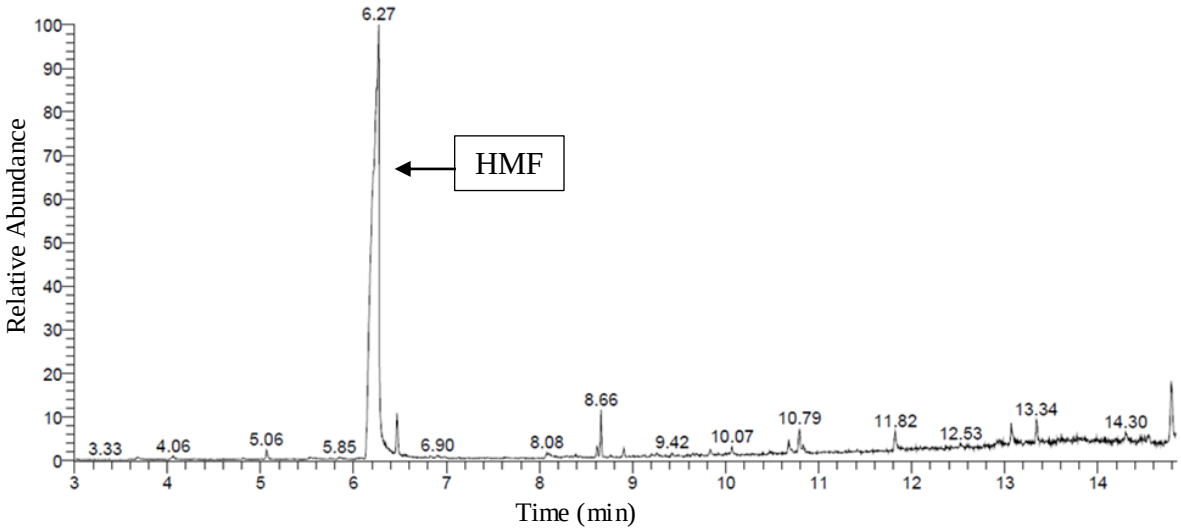


Figure 29. GC chromatogram of the reaction mixture obtained from the transformation of the dry MeOH extract of switchgrass to HMF using a 3 wt% catalyst loading in [EMIM]Cl after a 30 min reaction time.

HMF production using the dried 0.5 M H_2SO_4 plant extracts for comfrey and switchgrass was studied (Table 21). A 3 wt% catalyst loading for both $CrCl_3 \cdot 6H_2O$ and $CuCl_2$ was used in either [BMIM]Cl (entries 1 and 2) or [EMIM]Cl (entries 3 and 4) with a dissolution step of 30 min at 120°C followed by a catalytic step of 30 min at 140°C. Substrate loading was always 10 wt% of the reaction mixture. In [BMIM]Cl, 100% of the acid extracts were soluble for both comfrey and switchgrass (entries 1 and 2), while 92% (entry 3) and 88% (entry 4) of the extract for comfrey and switchgrass, respectively, were dissolved in [EMIM]Cl. Poor yields (≤2.31%) of HMF were obtained using all reaction conditions used to transform the acid extracts (entries 1 to 4). The highest yield for the transformation of the dry comfrey H_2SO_4 extract was 2.31% in [EMIM]Cl (entry 3), and the highest yield for the transformation of the dry switchgrass H_2SO_4 extract was 1.14% in [BMIM]Cl (entry 2).

Table 21. HMF production from the dry 0.5 M H_2SO_4 extracts for comfrey and switchgrass. Substrate loading was 10 wt% of the reaction mixture.

Entry	Dry 0.5 M H_2SO_4 Extract	Solvent	Catalyst 1	Catalyst 2	Dissolution step (min, °C)	Catalytic step (min, °C)	% dissolved biomass	HMF by GC (mg/g of extract)	Yield of HMF (%)
1	Comfrey	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	100	0.534	1.74
2	Switchgrass	[BMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	100	0.592	1.14
3	Comfrey	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	92	0.709	2.31
4	Switchgrass	[EMIM]Cl	$CrCl_3 \bullet 6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	30, 120	30, 140	88	D[a]	D[a]

[a] D: detected (<1% yield of HMF).

4. Discussion

The goals of this research included developing methods to extract sugars from lignocellulosic materials using different types of treatments, including acid and base treatments, and solvent extraction. These sugar extracts, as well as the biomass, were then used to produce HMF, a precursor to the biofuel DMF. In this section, the results will be discussed and compared to the literature. Each treatment, as well as the production of HMF from those treatments, will be discussed.

4.1. HMF Production using Glucose

Our reaction system, including the type of catalyst and solvents, and the reaction time, was tested using glucose as the substrate to assess the protocol's suitability for conversion to HMF. The conditions chosen for our reactions were similar to reactions found in the literature for conversion of glucose or cellulose to HMF [89, 90]. Table 22 compares the results we obtained from our experiments with results from the literature which used conditions similar to our reactions.

