

# Pre-treatments to enhance the enzymatic saccharification of lignocellulose: technological and economic aspects



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## ABSTRACT

Lignocellulose is rich in cellulose, the world's most abundant glucose biopolymer. There is much interest in exploiting lignocellulose as a potential source of low-cost sugars which could be used for producing second generation (2G) bioethanol by industrial fermentation. To hydrolyse the cellulose enzymatically, the lignin and other protective barriers must be removed using pre-treatments which mostly involve (hydro)thermal, physical (including pressure), and chemical technologies, either individually or in combination. The aim of this report has been to review the literature on the wide range of pretreatment technologies in relation to their functionalities and efficacies and relative economic benefits as indicated from published techno-economic assessments (TEAs).

Comparative TEAs suggest that the specific savings associated with any one pre-treatment are generally offset by increased costs in other parts of the process chain. Hence, the estimated cost of ethanol production (minimum ethanol selling price – MESP) within a single comparative study is often similar for different pre-treatment scenarios. It is also evident from very many TEAs that the most important and sensitive cost components for ethanol production are frequently identified as the feedstock price and the cost of enzymes. Other important criteria include the global price of oil and the impacts of the policy environment. Several pilot and commercial scale second generation facilities have been built and operated over the last 10 years. It would be useful to compare the conclusions above with the findings made in the real-world operating environment.

For the future, it will be appropriate to continue research to explore the potential of novel pre-treatments and processing scenarios, in conjunction with optimising the exploitation of co-products, whilst using TEAs to guide strategy. Although several emerging technologies, particularly those involved in solvation of lignocellulose, are scientifically exciting, it is likely to be challenging to identify any single pre-treatment technology that can sufficiently enhance economic viability of the biorefining process for biofuel production, particularly whilst competing with extremely low petroleum prices. Therefore, other primary products such as more valuable platform chemicals may be considered, even though this will create other biorefining challenges.

## EXECUTIVE SUMMARY

Lignocellulose, which is made up of structural plant cell walls, may be obtained from crop residues and wood. It is rich in polysaccharides including cellulose, which is the world's most abundant biopolymer consisting of long chains of glucose, many of which are aligned in crystalline arrays. There is much interest in liberating lignocellulosic glucose and other sugars to provide low-cost, renewable substrates for the production of bioethanol by industrial fermentation. The use of these non-food sugars is loosely described as "Second Generation" or "2G" biorefining and the biofuels produced are thus referred to as 2G biofuels. To hydrolyse the cellulose enzymatically, the lignin and other protective barriers must be removed. This may be achieved using pre-treatments which mostly involve (hydro)thermal, physical (including pressure), and chemical technologies, either individually or in combination. The pre-treatments help to break down the structural barriers within the lignocellulose, permitting exogenously applied cell wall degrading enzymes to hydrolyse the cellulose and any accompanying polysaccharides. However, pre-treatments differ considerably in their efficiencies and costs, both operational and capital. The aim of this report has been to review the literature for the wide range of pretreatment technologies that are either established or in development, in relation to their functionalities and effectiveness. The report, through assessment of published techno-economic assessments (TEAs) of model processes, compares the estimated relative economic benefits of key pretreatments at industrial scale.

From the limited number of TEAs that have compared different pre-treatment processes, it is reasonable to conclude that the specific savings associated with any one pre-treatment are generally offset by increased costs in other parts of the process chain. Hence, the estimated cost of ethanol production (minimum ethanol selling price – MESP) is often similar. If one pre-treatment could be identified as the most economically beneficial, it is probably the well-established hot, dilute acid (HDA) process, but not as a clear winner. Probably of greater

significance, though, is the observation that many TEAs identify feedstock price and the cost of enzymes as the most important and sensitive cost components for ethanol production. Other important criteria include the global price of oil and the impacts of the policy environment. Several pilot and commercial scale second generation facilities have been built and operated over the last 10 years. It would be useful to compare the conclusions above with the findings made in the real-world operating environment.

For the future, research should continue to explore the potential of novel pre-treatments and processing scenarios, in conjunction with optimising the exploitation of co-products, whilst bearing in mind the above conclusions. The complexity and integrated nature of the biorefining process and the variability and heterogeneity of feedstocks indicate that it will be challenging to identify any single pre-treatment technology that can sufficiently enhance economic viability of the biorefining process for biofuel production, particularly whilst competing with extremely low petroleum prices. Therefore, other primary products such as more valuable platform chemicals may be considered, although these may result in other biorefining challenges that will have to be addressed.

## 1.0 Introduction

For tens of thousands of years, mankind has exploited biomass as a source of food, fuel and materials. During the last century, modern science and technology has facilitated the development of sophisticated biorefining technologies to convert biomass to renewable fuels and chemicals. Examples include large-scale anaerobic digestion of agri-food chain waste streams of plant and animal origin to produce biogas (methane), and the industrial manufacture of bioethanol through the fermentation of sugars from starch or sucrose, derived from food or animal feed crops. The latter example is generally known as “First Generation”, or 1G biofuel production and employs methods and technologies similar to those used in the brewing and distillery industries. Platform chemicals such as citric acid and lactic acid are also important emerging bio-based products of industrial fermentation (Khoo et al., 2016). 1G bioethanol production has, in part, been promoted in response to the mounting challenge to further reduce the use of petrochemical fossil products. However, there is a growing concern that its prolific use of starch and other food crops may impact directly on food production. Therefore, substantial research has also been carried out to exploit sugars derived from lignocellulose, the major structural component of the inedible parts of crops and woody biomass.

Lignocellulose is an important biorefinery feedstock because it is rich in polysaccharides such as cellulose, the world’s most abundant biopolymer (Coughlan, 1990), consisting of long chains of glucose (see Section 1.2). There is much interest in liberating lignocellulosic sugars to provide cheaper, renewable substrates for industrial fermentation. This approach, in which non-food sugars might be exploited, is loosely described as “Second Generation” or “2G” biorefining [(Naik et al., 2010) and references therein]. The biofuels produced are thus referred to as 2G biofuels. 2G biorefining has the potential to add value to under-exploited lignocellulosic wastes and co-products from agri-food production and forestry, and to encourage the production of lignocellulose-rich “energy crops” thereby enhancing agricultural wealth creation. However, in addition to the highly sought glucose, lignocellulose

is rich in lignin and non-cellulosic cell wall polysaccharides, especially the hemicelluloses (see Section 1.2) which protect the cellulose from enzymatic degradation. To hydrolyse the cellulose, particularly with the use of enzymes, the lignin and other barriers must be removed. To tackle this problem, a wide range of pre-treatments have been developed. These mostly involve (hydro)thermal, physical (including pressure), and chemical techniques, either individually or in combination. Pretreatments are used to break recalcitrant lignin and other cell wall chemical bonds thereby permitting fractionation of cell wall components and allowing the enzymatic hydrolysis of cellulose and non-cellulosic polysaccharides. The glucose and other monosaccharides produced may then be fermented using yeast, fungi, or bacteria.

Although straightforward in principle, different pretreatments have very different effects on the cell wall components and this is further complicated by the variations in composition of different lignocellulose substrates. In addition, the economic viability of 2G biorefining requires that as many as possible of the lignocellulosic components must be effectively exploited. Cellulosic glucose and hemicellulosic sugars often differ in their fermentation characteristics and the exploitation of lignin requires completely different approaches.

The aim of this report has been to survey a range of pretreatment technologies that are either established or in development, in relation to the exploitation of generally available feedstocks. The report serves to review the pretreatments and then comparatively assess (at a high level through a review of the literature) the potential economic benefits of such pretreatments at industrial scale. This information will help in understanding how to strategically exploit lignocellulosic feedstocks to produce platform chemicals and biofuels, in conjunction with the optimal use of co-products (such as lignin) from the biorefining side-streams.

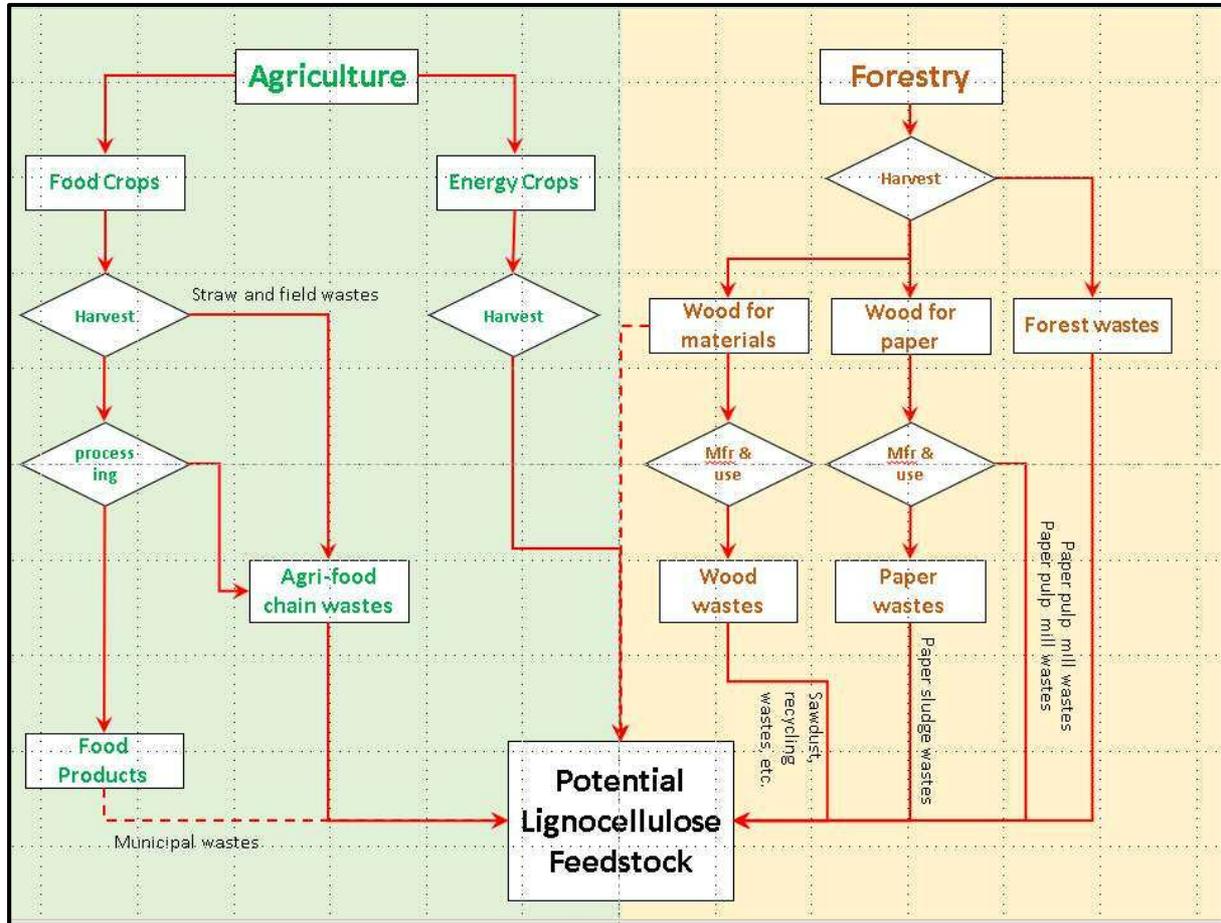
## **2.0 Lignocellulose as a biorefinery feedstock.**

### **2.1 Sources and global production of lignocellulose feedstocks**

Many studies have been carried out to assess the quantities and availabilities of various lignocellulosic biomass sources for fuel and energy production at national (Jiang et al., 2012; Scarlat et al., 2010) and global levels (Kim and Dale, 2004; Perea-Moreno et al., 2019; Tye et al., 2016; Vlosky and Smithhart, 2011). The global production of biomass has been estimated to range between 10 and 50 billion tons annually of which dry lignocellulose comprises approximately half (Claassen et al., 1999; Galbe and Zacchi, 2002; Zhao et al., 2012). Particular interest has been shown in the availability of lignocellulose for bioethanol production in order to replace 1G technology (Mabee et al., 2011), though classifications differ according to the nature and scope of the studies. Estimates have also been made on the availability of biomass sources over and above what is already used in other activities such as production of food, energy and materials (Slade et al., 2011). Others have focused on the sustainability of using lignocellulosic biomass for biorefining (Nanda et al., 2015).