Table 22. Comparison for the production of HMF from glucose to the literature.

Entry	Substrate	Solvent	Catalyst 1	Catalyst 2	T (°C)	Time (min)	Yield (%)	Reference
1	Cellulose	[EMIM]Cl	$CrCl_3$ (3 wt%)	$CuCl_2$ (3 wt%)	140	10	37.7	[90]
2	Glucose	[BMIM]Cl	$CrCl_3$•$6H_2O$ (25 mol%)	-	120	120	65.0	[89]
3	Glucose	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	140	30	50.0	Our research
4	Glucose	[EMIM]Cl	$CrCl_3$•$6H_2O$ (3 mol%)	$CuCl_2$ (3 mol%)	140	30	31.0	Our research
5	Glucose	[BMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	140	30	24.5	Our research
6	Glucose	[EMIM]Cl	$CrCl_3$•$6H_2O$ (3 wt%)	$CuCl_2$ (3 wt%)	140	30	24.4	Our research
7	Glucose	[BMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	140	30	48.7	Our research
8	Glucose	[EMIM]Cl	$CrCl_2$ (3 mol%)	$CuCl_2$ (3 mol%)	140	30	31.0	Our research

Although Hussein *et al*. (2013) used cellulose as a substrate, the conditions used for production of HMF are the closest to the conditions used in our experiments [90]. Hussein *et al*. (2013) obtained a yield of 37.7% of HMF from cellulose (entry 1) [90]. Here, we can expect a higher yield when using glucose due to the simplicity of the substrate. In this case, using similar conditions as Hussein *et al*. (2013), but with a reaction time of 30 min to match the reaction time for the conversion of untreated biomass, we have obtained a 50.0% yield in the ionic liquid [BMIM]Cl (entry 3), and 31.0% in the ionic liquid [EMIM]Cl (entry 4). The highest yield of HMF of 50.0% obtained using [BMIM]Cl with 3 mol% catalyst loading of $CrCl_3 \bullet 6H_2O$ and $CuCl_2$ (entry 3) is comparable to the literature where yields of HMF produced from simple feedstocks (which includes glucose and fructose) varies from around 50%, to nearly 100% [55, 75, 91, 92, 95, 97-101]. Our results are comparable to the literature, and suggest [BMIM]Cl is a more suitable solvent for conversion of glucose to HMF.

The replacement of $CrCl_3 \bullet 6H_2O$ by $CrCl_2$ (entries 7 and 8) was studied because H_2O can reduce the solubility of the biomass in the ionic liquid, reducing the conversion of the substrate to HMF [56, 64, 68, 85]. Although the system can tolerate H_2O concentrations up to 1% of the reaction mixture, restricting H_2O when possible is important since H_2O is also produced in the reaction by dehydration of fructose [76, 87]. Here, the use of $CrCl_2$ instead of $CrCl_3 \bullet 6H_2O$ did not significantly change the yield of HMF produced from the reaction (entries 7 and 8). Both catalysts are therefore adequate for production of HMF.

Using a catalyst loading of 3 wt% (entries 5 and 6) instead of 3 mol% (entries 3 and 4) based on the amount of substrate decreased the yield of HMF from 50.0% to 24.5% in [BMIM]Cl (entries 3 and 5) and from 31.0% to 24.4% in [EMIM]Cl (entries 4 and 6). This seems to indicate a decrease in the yield even though the total catalyst loading is very similar

for both mol% and wt% catalyst loading. For glucose, 3 mol% loading of each catalyst was equivalent to 45 mg of $CrCl_3 \cdot 6H_2O$ and 22 mg of $CuCl_2$ (total=67 mg) per g of glucose. For a 3 wt% loading, 30 mg of $CrCl_3 \cdot 6H_2O$ and 30 mg of $CuCl_2$ (total=60 mg) were used per g of glucose. Therefore, the reaction system is sensitive to the amount of each catalyst used, even if the total amount of the catalysts is very close for each. This is relevant when it comes to the catalyst loading for treated and untreated biomass. A large portion of the biomass is not made of hydrolysable sugars, the mol% loading will be much smaller than the wt% loading when using lignocellulosic biomass. The catalyst loading can be estimated from the amount of sugars present in the extract being used. For example, when using the MeOH extract as a substrate for HMF production, we can refer to the amount of sugars in the dry extract. For comfrey, 300 mg of total sugars is found per g of dry MeOH extract, 3 mol% is therefore equivalent to 13.3 mg of $CrCl_3 \cdot 6H_2O$ and 6.7 mg of $CuCl_2$ (total=20.0 mg) per g of dry extract (based on glucose units; refer to Table 16 for all catalyst loadings based on mol%). In comparison, when using 3 wt% loading based on the amount of dry MeOH extract used, 30 mg of $CrCl_3 \cdot 6H_2O$ and 30 mg of $CuCl_2$ are used (total=60 mg). Therefore, the total amount of catalyst being used varies much more between 3 mol% and 3 wt% for biomass compared to the transformation of glucose. This means that transformation of glucose to HMF may not be directly compared to biomass transformation due to the differences in catalyst loading, and wt% may be more advantageous to use as a catalyst loading for biomass even though yields were higher using a 3 mol% loading for glucose. This observation is supported in research literature as catalyst loading with glucose is usually calculated using mol%, whereas catalyst loading for cellulose and biomass is usually calculated using wt% (Tables 1 and 2), although methodology still varies between the two [55, 75, 86, 90-92, 94, 98, 99, 101].

4.2. Soluble Sugars in Comfrey and Switchgrass

Comfrey is composed of 22.4±0.2% of cellulose, 9.6±0.7% of hemicellulose, 6.9±1.1% of lignin, 14.2±0.3% of ash and 46.9±0.5% of other components including soluble sugars, organic acids, proteins and lipids (Figure 13) [30]. Switchgrass, on the other hand, is composed of 40.1±1.7% of cellulose, 30.3±0.9% of hemicellulose, 7.2±0.3% of lignin, 5.5±0.4% of ash and 17.0±2.0% of other components including soluble sugars, organic acids, proteins and lipids (Figure 13) [30]. In both cases, the majority of the biomass is therefore composed of cellulose, hemicellulose, and lignin, meaning that most sugars will neither be soluble nor extractable using common organic solvents. Using the soluble fraction of sugars will eliminate the need for a cellulose breakdown step before the conversion to HMF. Furthermore, simple sugars (such as glucose and xylose commonly found in plants) have been shown to be more easily converted to HMF, coincidentally with higher yields [55, 75, 86, 91, 92, 95, 97-101]. Therefore, there are advantages in trying to convert simple soluble sugars found in the plant, even if those sugars only make up a small portion of the material. Here, the MeOH extraction was used in order to extract the soluble sugars of the plants.

4.2.1. Total Sugars and Reducing Sugars in the MeOH Extracts of Comfrey and Switchgrass

As expected, only a small portion of the biomass could be dissolved in MeOH. For comfrey, 19.6±11.1% of the biomass was dissolved compared to 17.7±2.3% for switchgrass. These numbers reflect the amount of cellulose, hemicellulose and lignin contained in each plant, which are not soluble in the MeOH. These numbers are also reflected in the proportion of sugars obtained in comparison to the used DW. For comfrey, 47.0±13.1 mg of total sugars/g of DW was reported, compared to 34.7±4.8 mg of total sugars/g of DW for

switchgrass (Table 9, Table 10, and Figure 18). When converted into a percentage, only 4.70±1.31% of comfrey is composed of soluble sugars (Table 9), and 3.47±0.48% of switchgrass is made of soluble sugars (Table 10). This is problematic on the industrial scale, since wasting over 80% of the biomass would not be tenable if there was no alternative ways to recover and use the materials. However, the MeOH extraction did produce an extract which was high in sugars, even though only a small fraction of the biomass was actually dissolved in the solvent. The extracts contained 300±60 mg of total sugars/g of dry extract for comfrey (Table 9), and 202±16 mg of total sugars/g of dry extract for switchgrass (Table 10). These extracts therefore contain 30.0±6.0% and 20.2±1.6% of sugars, respectively (Tables 9 and 10). Whether or not we can convert those sugars to HMF will give important information concerning to suitability of our feedstocks for production of HMF, and whether or not our feedstock may be used commercially in the future for bioenergy production. These results coincide with those reported by Godin *et al*. (2010), with switchgrass having a smaller soluble sugar fraction than comfrey [30].

In the case of comfrey, the amount of reducing sugars was the same as the amount of total sugars (Table 9), indicating that all sugars in the MeOH extract for comfrey were reducing. In the case of switchgrass, the reducing sugar content was lower than the total sugar content, with the extract having 91.9±1.6 mg of reducing sugars/g of dry extract compared to the 202±16 mg of total sugars/g of dry extract (Table 10). Therefore, over half of the sugars obtained from the MeOH extract of switchgrass are non-reducing. These results indicate the suitability of the extract for fermentation in future studies, as yeast-based fermentation requires reducing sugars for conversion into biofuel products [127].

4.2.2. HMF Production from the MeOH Extracts of Comfrey and Switchgrass

For the production of HMF from the MeOH extract, the catalyst loading of 3 wt% produced a higher amount of HMF (6.04% yield for comfrey under our best conditions, entry 1, Table 20) compared to the catalyst loading of 3 mol% (<1% HMF yield for comfrey using optimum conditions, entry 3, Table 20), which can be attributed to the fact that a higher amount of catalyst was used when loading at 3 wt% (Table 20). These results were the reverse of the results obtained from the conversion of glucose to HMF, where the 3 mol% catalyst loading gave better results.