There are numerous sources of lignocellulosic biomass and these can be broadly categorised into wood and non-wood (mostly agriculturally-produced) biomass (Tye et al., 2016). Sources are summarised diagrammatically in Figure 1. Some of the most attractive sources of lignocellulose for biorefining are the cellulose-rich waste streams derived from agri-food production and forestry process and supply chains. Such waste streams may also be classified into primary, secondary and tertiary wastes (Rutz and Janssen, 2008). Primary wastes arise directly from the harvesting of food crops (straw, leaves, stalks) or from wood thinning in commercial forestry. This type of waste is abundant but requires recovery which may be costly. Furthermore, its removal may denude the replenishment of soil and be environmentally deleterious in the long term. Secondary wastes are produced by food processing or from saw, paper and sugar mills. Tertiary solid waste comprises a large category of waste formed after biomass products have been utilised. This includes organic

municipal solid waste (MSW), demolition wood, sludge and paper etc (Rutz and Janssen, 2008). Further consideration of biomass sourcing is given by the European Union (EU) (European Parliament, 2018).



**Figure 1:** Schematic diagram showing sources of lignocellulose for biorefining

### 2.1.1 Wood biomass

This comprises both hardwood and softwood which is used mainly in the production of materials for the manufacture of structural products such as building materials and paper or used to produce energy by burning. Wood biomass is produced in very large quantities. Around the turn of the century, the estimated global use of round wood and wood fuel was about 3.27 Bn m<sup>3</sup> per annum (Parikka, 2004). However, whilst wood is rich in cellulose, it is denser and tougher compared with non-wood lignocellulose and for biorefining requires

greater energy for size reduction (Tye et al., 2016). Examples of wood biomass considered for biorefining include wood and wood waste streams from willow (Budsberg et al., 2012), spruce and birch (Mirahmadi et al., 2010), eucalyptus (Romaní et al., 2010), oil palm (Jeon et al., 2014). Some wood biomass may be produced in the form of short-rotation coppice (SRC) energy crops (Hinchee et al., 2009).

### *2.1.2 Non-wood lignocellulosic biomass:*

This is generally produced through agricultural activity and comprises industrially relevant native plants, or agricultural residues and agri-food chain waste streams produced during the production of food and feed (Figure 1).

Herbaceous crops include “energy crops” such as miscanthus, switchgrass and costal bermudagrass. Annual global production of each is in the order of 200-300 million tonnes (Tye et al., 2016). Other crops grown for natural non-wood fibres include cotton, hemp, flax and jute. However, apart from cotton fibre (25M tonnes pa), the quantities produced are relatively small on a global basis.

Agricultural wastes include field wastes and straw from rice, sugar beet tops, sugarcane bagasse, sorghum, corn, wheat, barley, oats, oil-seed rape and oil-palm biomass. The most abundant sources globally are sugarcane bagasse (over 1Bn tonnes pa), rice straw (over 650 M tonnes pa), wheat straw (400-500 M tonnes pa) and corn stover (over 350 M tonnes pa) (Tye et al., 2016).

Additional food processing-derived lignocellulose-rich waste streams include brewers’ spent grains, underutilised wheat bran, rice bran, shells from nuts (e.g. peanuts, almonds), corn cob waste, olive, avocado and mango stones. Whilst significant as co-product streams for local industries, their levels of production are relatively much less than primary agricultural waste streams. However, they are often geographically concentrated at the locations of the processors (e.g. sugar-cane bagasse, oil palm bunches and beet pulp) and this may be

economically advantageous for local biorefining. Non-wood lignocellulosic biomass is generally of lower density compared to wood biomass. Since many examples are low in value or are waste streams, they are attractive as lignocellulose feedstocks. However, the lower density results in higher transport costs.

### *2.1.3 Other cellulose-containing waste streams:*

Cellulose may also be obtained from waste streams derived from the paper and cardboard industries. Whilst not the focus of this review, such waste streams are attractive because the paper and card production processes (analogous to highly effective pre-treatments) involve the removal of much, if not all, of the lignin from the original wood sources. High concentrations of cellulosic ethanol may be achieved from the biorefining of such waste streams (Al-Azkawi et al., 2019; Elliston et al., 2015, 2014) which will have a significant impact on distillation energy requirements. Much cellulose-derived fibre may also be found in municipal waste, though its recovery may require extensive processing and fractionation (Meor Hussin et al., 2013).

**The potential feedstocks highlighted above are all described as lignocellulose. However, that single description belies considerable differences in chemistry, structure and physical properties. Such differences will influence the nature and economics of the processes needed to optimally liberate cellulosic glucose and other sugars, and on the types of product that will be obtained from any biorefining process.**

## 2.2 General structure and composition of lignocellulose

Like all advanced life forms, plants comprise a broad hierarchy of structures. The plant, whether it be a tree or a seedling, is made up of various organs including leaves, stems and roots. These in turn are made up of different cell types, each of which are surrounded by a plant cell wall and separated from each other by a middle lamella layer. The cell wall provides structural support to plants, as well as serving as a protective layer. The nature of the cell wall, its physical and chemical structure, its shape and form and material properties, reflect the function of the cell and its stage of maturity.

In growing plant tissues, the slightly elastic properties of the thin primary cell walls (approximately 1  $\mu\text{m}$  thick), in conjunction with the internal semi-permeable plasma membrane, can counter the osmotically driven uptake of water into the cell (Brett and Waldron, 1990). This creates turgor pressure without which the tissues will wilt. The turgor pressure drives cell expansion during which the cell walls controllably extend, thereby facilitating plant growth. Such primary cell walls are highly hydrated. A typical primary cell wall has approximately 3-6 layers cellulose microfibrils and these are embedded in a matrix of non-cellulosic polysaccharides, predominantly pectins and hemicelluloses, along with various glycoproteins and some phenolic moieties (Brett and Waldron, 1990).

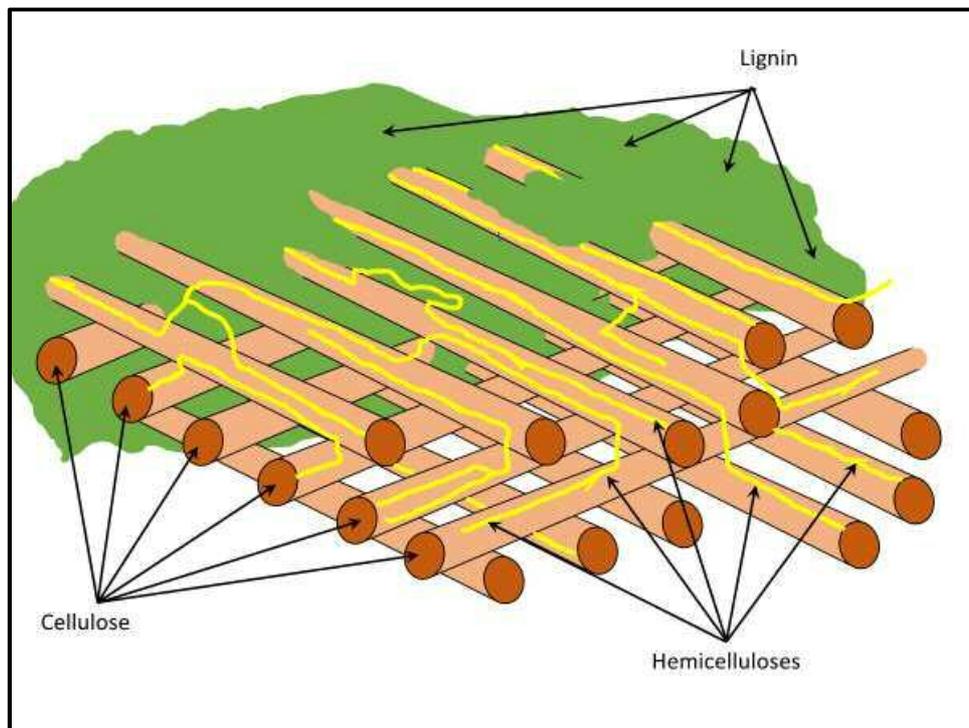
In older, more mature structural cell walls such as in mature and more woody stems, the cells may be very elongated (many cm). Each cell will have in addition to the middle lamella and primary wall, a thick internal secondary cell wall which itself can comprise sublayers (the outer S1, middle S2 and inner S3 layers (Timell, 1964)). These secondary walls are rich in cellulose and hemicelluloses and are generally highly lignified.

Cellulose is found in the form of microfibrils which comprise long parallel chains of (1-4)-linked  $\beta$ -glucose, that are often hydrogen bonded to one another. The chains can be arranged in several ways; cellulose in different regions of the microfibril can be amorphous or can occur in one of several crystalline forms (Nishiyama et al., 2003). Pectic

polysaccharides are predominantly located in the middle lamella and primary cell walls and are rich in galacturonic acid. The hemicellulosic polysaccharides are diverse in structure and comprise a range of sugars including pentoses such as L-arabinose and D-xylose, hexoses such as D-glucose, D-galactose and D-mannose and D-glucuronic acid. Some sugars, for example D-xylose, may also be acetylated. The hemicelluloses, which are considered to coat the microfibrils, are commonly branched and cross-linked both to each other and other wall components (Brett and Waldron, 1990) and their compositions vary with wall type and plant species. Lignin is an amorphous polyphenolic material and consists of highly cross-linked phenolic subunits. The principle ones are para-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) subunits and the ratios of these will differ in different types of plant. Lignified cell walls are highly rigid and impart great tensile and compressive strength to support the plant (some trees grow to several hundred feet). It is these secondary cell-walled cell-types, rich in cellulose, hemicelluloses and lignin, which make up the bulk of lignocellulosic feedstocks. The lignin component not only provides structural support, it is also highly water resistant and protects the cells from the many bacterial and fungal plant pathogens. Such pests produce cell wall degrading enzymes designed to break-down the cell wall components and dissolve a passage into the plant tissues where they can thrive and multiply. Interestingly, many industrial enzymes developed by biotechnology companies for use in biorefining to break down the cellulose and other cell wall polysaccharides are derived from such micro-organisms. The lignin serves to protect the celluloses and hemicelluloses from such enzymatic degradation.