Studying both comfrey and switchgrass, we have uncovered that the ideal reaction time and the ideal solvent were dependent of the biomass type. For comfrey, the highest yield of HMF of 6.04% was obtained after a 15 min catalytic step in [BMIM]Cl (entry 1, Table 20), whereas, for switchgrass, the highest yield of 18.0% was obtained after a 30 min catalytic step in [EMIM]Cl (entry 9, Table 20). In the case of comfrey, using [EMIM]Cl, the production of HMF decreased, which was the opposite result for switchgrass when using [BMIM]Cl. The dissolution of the MeOH extract did not seem to be affected by the ionic liquid used for either plant species (Table 20). The conditions used for production of HMF therefore differed depending on the nature of the feedstock. Using $CrCl_2$ instead of $CrCl_3 \cdot 6H_2O$ (entries 2 and 10, Table 20) also did seem to have a slight effect on actual production of HMF, with slightly reduced yields under the optimum conditions (6.04% to 4.93% for the comfrey extract, and 18.0% to 14.1% for the switchgrass extract), although the differences might not be significant.

Comparison in the formation of HMF from our glucose controls to the formation of HMF from the biomass is difficult due to the differences in optimum reaction conditions. In comparison to the reactions using glucose as a feedstock, the best conditions for production

of HMF from glucose in [BMIM]Cl (entry 3, Table 22) gave a yield of 50.0% of HMF, while in [EMIM]Cl (entry 4, Table 22), the yield was 31.0%. These numbers are much higher than the best yields obtained from the MeOH extract of comfrey (6.04%) and the MeOH extract of switchgrass (18.0%). However, the highest yields for glucose were obtained using a catalyst loading of 3 mol%. When compared to a 3 wt% catalyst loading, the yield of HMF from glucose were 24.5% and 24.4% in [BMIM]Cl and [EMIM]Cl, respectively (entries 5 and 6, Table 22). Therefore, using [EMIM]Cl, the highest yield of HMF for the MeOH extract of switchgrass of 18.0% (entry 9, Table 20) was approaching the yield of HMF from glucose obtained under the same conditions with a 3 wt% catalyst loading and a reaction time of 30 min at 140°C (entry 6, Table 22). The maximal yield obtained from switchgrass or comfrey is not expected to surpass the yields obtained from glucose due to the complexity of the plant material which contains more complex sugars compared to glucose.

4.2.3. Comparison to the Literature

In the literature, conversion of glucose and fructose to HMF usually range from approximately 50 to nearly 100%, although differences in reaction conditions make the absolute comparison of the results difficult [55, 75, 86, 91, 92, 95, 97-101].

Comparison to the literature for the conversion of comfrey and switchgrass to HMF is difficult since, to our knowledge, no plant MeOH extract has been tested for production of HMF. Furthermore, comfrey has not been used as a substrate, and production of HMF from switchgrass was reported in only two studies. The 5-hydroxymethylfurfural was produced from switchgrass in a H_2O/THF mixture with $AlCl_3 \cdot 6H_2O$ with a yield of HMF of 21% [104]. In a mechanistic study, HMF was produced in a yield of 4.5% from switchgrass when placed in a solution of 1% H_2SO_4 for 2 min [105]. The conditions used in those studies are widely

different than the conditions used in the current research, making comparison difficult. In addition, in the first study the yield reported was based on the hexose content of switchgrass, while in the second study the yield reported was based on the glucose content [104, 105]. The current study reports yield based on total sugar content in the dry MeOH extract. The best yield of 18.0% in [EMIM]Cl with 3 wt% loading of $CuCl_2$ and $CrCl_3 \cdot 6H_2O$ after a 30 min dissolution at 120°C and a catalytic step of 30 min at 140°C rivals the findings in the literature (entry 9, Table 20). Furthermore, to the best of our knowledge, this study is the first to show that HMF can be produced from switchgrass using ionic liquids and metal halide catalysis, an observation also true for the conversion of comfrey. The best yield produced from comfrey is modest (6.04%), but is the first confirmed amount of HMF produced from this feedstock (entry 1, Table 20).

4.3. Acid and Base Treated Comfrey and Switchgrass

4.3.1. Total Sugars and Reducing Sugars in the Acid Extracts of Comfrey and Switchgrass

Different treatments were tested in an attempt to hydrolyse cellulose and hemicellulose in the studied plants. Comfrey contains 22.4±0.2% of cellulose, and 9.6± 0.7% of hemicellulose, while switchgrass contains 40.1±1.7% of cellulose, and 30.3± 0.9% of hemicellulose (Figure 13) [30]. Therefore, extraction treatments are expected to yield higher amounts of hydrolysable sugars from switchgrass than from comfrey.