Hence, the cell walls that comprise lignocellulose are highly complex in structure and their key components differ from cell-to-cell and from species-to-species. A schematic diagram of the main components in lignocellulose is shown in Figure 2. A typical example of lignocellulose composition is provided for corn stover (Humbird et al., 2011) in which the cell-wall sugar components make up about 65% (dwt) of which glucan contributes about 37%, and lignin about 18%. An example of variation in composition between tissues and across

varieties of a single species of feedstock lignocellulose is exemplified for wheat straw (Collins et al., 2014).



**Figure 2:** schematic diagram of lignocellulose at the cellulose microfibril scale, showing the interactions with lignin and hemicelluloses.

## 2.3 The role of pretreatments in the biorefining of lignocellulose

### 2.3.1 Basis for recalcitrance of lignocellulose

Monosaccharides may be obtained from lignocellulosic cellulose and hemicelluloses by hydrolysis. The earliest approaches involved chemical hydrolysis using hot, concentrated acids such as sulfuric or hydrochloric acids (Ruttan, 1909). Acid hydrolysis has been used for over a century in the production of wood ethanol. It was exploited from the mid 1930's in the USSR (Rabinovich, 2010) and in Germany using the Rheinau-Bergius process (Jung and Kim, 2015). However, it is generally considered to be very expensive due to the need for

acid-resistant processing facilities and the need to recover the large quantities of acids used - for example by distillation as in the case of hydrochloric acid (Bergius, 1937). The use of modern biotechnology to produce enzymes that can effectively degrade polysaccharides within a biorefinery is believed to be more environmentally and economically attractive. However, for enzymes to reach the polysaccharides, particularly cellulose, the protective barriers of lignin and hemicellulose must be overcome. The resistance to enzymolysis, i.e. the “recalcitrant” nature of lignocellulose is determined by its structure at all length scales. Pretreatments are designed to diminish or eliminate the structural and steric impediments at one or more of these different levels. The main elements of recalcitrance are as follows:

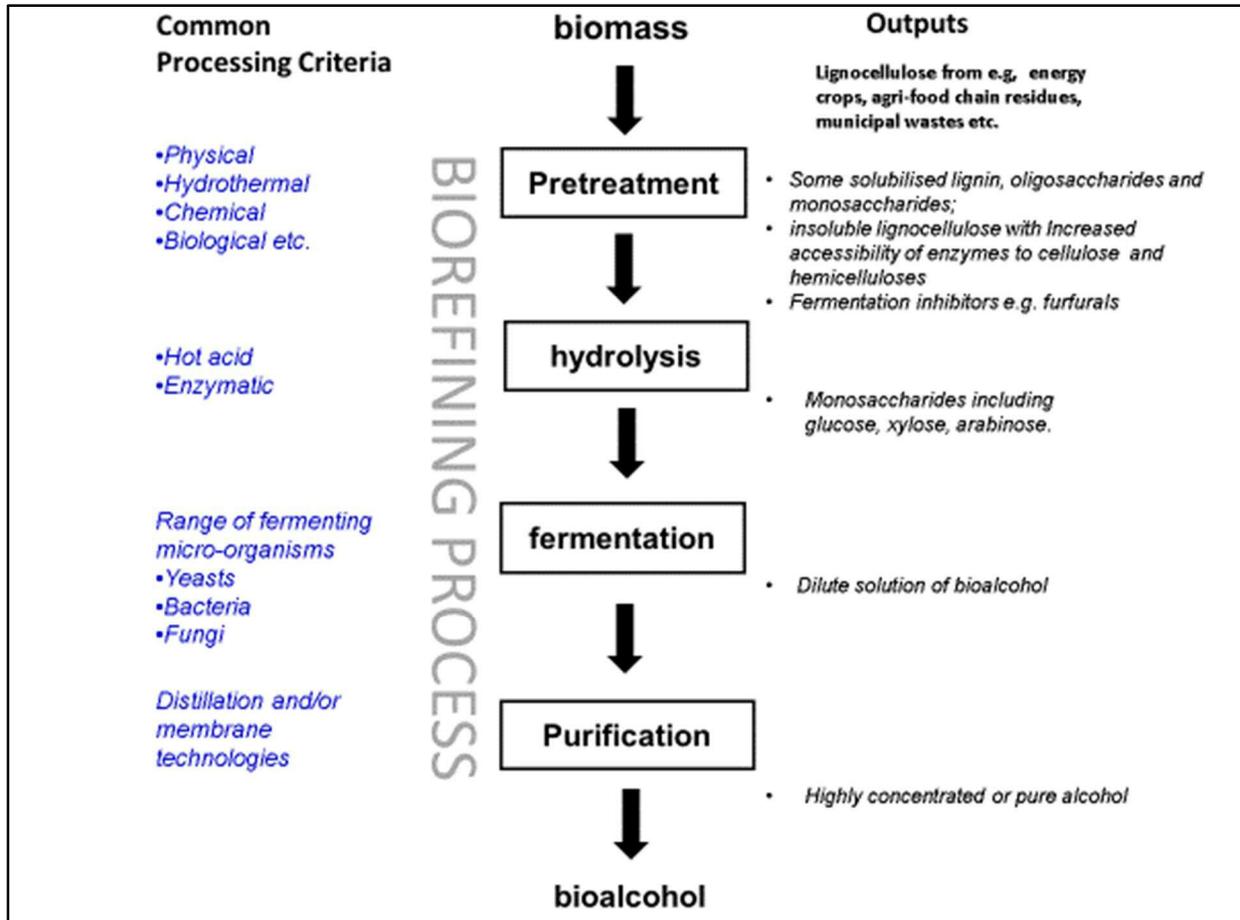
- 1) Crystallinity of cellulose: the heterogeneity in structure and particularly crystallinity is thought to provide resistance to enzymatic hydrolysis (Gross and Chu, 2010), although there appears to be considerable conflicting evidence (Grethlein, 1985; Taherzadeh and Karimi, 2008).
- 2) Hemicellulose structure: the hemicelluloses coat, surround and thereby protect the cellulose microfibril and their removal will increase accessibility of cellulose to enzymatic hydrolysis. Enzymolysis of all polysaccharides will also be enhanced by reductions in their degree of polymerisation (DP), interpolymeric cross-linking, acetylation and by their solubilisation.
- 3) Lignin: this coats cellulose and hemicellulose acting as a direct barrier; it can also irreversibly bind cellulases preventing them from reaching the cellulose (Shi et al., 2015; Yoo et al., 2020). Hence its removal will also increase efficiency of enzymolysis.
- 4) Acetyl groups: present on hemicelluloses, these can interfere with enzyme accessibility (Bhatia et al., 2020); their release increases the levels of organic acids that can inhibit enzymolysis and downstream fermentation processes.
- 5) Ester linkages: these form cross- links between hemicelluloses (as in the case of diferulic acids) and between hemicelluloses and lignin (Merali et al., 2013);

- 6) Endogenous proteins: these may have either positive or negative impacts on enzymolysis (Bhatia et al., 2020).
- 7) Other structural components: these include organic materials such as cutin, suberin, waxes, lipids etc and inorganic materials such as silica (found at significant levels in grass lignocellulose).
- 8) Porosity: the cross-linking of the cell wall components affect porosity of the cell wall network and cellulases are only able to penetrate pores wider than 5.1nm (Grethlein, 1985).
- 9) Particle size: this will affect the surface area to volume ratio and therefore the accessible surface area (ASA) available to the enzymes. Breaking up the lignocellulose will create fracture surfaces which may increase accessibility of cellulose within the lignified matrix.
- 10) Nature and composition of the enzyme cocktail: accessibility of cellulose by cellulases may be dependent on the presence of accessory enzymes that can hydrolyse hemicelluloses, acting as concurrent pretreatments.

### 2.3.2. Biorefining process overview

A simple schematic overview of the biorefining of lignocellulose to produce biofuels and biochemicals is shown in Figure 3. The main steps include the provision of lignocellulosic biomass, the pretreatment of the lignocellulose to reduce recalcitrance, hydrolysis of the component polysaccharides (cellulose and hemicelluloses), fermentation of the sugars to create new products and recovery of the products using, for example, distillation in the case of fuel alcohols. There are very many permutations possible in such a process. These should reflect the variability in lignocellulose feedstock chemistry, the range and impacts of pretreatments available augmented by the efficacy of different enzyme cocktails, and the wide range of fermenting micro-organisms and potential biochemical products. Some of the steps may be combined, for example enzymatic saccharification and fermentation may be

carried out together as “simultaneous saccharification and fermentation” (SSF). Furthermore, the process may involve separation of pre-treatment-solubilised hemicelluloses from the cellulose followed by separate hemicellulose and cellulose biorefining processes.



**Figure 3:** a simple overview of a lignocellulose - to - bioalcohol process scheme.

The final design will be a balance between the price that can be set for the final product in relation to the economic performance of the integrated unit processes along with the cost of the facility and raw (biomass) materials (see Section 4). It is important to note that by its very nature, the process is complex and highly integrated. Optimisation of, or changes to any unit process can have a considerable bearing on the efficiencies of other unit processes. For example, increasing the intensity (severity) of a pretreatment may increase the efficiency of the enzyme hydrolysis step. However, it is also likely to increase the levels of chemical

breakdown products (see below) many of which are toxic to fermenting microorganisms and will reduce the efficiency of fermentation (Field et al., 2015; Wu et al., 2017). Furthermore, reflecting the wide variation in feedstocks (Figure 1), different types of lignocellulose will respond differently to pretreatments and enzymolysis. A process design and operation that is optimised for one lignocellulosic feedstock may not work efficiently for a different feedstock. This has implications for feedstock availability and supply chain optimisation. Early 2G biorefinery designs and pilot facilities e.g. (Aden et al., 2002; Humbird et al., 2011) have been based on the use of locally available large tonnage agricultural residues such as yellow poplar, corn stover and switchgrass, (NREL), sugarcane bagasse and wheat straw (see Section 4). All these variations make the prediction of process efficiency for other feedstocks and process schemes highly challenging. However, advances in process modelling software in conjunction with techno-economic analysis (TEA) has provided a basis for strategic decision making (Section 4).

## 3.0 Types of pre-treatment

### 3.1 Size reduction

#### *3.1.1 Introduction and background*

Particle size reduction is one of the oldest technologies used in the exploitation of lignocellulose, having been used in the paper pulping industry for over a hundred years (Liao et al., 2020a). In 2G biorefinery models it is employed as a first stage to break down raw biomass into manageable particles suitable for transport (including densification), further pretreatments and movement within a biorefinery process system (Davis et al., 2013). However, much research has also been carried out to develop this approach for the pre-treatment of lignocellulose to enhance enzymatic saccharification. The approach increases the surface area-to-volume of the biomass and may, through internal fracturing, enhance porosity.

A wide range of small and large-scale milling and comminution technologies are available that include hammer mills, vibratory rod mills, compression mill, duel-roller mills, colloid mills, disk mills, freeze mills, wet and dry ball mills, tumbling, planetary and vibratory ball mills jet mills and pin mills (Mandels et al., 1974; Pew and Weyna, 1962; Silva et al., 2012) [and references therein]. Some of these, like hammer mills, generally reduce particles to the centimetre or sub-millimetre scales. Other, more intense milling technologies such as disk mills and ball mills can break down biomass further towards the 100µm level or below.

### *3.1.2 Effects of mechanical disruption on lignocellulose*

Intense mechanical disruption of lignocellulosic can increase the efficiency of enzymatic saccharification. Early studies highlighted the potential importance of reducing particle size and porosity (increasing surface area to volume) and cellulose crystallinity (Vaidya et al., 2016). As an example, Silva et al (Silva et al., 2012) subjected wheat straw to a series of sieve-based cutting and centrifugal grinding methods, reducing the particle sizes sequentially to a subcellular 50µm. Further disruption using ball milling and jet milling reduced particles to less than 10µm through disruption of cell wall structure. The decrease in particle size was accompanied by an increase in saccharification efficiency and the authors reported up to 72% glucose yields. They also highlighted the decrease in cellulose crystallinity in the very fine particles and suggested a relationship with increased saccharification. Other studies on disrupting Douglas fir wood-chip lignocellulose to 30µm using a vibratory ring and puck mill (Jiang et al., 2016) resulted in the disintegration of fibres and fibre bundles and increased enzymatic saccharification of glucose to 40% of the theoretical yield. More intense milling to particle sizes of less than 30µm further increased surface area, reduced cellulose crystallinity and increased glucose yields to over 70% of the theoretical yield. Further studies on the same materials (Jiang et al., 2017a) highlighted cell wall disruption and intra-wall fracture and the importance of moisture content on fracture properties.

Whilst reducing particle size is often associated with enhanced saccharification, its effect on, and the role of, cellulose crystallinity is not completely clear. Wet and dry ball milling studies (Rivers and Emert, 1987) and comparison of different ball milling types (H. J. Kim et al., 2013) have demonstrated that milling to achieve high levels of enzymatic saccharification is not always accompanied by similar reductions in cellulose crystallinity. Such observations had led some researchers (Vaidya et al., 2016) [and references therein] to suggest that the underlying changes in particle morphology are more important than changes in crystallinity or particle size. However, recent studies on micronized wood compared cellulose hydrolysis

with a range of analytical data using principal components analysis. The results indicated that surface accessibility and porosity were the major factors positively affecting enzymolysis, and cellulose crystallinity was a negative factor (Jiang et al., 2017b).

### *3.1.3 Advantages and disadvantages*

A key advantage of size-reduction pre-treatments is that they result in little change to the chemical nature and composition of the lignocellulose. Unlike high severity thermochemical pre-treatments, milling does not extensively degrade cell wall polysaccharides and thus has the potential to permit higher yields of sugars (particularly hemicelluloses that are more prone to thermochemically-catalysed degradation). Thus, milling leads to much lower levels of sugar degradation products such as furfurals (H. J. Kim et al., 2013) that can inhibit fermentation and enzymolysis. Elimination of the need to remove inhibitors also means fewer processing steps prior to enzymatic hydrolysis and fermentation. In addition, mechanical disruption can negate the need for harsh chemicals that damage and corrode the equipment and then must be disposed of with environmental consequences. Hence, many researchers consider size reduction to be an “eco-friendly” technology. However, milling pre-treatment is acknowledged to be energy intensive, particularly the technologies such as ball milling that are used to obtain ultrafine particles. Indeed, much of the research on milling pretreatment provides data on energy consumption per Kg sugar (see below).

Nevertheless, some authors have attempted to enhance efficiency of milling pre-treatment approaches by utilising several milling units sequentially, each optimised to a specific milling size range. For example Wang et al (Wang et al., 2018) pretreated wood chips using a hammer mill to achieve particle sizes between 7810 and 700  $\mu\text{m}$ , followed by an air classifier mill (ACM) to reduce particles to less than 114 $\mu\text{m}$ . Finally, ultrafine milling made use of vibratory tube mills for wood cellulose amorphization. The results indicated an energy

consumption of about 1.5 kWh/kg and resulted in a 70% sugar yield on enzymatic saccharification.

Scalability of some milling technologies for industrial pre-treatment is often considered to be challenging. Many coarse-milling technologies such as hammer milling and knife milling are readily scalable to large industrial size and already exist. However, although much research on ball milling has highlighted its potential as a functional pretreatment for enzymatic saccharification, it is often discounted as a realistic approach because of the high energy costs involved and the challenging requirement for industrial-scale operations (Liao et al., 2020a). Nevertheless, some research has suggested that biorefining could employ relatively energy-efficient ball mills used in the cement industry for production of clinker. With some constraints, researchers have estimated that the cost of the biomass milling pre-treatment component for ethanol production could be limited to 85 euros per tonne final product, i.e. about 10% of the market price (Liao et al., 2020a; Meine et al., 2012).

#### *3.1.4 Combination with other pre-treatments*

Although one of the attractive features of milling pretreatments is the potential to avoid the use of environmentally unfriendly and corrosive chemicals, significant research has been carried out into the use of milling in combination with other pretreatment approaches. The most straightforward involve the addition of catalytic chemicals to the milling medium. For example, Shi et al evaluated the effects of removing lignin from corn straw using ozonolysis and ball milling either sequentially or in combination and found the latter produced a synergistic improvement in saccharification and release of glucose and xylose (Shi et al., 2015). They also suggested that using mechanical pretreatments in combination with other pretreatment approaches might reduce energy use. Schneider et al., evaluated the addition of a solid chemical catalyst potassium pyrosulfate ( $K_2S_2O_7$ ) which enhanced recovery of xylose and xylo-oligosaccharides from barley straw (Schneider et al., 2017). Milling has also

been assessed in advance of chemical pre-treatments. Qu et al., evaluated the impact of ball milling on wheat straw followed by extraction in mild alkali. This had the effect of removing much of the lignin and hemicelluloses and enabled enzymolysis to release up to 98% glucose (Qu et al., 2017). A combination process involving deacetylation and mechanical refining (DMR) was reported recently which used alkali treatment in conjunction with various milling technologies (Chen et al., 2019, 2016). This permitted effective delignification and permitted high solids loadings for saccharification, resulting in high ethanol concentrations (see Section 4). Milling has also been combined with other physical pretreatments, for example in advance of extrusion (Gu et al., 2018). Some research has also attempted to integrate size reduction with saccharification. Using a bead-milling process, cedar wood lignocellulose has been effectively used to demonstrate a simultaneous enzymatic saccharification and comminution process (SSEC) (Navarro et al., 2018).

### *3.1.5 Ball milling of pure cellulose*

In addition to its effects on lignocellulose, a significant body of research now exists on the impacts of milling on cellulose, both alone and in combination with catalytic additives and other physical pretreatments. This approach is often termed reactive ball milling and mechanocatalysis and can lead to the direct hydrolysis of cellulose to produce sugars and oligosaccharides, or enhance subsequent chemical or enzymatic saccharification (Liao et al., 2020a; Peng et al., 2013; Wu et al., 2018). Catalysts used include mineral acids such as sulfuric and hydrochloric acids, but also organic acids and solid acidic catalyst may be employed. Additional combined physical pretreatments include microwave irradiation (Peng et al., 2013). Whilst mostly academic in nature and probably not readily applicable to the pretreatment of lignocellulose in biorefining, such research is providing more information on the impact of structural changes on the hydrolysis and properties of cellulose.

## **3.2 Extrusion pre-treatment**

### *3.2.1 Introduction and background*

Exploited by many industries, extrusion processing is used to force a softened or melted material through a die of fixed cross-section. Familiar examples of extruded materials include polymers such as extruded plastics, metals including extruded aluminium and foodstuffs for example extruded snack foods. Extrusion of plastics and food materials typically use screw-fed extruders. These extruders commonly consist of a long barrel containing a single or dual screws. Raw materials such as plastic pellets or starchy food materials of a suitable particle size are fed via a hopper into one end of the barrel. The rotating screw(s) drive the material along the barrel where it is heated by a combination of heating elements within the body of the barrel and friction between the extruder and the material. Overheating may be prevented by the provision of cooling systems within the barrel. Usually, some or all the material melts. The rotating screw(s) will also provide mixing and create high pressure and shear forces that may change the material.

The processed material continually moves along the barrel to the die where it will be forcibly extruded. In the case of starchy snacks, the instant reduction in pressure permits hot pressurised water within the starchy melt to vaporise as steam, creating bubbles in the melt, thus producing an expanded starch snack. Many materials are processed by extrusion and over 20 years ago, studies on the extrusion of plant cell wall matrices showed that plant cell walls can be severely disrupted (Ng et al., 1999). Thus, it is not surprising that such a versatile and continuous thermophysical process came to the attention of the 2G biorefinery community as a possible means to pre-treat lignocellulose.

### *3.2.2 Effects of extrusion on lignocellulose*

The impact of extrusion on lignocellulosic biomass is complex and not fully understood (Zhang et al., 2016). Many process parameters may be controlled including residence time, temperature and mixing (Karunanithy and Muthukumarappan, 2011). These are particularly influenced by the screw configurations which, in the case of dual screw extruders, can convey, knead and mix and induce reverse flow, thus creating high pressure and shear. Such processing will impact on most of the criteria that underly recalcitrance and many studies have demonstrated that extrusion can improve enzymatic saccharification of lignocellulosic biomass. For example, optimizing single screw extrusion parameters for switchgrass resulted in an increase in yield of sugars to over 40% of the total sugars (Karunanithy and Muthukumarappan, 2011). Twin-screw extrusion studies on corn stover demonstrated that saccharification of glucan could be increased by about 2 fold to nearly 50% (Zhang et al., 2012). More recently, studies on pre-treating corncobs in a twin-screw extruder with a range of screw elements showed that extrusion alone could increase the efficiency of enzymatic saccharification of glucans from 12% in the raw material to about 22% in the extruded cob (Zheng et al., 2015). Modifications to the lignocellulose resulting from the effects of temperature and shear forces include changes in cellulose crystallinity, decreases in particle size and porosity, possible redistribution of lignin (Zheng et al., 2015) and microfibrillation which increases the specific surface area (SSA) (Zhang et al., 2012).

### *3.2.3 Advantages and disadvantages*

Extrusion processing for biomass pretreatment is potentially attractive because it is a highly versatile and continuous process that permits monitoring and control of the key parameters such as material flow, screw speed, moisture content (steam and liquids may be injected), temperature and pressure. It is also a high solids pretreatment method (Zhang et al., 2016), thus reducing the production of effluents and may be environmentally beneficial compared

with other pre-treatments. Indeed, it is promoted as a technology for biomass fractionation whilst using lower temperatures and fewer chemicals, limiting biomass degradation and reducing the production of breakdown products and fermentation inhibitors (Duque et al., 2017). In addition, there is a long history of research and development in extruder technology along with a global industrial manufacturing base. However, the above examples and many other studies have generally demonstrated that extrusion alone does not appear to enhance the enzymatic saccharification of lignocellulose to the same degree as other thermophysical and chemical pre-treatments (Duque et al., 2017). This may be because of the relatively short residence times in extrusion (seconds or minutes) possibly resulting in a relatively lower severity pretreatment (see below for further information on severity). However, extrusion processing is readily adapted to combination processing through, for example, the inclusion of chemicals which can considerably enhance saccharification.

#### *3.2.4 Combination with other pre-treatments*

Extrusion pre-treatment lends itself to combination processing, either additionally or sequentially. A range of combination pre-treatments involving extrusion (see below) have been considered and often patented (Duque et al., 2017).