A series of tests were performed on comfrey to find the best conditions for hydrolysis. The 0.5 M H_2SO_4 treatment produced more sugars than the 0.1 M H_2SO_4 treatment, while incubation of the sample before the autoclave did not affect the yields (section 3.1.3). Thus, the 0.5 M H_2SO_4 treatment without incubation prior to autoclave was used for the switchgrass

sample experiments. A large amount of biomass was hydrolysed by 0.5 M H_2SO_4 for both comfrey and switchgrass. In the case of comfrey, 57.2±3.2% of the biomass was dissolved using the treatment. The high percentage of hydrolysis is reflected in the higher amount of sugars per g of DW obtained from the H_2SO_4 treatment compared to the MeOH extract, with 130±18 mg of total sugars/g of DW, representing an extraction rate of 13.0±1.8% for the sugars from the DW (Table 11). These results are higher than the soluble sugars extracted by the MeOH for comfrey, amounting for 4.70±1.31% of sugars in the extract (Table 9). Therefore, the acid treatment is more suitable for the utilisation of a larger amount of the sugars found in the plant. Compared to the cellulose content for comfrey (22.4±0.2%), a little over half of the cellulose is broken down into simple sugars using this treatment, not including the hemicellulose portion [30]. For the sugars in the hydrolysed fraction only, results only account for 230±29 mg of total sugars/g of hydrolysed biomass (Table 11), an extract that is less concentrated in sugars than the MeOH extract which contained 300±60 mg of total sugars/g of extracted biomass (or dry extract) (Table 9). This observation is somewhat misleading, because, although there appear to be less sugars in the acid extract which may be converted to HMF, more sugars in total from the plant material are utilized as a larger amount of the biomass is dissolved in the acid. This result means that a larger quantity of the extract is obtained per DW used. Use of an acid treatment may therefore be more viable on an industrial scale with access to a portion of both the cellulose and the hemicellulose fraction of the plant biomass for conversion to HMF.

The use of three consecutive acid treatments on comfrey did not significantly increase the amount of sugars obtained per DW of biomass (Table 13). Therefore one 0.5 M H_2SO_4

treatment is sufficient to obtain the sugars hydrolysable under acid conditions. Different treatments may be required to obtain a more significant portion of sugars.

In the case of comfrey, the amount of total sugars and the amount of reducing sugars obtained were the same, meaning that all extractable sugars were reducing (Table 11).

The results of this study are in agreement with the literature, with treatment of switchgrass yielding more sugars than comfrey (Figure 19). In this case, the dissolution of the biomass using the 0.5 M H_2SO_4 treatment was 44.2±7.2%, a result slightly lower than with comfrey for the same treatment. This dissolution result is much higher than the dissolution result for switchgrass in MeOH, as reflected in the sugars obtained being 189±3.7 mg of total sugars/g of DW (Table 12), equivalent to nearly half of the 40.1±1.7% of cellulose found in switchgrass [30]. For switchgrass, the amount of sugars found in the extract was 425±13 mg of total sugars/g of hydrolysed biomass (Table 12), higher than the 202±16 mg of total sugars/g of extracted biomass for the MeOH extract of switchgrass (Table 10). Thus, the H_2SO_4 extract was therefore the most concentrated in sugars for switchgrass, and utilized a larger portion of the biomass compared to the MeOH extract. Once again, reducing sugar amounts were also equal to the total amount of sugars (Table 12).

4.3.2. Total Sugars and Reducing Sugars in the Base Extract and the Combination of Treatments for Comfrey

The base treatment was found to be a poor choice of treatment for comfrey sugar extraction. Little of the biomass was dissolved in the NaOH (19.4±1.0%), and only 2.08±1.2 mg of total sugars/g of DW were recovered using this treatment (section 3.1.4.), approximately half of that obtained from the MeOH extract, which contained soluble sugars only. Therefore, not only does the NaOH treatment hydrolyse very little cellulose or hemicellulose, it also

potentially degrades soluble sugars. The treatment conditions are possibly too harsh, accounting for the low quantity of sugars obtained after treatment. Even the extract itself contains a mere 5.95±0.4 mg of total sugars/g of hydrolysed biomass, the lowest reported yield in this study (section 3.1.4). Due to the low amount of sugars obtained from the NaOH treatment, the extract was not further tested for conversion to HMF, since both the MeOH extract and the H_2SO_4 extracts had a better potential for production of HMF.

The combination of treatments was also deemed non-viable for production of HMF, since the same amount of sugars was obtained after three treatments (MeOH, H_2SO_4, and NaOH) compared to a single H_2SO_4 treatment, even though a slightly larger amount of the biomass could be dissolved (Table 13, Figure 20). Therefore, this research suggest the use of more costly and potentially corrosive materials produce the same amount of sugars which can be obtained after a single H_2SO_4 treatment.