*Reactive extrusion:* The inclusion of additional chemicals and catalysts in the extrusion process is often termed “reactive extrusion” and can significantly improve downstream saccharification of lignocellulose. Examples include the addition of alkali, acids and enzymes.

*Alkali extrusion:* Addition of alkali into the extrusion process has been investigated by many researchers (Cha et al., 2015; Coimbra et al., 2016; Duque et al., 2018, 2014, 2013; Liu et al., 2013; Negro et al., 2015) and has resulted in significant improvements in enzymatic saccharification. For example, an integrated alkaline-extrusion pre-treatment developed for barley straw increased the enzymatic saccharification yields of glucose and

xylose respectively to 90% and 71% of the theoretical yields (Duque et al., 2013). The pre-treatment involved extrusion at moderate temperatures (100°C) and alkali (approximately 5% w/w with respect to biomass) followed by a washing step to remove the alkali and neutralise the residue. Very low levels of sugar-derived breakdowns products were observed and the process was accompanied by solubilisation of much of the lignin and hemicellulosic components. Subsequent studies assessed the benefit of neutralising the alkali within the extrusion process to reduce the number of processing steps, followed by saccharification (Duque et al., 2014). In this case glucose and xylose yields were lower (circa 70% and 60% respectively). It is likely that the effect of alkaline extrusion is to combine the effects of both thermo-physical changes of extrusion (fibrillation, particle size reduction and an increase in SSA) with those of alkali treatment alone (solubilisation of some lignin components and hemicelluloses, disruption of ester cross-links and glycosidic and other linkages within the polysaccharide lignin matrix along with fibre swelling) (Duque et al., 2017). It is quite likely that some of these will also be synergistic through, for example, impacts on rheology and viscosity and the effects of alkali on cell separation.

*Acid catalysis:* Dilute sulfuric acid has been used to enhance extrusion pre-treatment on numerous occasions, over a range of temperatures and conditions. This approach has enabled significant increases in enzymatic saccharification yields, from modest increases of 20% in corn stover (Chen et al., 2013) and 29% in rape straw (Choi and Oh, 2012) to yields of 73.6% in poplar sawdust (T. H. Kim et al., 2013). The latter study highlighted the importance of enzyme concentrations used, with maximum saccharification requiring high enzyme doses. In related research, acid catalysed steam explosion has been used for xylose production (Zhang et al., 2014).

*Bioextrusion:* this uses enzymes as biocatalysts during extrusion. Unlike conventional enzymolysis which is generally carried out in dilute solution, this approach facilitates enzyme catalysis at high substrate concentrations and viscosities. One of the main areas in which bioextrusion was developed was for the processing of cereal starches, particularly with the

use of thermophilic amylases (Gatt et al., 2018). However, it has also been applied to the pre-treatment of lignocellulosic biomass where studies have highlighted the limitations of short residence times and only small increases in saccharification could be achieved. This has been addressed to some degree by continuing the saccharification process post bio-extrusion (Gatt et al., 2019).

Extrusion has also been integrated with other pre-treatment technologies such as steam explosion to provide a continuous process. This has been shown to enhance saccharification of corn stover to 89% whilst producing low levels of fermentation inhibitors (Chen et al., 2014). It has also been evaluated for sequential use with other pre-treatments including the pre-impregnation of feedstock with chemicals, the use of organic solvents, the adaptation of microwave technologies (Duque et al., 2017) and post-extrusion application of surfactants (Eckard et al., 2012).

### **3.3 Acid pre-treatments**

#### *3.3.1 Introduction and background*

During the late 19<sup>th</sup> and early 20<sup>th</sup> centuries much research was carried out to exploit biomass as a source of cheap sugars for industrial use. One of the earliest methods to hydrolyse lignocellulose for ethanol production involved the use of acids (Ruttan, 1909). Other industrial exploitation included the use of dilute acid hydrolysis to remove hemicelluloses that were converted to furfurals for use as platform chemicals, then separated from the cellulose. The latter was then hydrolysed in concentrated acid (between 10 and 30%) as a source of glucose for fermentation (Chundawat et al., 2010). A substantial body of knowledge was thus developed concerning the kinetics of polysaccharide hydrolysis, sugar degradation and the relative resistance of (crystalline) cellulose to dilute acid hydrolysis compared with other cell wall polysaccharides (Saeman, 1945).

### 3.3.2 Effects of acid pre-treatment on lignocellulose

In the context of modern biorefining, acid pre-treatments, usually at high temperature, are used to partially hydrolyse and solubilise lignocellulosic components, particularly hemicelluloses and some lignin, thereby increasing accessibility of cellulose to enzymolysis (Weil et al., 1998). Acid pre-treatment is probably the most commonly used industrial method for pre-treating lignocellulose (Arumugam et al., 2020). Acids may be added to the pre-treatment process in the form of mineral acids including sulfuric, nitric and phosphoric acids, or organic acids such as citric, formic or acetic acids (Liao et al., 2020a). The solid-to-liquid ratios vary, for example 10% (w/w) solids in 0.9-1.4% sulfuric acid (Zheng et al., 2009) and 30% (w/v) solids in 0-0.7% (v/v) sulfuric acid (Wang et al., 2011). The latter solids loading was also used in dilute acid pre-treatment designed for industrial scale (Humbird et al., 2011). Acid may also be produced within the pretreatment process. At high temperatures and pressures associated with pre-treatments, water behaves as an acid (Chundawat et al., 2010). In addition, hemicellulosic acetyl groups are cleaved under such conditions creating free organic (acetic) acid and this can lead to a decrease in pH and autohydrolysis. Hence, even if acid is not added to the pre-treatment processes such as native steam explosion or hot water treatments, the process may comprise a very dilute acid pre-treatment. This has been demonstrated during pre-treatment of corn fibre at up to 260°C and showed an increase in acidity to about pH = 3 (Weil et al., 1998). The pH will be dependent on the concentration of the acid and its relative strength, or PKa value, and the severity of the acid-pretreatment will be dependent on a combination of pH, time and temperature as described by the following equation (Chum et al., 1990) as summarised by Chundawat and colleagues (Chundawat et al., 2010):

$$\text{Log}(S) = \log(R_0) - \text{pH} \quad \text{where} \quad R_0 = t \times \exp [(T-100)/14.75]$$

$t$  = time,  $T$  = temperature, pH = acidity,  $S$  = "Combined Severity Factor".

For simplicity, acid pretreatments are often divided into dilute (low concentration) acid pretreatments and concentrated acid pre-treatments (Taherzadeh and Karimi, 2008).

#### *Pretreatment with dilute acids*

This most common involves low concentrations of sulfuric or hydrochloric acid (circa 0.5-5% w/v) at temperatures of between 160 °C and 220°C and hydrolysis times of between a few seconds and several minutes. Under such conditions it is possible to remove almost all of the hemicellulose which can either be separated from the cellulose, or included for downstream saccharification (Taherzadeh and Karimi, 2008). The pre-treatment can be readily carried out in batch modes, fed-batch or developed for continuous mode operations (Chundawat et al., 2010; Zheng et al., 2009). There have been very many studies on the effect of acid pre-treatment severities on a wide range of lignocellulose sources highlighting the variation in conditions needed. For example, Zheng and colleagues in a comprehensive study applied dilute acid pre-treatments to four different lignocellulose crops (two wood and two grass species) and investigated the impact on enzymatic saccharification (Zheng et al., 2009). By varying a range of pretreatment conditions including temperatures, acid concentrations and residence times, they found that the pre-treatment conditions that optimised saccharification yields required 165°C for 8 min at a sulfuric acid concentration of 1.4% (w/w). Their studies also highlighted the importance of saccharification conditions particularly substrate and enzyme concentrations. For example, high solids loadings led to higher glucose concentrations but lower overall digestibility due to sub-optimal levels of  $\beta$ -glucosidase, resulting in product inhibition of cellulase by cellobiose. This could be addressed by adding more  $\beta$ -glucosidase. Other studies on acid pre-treatment of corn cob lignocellulose with dilute sulfuric acid at up to 0.7% and at an optimal temperature of between 160 and 170°C for 30 min demonstrated high recoveries of glucose and xylose (Wang et al., 2011). Investigations on eucalyptus lignocellulose demonstrated 90% glucose saccharification yields after pretreatment at 200°C for only 2 minutes (Emmel et al., 2003).

### *Concentrated acid pre-treatments*

Mineral acids at high concentrations and temperatures have been used industrially to hydrolyse cellulose to glucose (Cuzens and Miller, 1997; Ruttan, 1909) and would probably be excessive as a pre-treatment prior to enzymatic saccharification. Under the correct conditions, complete hydrolysis using concentrated acids can achieve very high sugar yields in excess of 90% whilst apparently producing lower levels of degradation products (Janga et al., 2012). Organic acids have been exploited at high concentrations for example in the pre-treatment of sugar cane bagasse and poplar with 60% peracetic acid at ambient temperature, although for very long residence times (5-6 days) (Teixeira et al., 1999a, 1999b).

### *3.3.3 Advantages and disadvantages*

Due to the effective solubilisation of hemicelluloses and removal of some lignin (Cheah et al., 2020), hot, dilute acid pre-treatments are highly effective at enhancing enzymatic saccharification. They readily address some of the key criteria underlying recalcitrance. Furthermore, mineral acids are generally low cost. Depending on the severity, the solubilisation of hemicelluloses may even be extended to their total hydrolysis, reducing the need for hemicellulose-degrading enzymes (Ali et al., 2020; Hassan et al., 2018). It is not surprising that this pre-treatment is often chosen for industrial development (Humbird et al., 2011). Unfortunately, acid pretreatments also present some major challenges. The use of such corrosive chemicals requires the use of acid-resistant materials and alloys in the pre-treatment facility which increases capital expenditure (Ali et al., 2020). The acids also require neutralisation after the pre-treatment which may create waste streams such as gypsum (Mosier et al., 2005a), or costly recycling as in the case of high concentration acids (Janga et al., 2012). Furthermore, particularly at the higher acid concentrations and temperatures, solubilisation of hemicelluloses and lignin is accompanied by their degradation to produce a

range of breakdown products including furfurals and phenolics (Jönsson and Martín, 2016). If allowed to accumulate, they will inhibit the activity of saccharification enzymes and fermenting microorganisms, reducing the overall efficiency of the biorefining process. Conventional wisdom says that either they have to be removed (Kim, 2018) or there has to be a balance struck between the severity of the pre-treatment and the final ethanol yield (Cheah et al., 2020). However, modern biotechnological approaches such as the development of inhibitor-resistant strains of fermenting micro-organisms might be an alternative solution (Field et al., 2015; Wu et al., 2017). A further inconvenience may result from the relocation of partially solubilised lignin within the lignocellulose, continuing to create a barrier to enzymolysis.

#### *3.3.4 Combination with other pre-treatments*

Dilute acid pre-treatment is often synergistically included as a catalyst within the processing of a physical pre-treatment such as size reduction (e.g. milling and extrusion as highlighted above), steam explosion or hot water pretreatment (see below).

### **3.4 Steam Explosion Pre-treatment**

#### *3.4.1 Introduction and background*

Steam explosion (STEX) is one of the most traditional pre-treatments in biorefining and has been evaluated in many key types of biomass (Chundawat et al., 2010). It is generally classified as a physicochemical pre-treatment although it has some similarities to liquid hot water (LHW, a hydrothermal) pre-treatment. It has been described as one of the best pretreatment options because it is able combine both physical and chemical approaches (Pereira Ramos, 2003).

### *3.4.2 Effects of STEX pre-treatment on lignocellulose*

Steam explosion pre-treatment involves using high pressure steam to subject biomass to high pressures (20-50 bar) and temperatures (160-290°C) for several minutes. The treatment is terminated by explosive decompression to atmospheric pressure (Ryden et al., 2014). During the explosion phase, the solid material is often recovered as a damp or wet slurry using a cyclone. In small pilot systems, the steam released may be controllably quenched whilst in industrial settings it would be appropriate to recover the heat for re-use in other processes.

The high temperatures and low oxygen levels of the reactor result in several physicochemical changes in the lignocellulose that enhance enzymatic saccharification. For example, lignin, which blocks access of cellulases, binds to cellulases and prevents fibre swelling, becomes fluidised at temperatures which exceed the lignin glass transition point ( $T_g$ ), resulting in its movement away from the cellulose (Takada et al., 2019). Indeed, the lignin may relocate as droplets on the fibre surfaces. At the same time, hemicelluloses will undergo autohydrolysis at higher severities, thus liberating cellulose for enzymolysis. For example, in-depth studies on the effect of steam explosion on oilseed rape straw showed that saccharification was most strongly associated with the breakdown of xylan hemicelluloses (Ryden et al., 2014; Wood et al., 2014). Augmenting these effects, the high temperatures also result in cleavage of hemicellulose-lignin bonds (Cheah et al., 2020). Sánchez & Cardona (2008) reported in their review that pressures in the range 0.69-4.85 MPa and temperatures as high as 290°C can be used for uncatalyzed steam explosions and that the process could handle high solid loadings resulting in the hydrolysis of 80-100% of the hemicellulose present in the biomass (Sánchez and Cardona, 2008). Physical changes also occur in the biomass as shown by the increase in surface area of spruce lignocellulose (Liao et al., 2020a; Wiman et al., 2012). Interestingly, although the explosion part of the process is very vigorous, it appears to make no significant contribution to enhancing

enzymatic saccharification (Brownell et al., 1986). However, it does serve to rapidly terminate the process.

The amount of water in the steam exploded biomass will be dependent on the initial moisture content of the biomass, any added water and the amount of steam used to initially heat the raw lignocellulose within the pressure reactor. The latter will depend on the temperature and heat capacity of the biomass and the reactor equipment. The severity of the steam explosion will be directly related to the time and temperature and can be defined using the following land-mark equation (Overend and Chornet, 1987):

$$R_0 = t \times \exp [(T-100)/14.75]$$

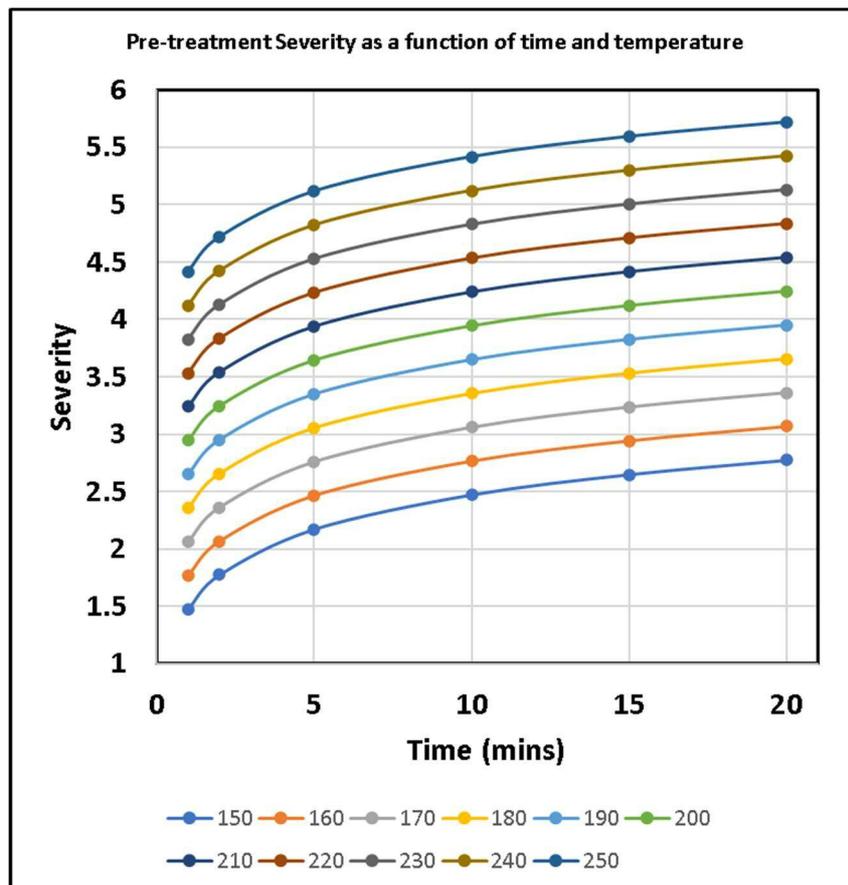
$t$  = time,  $T$  = temperature,  $R_0$  = Severity Factor.

Thus, pretreatments of similar severities can be achieved using either high temperature and short residence times or low temperature and long residence times as shown in Figure 4.

### *3.4.3 Advantages and disadvantages*

One of the key advantages of steam explosion pretreatment is that it can be employed for a very wide range of different feedstocks. Examples include poplar, aspen, eucalyptus wood, sugarcane bagasse, wheat straw, sweet sorghum bagasse and olive stones etc. (Sánchez and Cardona, 2008). It permits high solids loading (Liu and Chen, 2016) and is generally more effective for pre-treating hardwoods than softwoods (Haldar and Purkait, 2020; Zhu and Pan, 2010); acid-catalysed steam explosion for 1-10 mins at 190-210°C is typically required for hardwoods and 200-220°C is typical for softwoods (Zhu and Pan, 2010). The method can also be used without any added catalyst (acid or alkali), which avoids the need to neutralise and dispose of waste salt solutions and results in a relatively low environmental impact. Although significant capital investment is required, some reports indicate that it is relatively low compared with other pretreatments (Ali et al., 2020). Steam generation is

costly (Cheah et al., 2020), requiring much energy and specialist capital equipment. However, heat could be recovered downstream for use in other biorefining processes. Hence, the method is considered to use only moderate amounts of energy overall. Also, whilst hemicelluloses and other non-cellulosic component may be broken down by the pre-treatment, they should be retained with the damp solids rather than being lost in a liquid phase.



**Figure 4:** graph showing relationship between time and severity at different temperatures of steam explosion pre-treatment based on the above published equation (Overend and Chornet, 1987). This shows that a severity of e.g. 4 can be achieved by carrying out pre-treatments at a range of temperatures and times.

However, steam explosion pretreatment does create several important challenges. Steam explosion results in the degradation of hemicelluloses to produce fermentation and enzyme inhibitors including furfural, formaldehyde, acetic acid and formic acid. These are produced particularly at the most effective severities as shown in steam exploded rice straw and husk (Wood et al., 2016). Of course, such inhibitors may be separated from the pre-treated solids by washing, but this will remove the solubilised hemicelluloses which would require separate processing. At an operation level, several important issues arise. Firstly, safety is an important consideration when increasing the size of the operation to industrial scale and if catalysts are to be included (see below), the capital investment will have to account for the use of resistant materials and alloys such as specialist stainless steel. Secondly, in relation to the economics of the process, much steam explosion research utilises small pilot-scale batch processing. However at larger scale, this can create economic hurdles as highlighted by the industrial companies Granbio, CTC-Piracicaba and Praj who use steam explosion treatment method for their industry scale SCB treatments (Silveira et al., 2018). A continuous, 100 tonne/day STEX process has been previously built and patented (Pschorn et al., 2009), although operational data is not readily available. Continuous steam explosion processes may utilise screw feeders/augers and highly pressurised compartments, each becoming pressurised as the pre-treated biomass is constantly fed towards a product receiver tank, leading to smaller and more regular steam explosions (Stelte, 2013). The components for such a system would need to be built out of high-quality materials because high pressures would need to be maintained within the system indefinitely.

#### *3.4.4 Combination with other pre-treatments*

Although highly effective alone, steam explosion is frequently augmented with the addition of chemical catalysts, both acids and alkalis, even though this will require additional processing inputs to facilitate neutralisation and disposal of wastes. For example, willow biomass was milled and impregnated with dilute acid (circa 1% w/v H<sub>2</sub>SO<sub>4</sub> or 1% w/w SO<sub>2</sub>) prior to steam

explosion over a range of severities (Eklund et al., 1995). This pre-treatment increased the enzymatic saccharification yield of glucose from 67% to 95% of total glucan available, benefiting from the effects of steam explosion and dilute acid pre-treatments but at a high solids load. Steam explosion pre-treatment combined with pre-impregnation of biomass with alkali was used recently to demonstrate enhanced xylose extraction and enzymatic saccharification of cellulose from sugarcane bagasse and aspen wood (Mihiretu et al., 2019). This would be expected to enhance lignin dissolution along with hemicellulose fractionation. Many other combination approaches have been evaluated either with other chemicals such as ozone, CO<sub>2</sub> and hydrogen peroxide, or in conjunction with other physical processes such as extrusion, super-fine milling, supercritical CO<sub>2</sub> extraction and biological pretreatments (Haldar and Purkait, 2020; Kumari and Singh, 2018; Liao et al., 2020a; Zheng et al., 2014).

### **3.5 Liquid hot water pre-treatment**

#### *3.5.1 Introduction and background*

Liquid hot-water (LHW) pre-treatment, like steam explosion, is one of the traditional methods used to enhance saccharification of lignocellulose. It is conceptually straightforward and in its normal state involves no added chemicals.

#### *3.5.2 Effects of LHW pre-treatment on lignocellulose*

LHW pretreatment involves extracting lignocellulose in water at high temperatures (from about 160°C to about 240°C) for up to about 20 minutes. The extraction occurs in pressure vessels to maintain the water in a liquid state. This results in the autohydrolysis and solubilisation of hemicelluloses and the melting and solubilisation of some of the lignin,

thereby increasing enzymatic digestibility of the cellulose. To terminate the extraction the reaction slurry is cooled to below 100°C. Hence, heating and cooling periods contribute towards the overall severity of the pre-treatment. Using this approach and in the absence of catalysts, higher quantities of hemicellulose (circa 80%) can be recovered in comparison with steam explosion pre-treatments (Laser et al., 2002). Other research has shown that xylose recoveries of over 80% and glucan saccharification yields of 96% could be achieved with LHW pre-treatments (Pérez et al., 2007). Conventional LHW processes were carried out in batch reactors. However, flow-through reactors have also been developed in which the LHW is pumped through biomass to extract the solubles prior to the saccharification stage. In this way, the impact of parameters such as flow-through rate have been found to be important (Liu and Wyman, 2004). LHW pretreatment is extremely scalable and very small reactors of only 20ml or less may be used for laboratory research. This has permitted high throughput pre-treatment of large numbers of samples allowing relatively large data sets to be produced (Collins et al., 2017; Wood et al., 2017). For example, studies on wheat straw derived from 89 cultivars, showed that pre-treatment impact, saccharification potential, production and release of inhibitors, are all significantly influenced not only by the pre-treatment conditions, but by the compositions of the different tissues, particularly the stem-to-leaf ratios, which varied considerably across the varieties assessed (Collins et al., 2017). This highlights the difficulty in optimising any biorefining process when such heterogeneity exists even within one feedstock. Further research on the solubilisation of cell-wall components during pre-treatment has also highlighted the release of important hemicellulose cross-linking phenolics such as di-ferulic acids (Merali et al., 2016, 2013). Such components have been implicated in cell adhesion particularly in monocot plants, and their release during LHW pretreatment of wheat straw is accompanied by the loss of lignocellulose structure and cell separation (Merali et al., 2016).