4.3.3. HMF Production from the Acid Extracts of Comfrey and Switchgrass

Using the 0.5 M H_2SO_4 plant extracts, HMF was produced. The 3 wt% catalyst loading of $CrCl_3 \cdot 6H_2O$ and $CuCl_2$ was chosen as these conditions produced the highest amount of HMF when using the MeOH extract (entries 1 and 9, Table 20). In this case, most of the dry extract was dissolved in [EMIM]Cl (88% of switchgrass and 92% of comfrey, entries 3 and 4, Table 21), while all of the biomass was dissolved in [BMIM]Cl (entries 1 and 2, Table 21). Dissolution in ionic liquid was therefore superior for the dry acid extract compared to the dry MeOH extract. Lower yields of HMF were produced. Comfrey yielded 1.74% of HMF in [BMIM]Cl (entry 1, Table 21) and 2.31% of HMF in [EMIM]Cl (entry 3, Table 21), while switchgrass yielded 1.14% of HMF in [EMIM]Cl (entry 4, Table 21) and

<1% of HMF in [BMIM]Cl (entry 2, Table 21). The 5-hydroxymethylfurfural conversion was therefore more difficult using the H_2SO_4 extract compared to the MeOH extract, with little difference between the two ionic liquids used. The sugars obtained in the H_2SO_4 extract are possibly more complex and, therefore, are harder to convert to HMF compared to the soluble sugars found in the MeOH extract. Furthermore, prior to the reaction, the acid extracts were neutralized using NaOH, with a large quantity of Na_2SO_4 being produced as a by-product. Salts such as LiCl are sometimes used in reactions to produce HMF, but few studies have focused on utilizing other salts in the reactions [86, 93, 97]. To our knowledge, the effect of Na_2SO_4 on the reaction has not been studied. Thus the effect of the salt on the reaction to potentially decrease to amount of HMF produced is not known.

4.3.4. Comparison to the literature

No studies have documented the production of HMF from 0.5 M H_2SO_4 extracts of switchgrass or comfrey. Therefore, although yields are low, this study has been able to confirm that these extracts could potentially be useful in producing HMF compared to untreated biomass. However, a few studies have used 0.5 M HCl extracts to produce HMF in a [OMIM]Cl/EtOAc mixture using girasol tubers, potato tubers, acorns, and chicory roots as feedstocks (Table 2) [108-110]. In those studies, yields varied from 50.9% for the chicory root, to 58.7% for the acorn [109-110]. Although yields are high, those feedstocks are primarily composed of inulin, starch, and/or simple sugars, in contrast to our lignocellulosic material composed of primarily of cellulose and hemicellulose. For example, the girasol tuber contains 55.9% of inulin per DW, and potato tubers contain 58.4% of starch per DW [108]. The complexity of our materials therefore renders the transformation to HMF more difficult compared to the materials documented in the literature studies. Furthermore, in all studies

mentioned above, the extracts were not neutralised, nor dried, and the extracts were used directly for conversion to HMF. In some instances, a metal halide catalyst was not even required for the production of HMF [108, 110]. For the current study, the decision was made to dry the extract on the basis that H_2O restricts the reaction for production of HMF [56, 64, 68, 85]. Due to the large difference in yields between our feedstocks and the reported literature amounts, extracts which are not neutralized are possibly more suitable for conversion to HMF. This observation is supported by the use of acids as a catalyst in reactions to produce HMF [86, 100, 128]. The determination of whether the feedstocks used for the appropriateness of the reaction conditions were responsible for the low yields obtained is not possible. The best conditions found for the MeOH extracts conversion to HMF are possibly not the same as those needed for the conversion of the acid extract.

4.4. Untreated Comfrey and Switchgrass

4.4.1. Dissolution of Untreated Comfrey and Switchgrass

One of the issues related to production of HMF using complex biomass is that the biomass must first be dissolved, which is why different solvent combinations were tested in an attempt to dissolve the cellulose and hemicellulose fraction of the plant material. The simplest solvents used were the single ionic liquids: [BMIM]Cl, [EMIM]Cl, 2-methylpyridine *N*-oxide, and 3-picoline *N*-oxide. Dissolution of the biomass was fairly low, with dissolution varying from 27-53% for comfrey in ionic liquid, and 12-54% for switchgrass in ionic liquid (entries 1 to 7 and 10 to 13, Table 18). Due to the low dissolution rate, production of HMF is expected to be difficult since a large fraction of cellulose and hemicellulose are not soluble in the chosen ionic liquids for those two plants. Using a DMA-LiCl mixture with 60 wt% of

[BMIM]Cl did not increase the dissolution of comfrey or switchgrass, with dissolution remaining at 35% and 14%, respectively (entries 8 and 13, Table 18).

Dissolution was maximal when using a DMSO mixture with 10 wt% of [BMIM]Cl, with dissolution increasing to 68% for comfrey and 62% for switchgrass (entries 9 and 14, Table 18). However DMSO is a problematic solvent as extraction of HMF from the DMSO is difficult, and purification is complex. The discovery of a different solvent combination which does not utilise DMSO while maintaining the highest dissolution possible would be advantageous. The next section will discuss the need to find a better extraction method for HMF from the DMSO mixture.

Use of a different ionic liquid might also improve dissolution. For example, [EMIM]Ac has been proven to dissolve switchgrass completely after 3 h at 120°C [66]. However, this ionic liquid may not be suitable for HMF production. Further experiments need to be completed in order to find a suitable solvent to dissolve untreated biomass, while still being suitable for production of HMF.