### *3.5.3 Advantages and disadvantages*

LHW, like steam explosion, has been applied to many major sources of lignocellulose (Kumari and Singh, 2018) and, by removing the extraction liquor after the process, leaves a cellulose-rich residue for saccharification. Hence, it is a very suitable approach if separate recovery of hemicelluloses and other extractants is required for alternative exploitation. The process will result in the formation of fermentation inhibitors predominantly due to hemicellulose degradation and particularly at low pH. Hence, if the hemicellulose extract containing the inhibitors is not to be removed, then pH regulation during pre-treatment may be beneficial so as to reduce their production (Kumari and Singh, 2018).

It has been suggested that LHW pre-treatment is not particularly effective on high lignin lignocellulose (Haldar and Purkait, 2020) and may therefore benefit from inclusion of mild alkali.

### *3.5.4 Combination with other pre-treatments*

There are numerous examples in which LHW has been combined with chemical catalysts and these often closely align with the chemical pre-treatments considered in other sections. LHW and dilute acid or alkali pre-treatments overlap in concept as shown in studies on sugarcane bagasse (Yu et al., 2013). In this study, LHW was compared with hot, dilute HCl and NaOH treatments. Enzymatic saccharification was improved to about 72% (total sugars) for the LHW process and about 77% for the dilute hot chemical treatments demonstrating a synergistic benefit of the combined processes. Similar studies were carried out on rice straw where LHW was supplemented with a range of acids including dilute sulfuric, hydrochloric, phosphoric and oxalic acids, or dilute alkali (NaOH) (Imman et al., 2014). The impacts of these additives over a range of temperatures in relation to enzymatic saccharification efficiency and the production of fermentation inhibitors was assessed. Dilute, hot oxalic acid at 160°C was deemed to be the most effective resulting in over 91% recovery of

enzymatically hydrolysed glucan. Focusing on LHW and alkali, recent studies on bamboo lignocellulose have demonstrated the partial removal of hemicelluloses and lignin in 0.5% NaOH at 170°C, leading to enhanced enzymatic hydrolysis of cellulose (Yang et al., 2019) although total cellulose conversion was still only about 50%.

### **3.6 Alkali pre-treatments**

#### *3.6.1 Introduction*

Alkali is highly effective at addressing many of the recalcitrant properties of lignocellulose. Consequently, numerous forms of alkali pre-treatment have been developed. Conveniently, many alkali pre-treatments can be carried out in a laboratory setting using standard equipment. There is a large body of knowledge concerning the impact of alkali on plant cell wall chemistry because of its historical use in sequentially extracting constituent polymers for analysis of their structures, compositions and interactions. Such work led to a comprehensive classification of hemicelluloses from wood and non-wood lignocellulose (Timell, 1964; York et al., 1986) and contributed towards the development of cell wall structural models (Brett and Waldron, 1990; McCann et al., 1990). Understanding how to control the effects of alkali on cell walls also led to research to relate cell wall structure to function (Waldron and Selvendran, 1992).

#### *3.6.2. Effects of different alkali pre-treatments on lignocellulose*

The effects of alkali pre-treatment are wide-ranging. Very dilute alkali (e.g. 0.05M Na<sub>2</sub>CO<sub>3</sub>) at low temperature (0-4°C) will de-esterify pectic polysaccharides (which are generally low in lignocellulose feedstocks) (Brett and Waldron, 1990; Selvendran and Ryden, 1990). Alkali at ambient temperature and higher concentrations (e.g. 0.5 – 4M KOH) will solubilise

hemicelluloses, mainly by breaking hydrogen bonds with cellulose, but also through the cleavage of phenolic esters which can cross-link hemicelluloses with themselves and with lignin (Brett and Waldron, 1990). Hot alkali extractions will additionally result in oxidative degradation and depolymerisation of polysaccharides for example through “alkali peeling”. This involves the degradation of polysaccharide residues at the reducing end. Alkali can also cause cleavage of cellulose glucan chains (Knill and Kennedy, 2002). Depending on the conditions, alkali pre-treatment can saponify and remove acetate groups and uronic acids such as glucuronic acid; it can also break aryl-ether and carbon-carbon linkages within the lignin (Liao et al., 2020a; Ponnusamy et al., 2019) thus helping to solubilise both hemicellulose and lignin (Rajendran et al., 2018). In cereal straws, alkali pre-treatments can also solubilise and remove silica (Ali et al., 2020; Rezende et al., 2018) which can contribute to recalcitrance and also cause mechanical wear to biorefinery equipment. In addition to partially hydrolysing cellulose, alkali can also reduce and change its crystallinity (from Cellulose I to Cellulose II) and cause swelling of the microfibrils thus increasing the internal surface area. This is considered to increase the accessibility of the cellulose to cellulases (Liao et al., 2020a; Rajendran et al., 2018). Hence, alkali pre-treatments, through the degradation of numerous inter- and intra-polymeric linkages, have the capacity to solubilise much of the hemicellulose and lignin, cause cellulose to swell and alter its crystallinity, and increase cell wall porosity and available surface area. These collectively enhance the capacity for enzymatic saccharification of both insoluble cellulose and solubilised hemicelluloses. The degree to which these changes occur are dependent on the type and concentration of alkali, the temperature, the processing method and the source of lignocellulose. Because alkali pre-treatments operate at very high pH, significant washing and neutralisation steps are usually required afterwards (except for ammonia, which can be removed by evaporation). In addition, the solubilised lignin and hemicelluloses may require further processing to ensure valorisation. A wide range of chemicals have been evaluated for use in alkali pre-treatments, the most common being NaOH, KOH, Ca(OH)<sub>2</sub> (lime) and ammonia. These can be applied in many process configurations and sometimes with added

oxidants such as O<sub>2</sub> or H<sub>2</sub>O<sub>2</sub> to improve delignification (Liao et al., 2020a). Examples of the most frequently considered methods are described below.

### 3.6.2.1 *NaOH pre-treatment at different concentrations and temperatures*

NaOH is one of the most widely investigated alkalis since it is readily available and easy to handle. It has been studied at a range of temperatures and concentrations. For example, Wu et al (Wu et al., 2011) assessed the effects of ambient and gentle heating on NaOH pre-treatment of two different sources of hammer-milled sorghum bagasse. The lignocellulose, at a loading of about 5% (w/v) was pre-treated in 0.5 – 2.5M NaOH at 25°C or 50°C for varying periods, washed, neutralised and saccharified with a commercial cellulase cocktail. The results showed that the alkali effectively solubilised over half the lignin within 30 min (up to about 50% in 0.5M NaOH and between 70- 80% in 2.5M NaOH) and much of the xylan hemicellulose. Saccharification of cellulose was increased from about 30% to over 90% in the best case and attributed mainly to the delignification effect. In a study on very low-temperature alkali pre-treatment, a mixture of NaOH (7% w/v) and urea (12% w/v) was observed to effectively pre-treat spruce wood chips at -15°C resulting in a 70% (of theoretical) glucose yield on enzymatic saccharification (Zhao et al., 2008). This was attributed to a loss of some of the lignin (circa 19%) and hemicellulose (circa 40%) along with tissue collapse. Interestingly, at ambient temperature and 60°C, the pre-treatment was far less effective. In a complementary study, spruce and birch lignocellulose were pre-treated with 7% w/w NaOH for 2h at between -15 °C and 100°C (Mirahmadi et al., 2010). Unlike the study of Zhao et al (Zhao et al., 2008) little if any lignin was removed at any temperature, although the hemicelluloses were reduced and cellulose crystallinity was lessened. However, the pre-treatments enhanced enzymatic saccharification and were more effective for the birch hardwood (over 80% glucose theoretical yield) compared with the spruce softwood (35%).

The variety of findings in these studies highlight the difficulty in elucidating the underlying mechanisms and thus the importance of optimising any pre-treatment for a specific lignocellulose source. Interestingly, it is also generally reported that alkali pre-treatments are much more effective on relatively low lignin grass and cereal-derived lignocelluloses compared with wood cellulose (Liu et al., 2020).

#### 3.6.2.2 Lime ( $\text{Ca}(\text{OH})_2$ ) pre-treatment

Calcium hydroxide ( $\text{Ca}(\text{OH})_2$ ) is a more mild alkaline pre-treatment often carried out at temperatures at or above 100°C. It is generally inexpensive to use, considered to be a relatively safe pre-treatment and can be partially recovered (up to 21%) through the carbonation of wash water with  $\text{CO}_2$  (Chang et al., 2001). Its use has been widely reported in the pre-treatment of a variety of lignocellulosic substrates including wheat straw (85°C for 3 hours), poplar wood (150°C for 6 hours, with 14 atm. oxygen), switchgrass (100°C for 2 hours) and corn stover (100°C for 13 hours) as reviewed by Mosier et al. (Mosier et al., 2005a). Lime has also been used in the pre-treatment of sugarcane bagasse. Under ambient conditions, the pre-treatment period is long (up to 192 hours) but can increase enzymatic digestibility from 20% to 72%. Increasing the temperature shortens reaction times; similar results were obtained by pre-treating at a temperature of 120°C with a reaction time of 1 hour (Mosier et al., 2005a). Lime on its own is not particularly good at removing lignin from high lignin biomass, compared with other alkali pre-treatments (Pang et al., 2011). However, its effectiveness may be enhanced by the addition of an oxidant. For example, high lignin poplar wood and newspaper were pre-treated using lime at 150°C with 14.0 bar absolute oxygen. This substantially increased the efficiencies of enzymatic saccharification (from 6% to 77% for pre-treated poplar wood) and was accompanied by the solubilisation of nearly 80% of the lignin and nearly 50% of the xylan (Chang et al., 2001). In a further example, lime pre-treatment of corn stover at 120°C for 4h reduced the lignin content by only about 12%. However enzymatic saccharification, using an enzyme loading of 10 FPU/g dry biomass,

achieved 60% glucan conversion and this was increased to 88% using higher enzyme loadings and longer digestion periods (Kaar and Holtzapple, 2000). Later studies (Kim and Holtzapple, 2005) showed that lime pre-treatment of corn stover in oxidative conditions (4 weeks with aeration at 55°C) removed over 87% of the lignin and most acetyl groups, and facilitated enzymatic glucan conversion at up to 91% using enzyme loadings of 15 FPU/g cellulose.

Interestingly, lime may also be used in the neutralisation of acid pre-treatments and has been reported to be effective at removing furfurals and HMF through precipitation as shown in the detoxification of spruce hydrolysate (Bhatia et al., 2020; Guo et al., 2013).