4.4.2. HMF Production from Untreated Comfrey and Switchgrass

For all attempted reactions, the yields of HMF from untreated biomass were either estimated to be below 1%, or HMF was not present (Table 19). Different conditions similar to those reported in the literature were studied. Starting with the conditions that yielded the best results for the MeOH and the H_2SO_4 extracts, we studied the conversion of untreated biomass in [BMIM]Cl or [EMIM]Cl with 3 wt% or 3 mol% catalyst loading of $CuCl_2$ and $CrCl_3 \cdot 6H_2O$, with varying dissolution and reaction times (Table 19). [BMIM]Cl and [EMIM]Cl, as well as other ionic liquids, were studied since we had previously shown with the MeOH extracts that the most suitable ionic liquids for production of HMF will be

dependent on the type of biomass used (Table 20). The same can be said about the comparison between a catalyst loading in wt% compared to a loading in mol%, which has previously influenced the yield of HMF produced (Tables 20 and 22). In all cases, HMF was either not detected, or it was detectable but in low concentrations (<1% yield) (Table 19). Hussein *et al*. (2013) used similar conditions to produce HMF from cellulose in a yield of 37.7% in [EMIM]Cl with a 3 wt% catalyst loading of $CuCl_2$ and $CrCl_3$ after 10 min at 140°C (entry 1, Table 22) [90]. In this case, the yield reported is clearly much higher than in our study, althought the substrate is much simpler than the substrates used in this research. Furthermore, purified cellulose readily dissolved in [EMIM]Cl [90]. The low dissolution in the ionic liquids for comfrey and switchgrass, as well as the complexity of the feedstock, can explain the low yield. Furthermore, based on multiple studies where acids were used as a catalyst in the reaction to produce HMF [86, 94, 95, 100, 106, 108-110], we tested the addition of multiple acids to the reaction mixture, including H_2SO_4, HCl, CH_3COOH, and TFA, but yields remained under 1% (Table 19).

We were not able to detect HMF after converting both untreated plants in a DMSO mixture with [BMIM]Cl (10 wt% of the reaction mixture) with 10 mol% catalyst loading of $AlCl_3$ for 9 h at 150°C (Table 19). However, as mentioned above, even if this reaction mixture offers excellent dissolution, it also provides difficulties for extracting HMF from the mixture. The results did not resolve the issue of either presence or non-extraction of HMF in the reaction mixture. Direct injection of the reaction mixture for quantification to the GC-MS was not possible. However, direct injection of the reaction mixture in an HPLC (high pressure liquid chromatography) enabled Xiao *et al*. (2014) to document a 54.9% yield of HMF from cellulose using the DMSO/[BMIM]Cl mixture with $AlCl_3$ after 9 h at 150°C [94]. If the low

yield is due to poor extraction, and not the lack of HMF in solution, a different extraction method might improve the yield of HMF quantified. Other methods studied for extraction of HMF in DMSO might prove more appropriate for our system. For example, a mixture of methyl isobutyl ketone (MIBK)/2-butanol (BuOH) (7/3 w/w) has been shown to extract 89% of the HMF in an aqueous mixture of TEACl and DMSO [100].

Finally, we tested for the conversion of untreated biomass in a DMA-LiCl/[BMIM]Cl mixture with $CrCl_2$ (10 mol%) and HCl (6 mol%) after a dissolution time of 24 h at 75°C and a reaction time of 2 h at 140°C. Once again, HMF was detected, but was below quantifiable amounts (Table 19). In comparison to the literature, cellulose was converted in a DMA-LiCl/[EMIM]Cl mixture with $CrCl_2$ (25 mol%) and HCl (6 mol%) with a reaction time of 2 h at 140°C in a yield of 54% [86]. Corn stover was also converted to HMF in a yield of 48% using similar conditions where the biomass is placed in a DMA-LiCl/[EMIM]Cl mixture with $CrCl_2$ (10 mol%) and HCl (6 mol%) (Table 2) [86]. Extraction levels cannot be directly compared with our studies due to the differences in solvents used. However, we see the same pattern where yields reported in the literature are higher, but they are also obtained from much simpler substrates compared to comfrey and switchgrass [86, 90, 92, 94, 100, 103-104, 106-110].

Conclusion and Future Research

In conclusion, we have demonstrated that sugars can be extracted from comfrey and switchgrass using different treatments, including MeOH treatment, acid hydrolysis, and base hydrolysis. Acceptable yields of extractable sugar were shown for both plants. Soluble sugars made up 47.0±13.1 mg of total sugars/g of DW of comfrey (Table 9), and 34.7±4.8 mg of total sugars/g of DW for switchgrass (Table 10). Using a 0.5 M H_2SO_4 treatment, extraction of a total of 130±18 mg of total sugars/g of DW of comfrey (Table 11), and 189±3.7 mg of total sugars/g of DW of switchgrass was possible (Table 12).

Extracts rich in sugars were produced from the MeOH extraction, with concentrations of 300±60 mg of total sugars/g of dry extract for comfrey (Table 9), and 202±16 mg of total sugars/g of dry extracts for switchgrass (Table 10). H_2SO_4 extracts contained 230±29 mg of total sugars/g of hydrolysed biomass of comfrey (Table 11), and 425±13 mg of total sugars/g of hydrolysed biomass of switchgrass (Table 12).

At the moment, the best yield of HMF was obtained using the soluble sugars found in each plant, which were extracted using the MeOH treatment. Maximum yields of HMF were 6.04% for comfrey and 18.0% for switchgrass (entries 1 and 9, Table 20). These yields remain lower than the yields obtained from transforming glucose to HMF in [BMIM]Cl (50.0%) or [EMIM]Cl (31.0%) under the best conditions (entries 3 and 4, Table 22). The yields of HMF from comfrey and switchgrass also are not comparable to the current literature data for production of HMF, which often documents yields of HMF of over 50% (Tables 1 and 2), although experiments reported in the literature often document use of simple feedstocks such as glucose, fructose, or purified cellulose [55, 75, 86, 91, 92, 94, 95, 97-101]. Even the biomass used, such as corn stover, are made up primarily of simple sugars and starch [86, 93,

103, 107-110]. As demonstrated by our results, simple sugars (contained in the MeOH extract), are more easily converted into HMF. Therefore, the exploration of biomass treatments will be important in the further development of an industry for biofuel production from complex biomass. Once again, this observation is supported by the fact that the conversion of untreated biomass to HMF offered yields below 1% (Table 19). A summary of our results is reported in Table 23.

Table 23. Summary of the results for sugar extraction, biomass dissolution, and HMF production using glucose, MeOH treated biomass, H_2SO_4 treated biomass, and untreated biomass.

Treatment	**Total sugars per DW (%)**	**Dissolved biomass (%)**	**HMF (mol%)**
Comfrey MeOH	4.70±1.31	19.6±11.1	6.04
Comfrey H_2SO_4 0.5 M	13.0±1.6	57.2±3.2	2.31
Untreated comfrey	-	≤49 in [EMIM]Cl	<1
Switchgrass MeOH	3.47±0.48	17.7±2.3	18.0
Switchgrass H_2SO_4 0.5 M	18.9±0.37	42.2±7.2	1.14
Untreated switchgrass	-	≤59 in [EMIM]Cl	<1
Glucose in [BMIM]Cl	-	100	50.0
Glucose in [EMIM]Cl	-	100	31.0

Few research groups seem able to produce HMF from substrate made primarily of cellulose and hemicellulose, and with a low content in soluble sugars. Therefore, even though yields were low, confirming that HMF can be produced from feedstocks such as comfrey and switchgrass is important for the future of the biofuel industry. This is especially true given the need to move away from using agricultural feed resources, such as corn stover, as a biofuel feedstock. Furthermore, even if HMF can be produced in high yields using glucose and fructose, and even cellulose, these feedstocks cannot be used on the industrial scale. Therefore

obtaining a small yield using complex biomass is a critical early step in building the industry of biofuel production using other molecules than EtOH.

At this point, future studies should focus on improving HMF yields from complex feedstocks. Improving dissolution of the material must also be considered, since current dissolution was rarely above 50% of the total available biomass. Analysis of the composition of the recovered plant biomass could prove useful in determining the quantity of remaining sugars in the plant biomass after dissolution in ionic liquids. The use of different solvents, co-solvents and catalysts must be explored in future work to find the most suitable condition for the formation of HMF from treated or untreated feedstock.

However, we may have reached the comfrey and switchgrass limits for the production of HMF. Therefore, exploration of other feedstocks may be studied for the production of HMF. For the industry in Northern Ontario, a focus should be put on using plants which produce a high amount of biomass, but which can also be grown in the Northern climate. The plant must grow in acidic soil potentially contaminated by mining and smelting activity, with bioaccumulation of metals being an added benefit for land remediation purposes. In this study, switchgrass was used to produce HMF, but different grasses might be more suitable for biofuel production depending on their composition. For example, grasses in the *Festuca* genus, commonly grown in North America, contain high amounts of sugars (34.0±1.2 % of cellulose) [30]. *Cannabis sativa* L. (hemp) has also been identified as a suitable biomass to produce biofuel, including bio-oil and biodiesel [129, 130]. Hemp has also been shown to contain high amounts of sugars, especially cellulose, which makes up 47.5±3.5% of the plant biomass [30]. Furthermore, hemp can be grown in Ontario's climate [131]. In all cases, the suitability of the plants to be grown in Sudbury and in a metal-rich soil, as well as the

suitability of the plant for HMF production, would have to be studied prior to building a local industry for biofuel production in Northern Ontario.

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