### 3.6.2.3. *Pre-treatments involving ammonia*

#### (i) *Soaking or steeping in aqueous ammonia (SAA)*

This pre-treatment involves soaking the biomass in aqueous ammonia for several hours to remove lignin and some hemicellulose, leaving the cellulose open to enzymatic conversion. An early example of this involved steeping corn cob lignocellulose in 2.9M ammonia at 26°C for 24h leading to the removal of between 89 and 90% of the lignin and resulting in a high enzymatic saccharification yield of glucose of up to 92%. This was successfully fermented using the SSF process to produce an 86% yield of ethanol (Cao et al., 1996). In a later example of higher temperature soaking, corn stover lignocellulose was soaked in aqueous ammonia (15-30 wt%) for up to 24h at between 40 and 90°C. The optimised treatments removed 62% of the lignin whilst retaining all the cellulose and most of the hemicellulosic xylan and increased glucan hydrolysis from 17% to 85% under SSF conditions (Kim and Lee, 2007).

(ii) Ammonia recycle percolation (ARP)

This alternative approach involves the flow of aqueous ammonia solution through a reactor containing the lignocellulose feedstock and has many permutations (Zhao et al., 2020). In a typical example, ammonia solution at a concentration of between 5 – 15 wt%, is fed through a packed column at high temperatures (160-180°C) with residence times of approximately 14 mins, the primary function of which is to remove lignin from the biomass, but leave the cellulose untouched (Mosier et al., 2005b). Ammonia can then be separated and recycled back into the process. For the pre-treatment of a corn cob/stover mixture (CCSM), it was reported that the conversion of cellulose to glucose was close to 100%, with slightly lower conversion rates for Switchgrass (Iyer et al., 1996). In another study, researchers using the ARP process reported a 70-85% decrease in lignin content of a corn stover feedstock and most was removed during the first 10 minutes. Approximately half of the hemicellulose component (xylan) was also solubilised whilst the glucan remained relatively intact (Kim et al., 2003). Although the approach has been around for over 20 years, ARP is still receiving attention and recent articles suggest the optimal conditions for corn cob and corn stover mixtures are a 10% ammonia concentration flowing at 1 mL/min, 170°C for 15-60 minutes (Zhao et al., 2020). This achieved a 74-80% removal of lignin and a reasonably high glucan conversion.

(iii) Low moisture anhydrous ammonia (LMAA)

LMAA involves reducing chemical and water input rates by using ammonia gas, but with residence times of hours to many weeks (Yoo et al., 2014; Zhao et al., 2020). The higher product yields facilitated by the glucan and xylan co-utilisation may not be worth the extra processing time (Zhao et al., 2020).

(iv) Ammonia fibre/freeze explosion (AFEX)

The AFEX process was developed nearly 40 years ago for the pre-treatment of grasses (Holtzapple et al., 1991; Zhao et al., 2020). The process involves soaking the lignocellulose

in liquid ammonia at high pressure (circa 15 bar) at about 50°C for approximately 15 min after which the pressure is released instantaneously (as in steam explosion). This causes the ammonia to flash off after which it can be recovered, compressed and re-used. Only about 1% of the ammonia is left in the biomass and this can serve as a source of nitrogen for downstream fermenting micro-organisms (Holtzapple et al., 1991). AFEX will reduce the lignin content in the biomass and may remove some hemicelluloses. It also reduces cellulose crystallinity and has been implicated in causing cellulose swelling, thus increasing the available surface area for saccharification (Mosier et al., 2005b; Peral, 2016). Unlike steam-explosion, AFEX pre-treatment occurs at much lower temperatures and pressures and results in very much less degradation. Indeed, very few fermentation and enzyme inhibitors are produced during AFEX as is the case in many alkali pre-treatments (Zhao et al., 2020). Use of anhydrous ammonia in AFEX means that high ammonia concentrations can be used for the extraction process, the only water being that from the biomass. If the biomass is sufficiently dry, the AFEX process will be a “dry – to – dry” process and the lack of inhibitors negates the requirement for a washing step. It is generally accepted that AFEX is most effective in enhancing enzymatic saccharification of low-lignin biomass such as grasses and herbaceous crops (Balan et al., 2009; Holtzapple et al., 1991; Zhao et al., 2020) but can be moderately effective on hardwoods (Mosier et al., 2005b). Suitable feedstocks for this process include aspen wood chips, bagasse, wheat straw, barley straw, rice hulls, corn stover, switchgrass, coastal Bermuda grass and alfalfa (Sánchez and Cardona, 2008). Although typical operating conditions fall in the range of 60-90°C, a residence time of 5-60 minutes and a pressure between 40 and 50 atm, it is possible to balance temperature against residence time. For example, 5 minutes at 90°C (Teymouri et al., 2005) and 8 hours at 40°C (Bals et al., 2012) both give reasonable glucan and xylan hydrolysis yields of 80-90% from corn stover (Zhao et al., 2020). The feed ratio will typically be 1 to 2kg ammonia/kg dry biomass, with a 40-80% ammonia concentration although this can differ.

#### 3.6.2.4 *The Kraft Process*

The Kraft process is an aggressive delignification process which has been used in the conversion of wood to wood pulp for over a century, having been invented in the late 1800's (Dahl, 1884). The process involves the batch or continuous digestion of wood chips at high pressure in a hot (145-170°C) solution of NaOH and sodium sulphide (Na<sub>2</sub>S), collectively known as "White Liquor". The hydroxide and hydrosulphide ions react with the lignin and split it into water soluble fragments. About 90% of the lignin can be removed using this process (Fernández-Rodríguez et al., 2018, 2016). The result is Kraft pulp (consisting of almost pure cellulose fibres) and black liquor. After washing the wood chips the black liquor can be concentrated by the use of an evaporator and subsequently combusted to make steam, which in turn is used to heat the initial process, reducing heating utility costs. Any wood can be broken down via this process; softwoods are generally preferred for the manufacture of paper because of their long fibres. However, hard woods and even agricultural residues can also be used, particularly in combination pre-treatments (see below).

#### 3.6.3 *Advantages and disadvantages of alkali pre-treatments*

Alkali pre-treatments have many advantages. They are generally highly effective and, compared with acid pre-treatments, cause less sugar degradation than acidic methods (Chen et al., 2017; Zhao et al., 2020). Furthermore, reactor costs for alkali treatments are reportedly cheaper than for acid treatments (Sánchez and Cardona, 2008). Of the different approaches, the use of Ca(OH)<sub>2</sub> is often considered to be the cheapest alkali pre-treatment (Cheah et al., 2020; Chen et al., 2017); whereas NaOH (and also KOH) are by far the most commonly used since they are more effective. Nevertheless, many researchers support the use of ammonia because its vaporisation makes the collection and recovery process much easier. Considering examples of specific variations in ammonia pre-treatment, the ammonia

and water inputs required for ARP are half of those required for SAA treatments (Zhao et al., 2020) and can remove up to 50% hemicellulose. They also enable very good glucan yields to be obtained at low enzyme loadings. ARP pre-treatments are well suited for agricultural residues and herbaceous biomass, although not so effective on hardwoods and unsuitable for softwoods. Finally, although used predominantly for pre-treating lignocellulose in advance of paper manufacturing, a particular advantage of the Kraft ammonia process is that the conditions also allow the digestion of pine chips, during which the resin can dissolve in the alkaline liquor. This resin can later be extracted in the form of 'tall oil', which can be a valuable by-product (Boncli, 2020; Tran and Vakkilainen, 2012).

Alkali pre-treatments also present several challenges. Although highly effective, a commonly-reported drawback is the conversion of alkali into unrecoverable salts (Mosier et al., 2005a). This can result in significant disposal costs if pollution of the natural environment is to be avoided. In the KRAFT process, whilst black liquor can be considered a very good fuel for producing heat, it is difficult to valorise it in any other way, although research continues. For example, hemicellulosic and lignin-derived components in black liquor may be used in producing porous materials (Foulet et al., 2015); pyrolysis gases produced from black liquor have been used to produce zinc nanosheets (Maciel et al., 2012). Black liquor is usually highly contaminated with carbohydrates, fatty acids and sulphur, making it unsuitable for further processes unless a purification step is included. A more suitable source of lignin for further value-added industrial processes can be found as by-products from alkaline or Organosolv processes (see below). It is important to note that some sources suggest that more research is needed to determine the extent at which airborne emissions from the Kraft process affect health of local communities (Hoffman et al., 2017).

#### 4.6.4 Combination with other pre-treatments

Alkali pre-treatments are readily combined with other pre-treatments such as with LHW (see above) – indeed, it could be said that (hot) aqueous ammonia extractions are all a form of LHW pre-treatment. However, there are additional examples such as the combination of aqueous ammonia with glycerol mixtures (AAWG) with an added focus on ammonia recovery for reuse in the system. Optimal conditions reported for this process were a 9.5%  $\text{NH}_3$  solution at 180°C for 1.78 hours, which resulted in a 70.9% lignin removal, 0.45g/(g biomass) total fermentable sugar yield (Shi et al., 2019). The distillation used to recover ammonia managed to extract approximately 30% of the ammonia for reuse. AFEX pre-treatment has been combined with pre-soaking biomass in hydrogen peroxide which helps in the processing of more highly recalcitrant biomass such as bamboo shoots and woody biomass. The peroxide pre-soaking removes a good deal of lignin, allowing for greater ammonia penetration prior to the pressure explosion. This technique increases glucan and xylan conversion yields by approximately 10% (Zhao et al., 2020). Similarly, ozone has been used in combination with aqueous ammonia treatment, with the optimal conditions for rice straw processing being a 90-minute ozonation period followed by a 9-hour SAA treatment. This considerably enhanced the subsequent production of biogas by about two-fold (Ai et al., 2019). An example of ARP combined with another pre-treatment includes sequential LHW followed by ARP. Here, the LHW treatment at 190°C for 30 minutes can remove approximately 92-95% of the xylan, subsequent ARP treatment at 170°C for 1 hour can then remove 75-81% of the lignin, leading to a treated solids glucan recovery of 78-85% (Tae and Lee, 2005). However, many of these combination processes have been performed only at laboratory scale and have not considered the recovery of hemicellulose or ammonia, so the extent to which these chemicals can be extracted/reused is unclear. It is clear that such extraction will be an important matter if industrial scale-up is to be considered (Zhao et al., 2020).

### **3.7 Solvent pre-treatments**

#### *3.7.1. Organosolv pre-treatment*

##### *3.7.1.1. Introduction and background*

The Organosolv process was invented in the late 1960's (Kleinert, 1968) and developed as a less polluting alternative to the Kraft process to produce pulp for use in paper manufacture. It has also been investigated as a pre-treatment for the biorefining of lignocellulose to bioethanol although the process is expensive compared with most other leading pre-treatment processes. Nevertheless, it is attractive because it is able to separate the key components from lignocellulose without causing undue chemical damage, thereby enhancing the possibility of adding value with quality co-products (Zhao et al., 2009).

##### *3.7.1.2. Effects of Organosolv pre-treatment on lignocellulose*

Organosolv pre-treatments involve the use of organic solvents such as ethanol, ethylene glycol, formic acid or acetic acid, with or without water, to partially depolymerise and solubilise hemicellulose and lignin from lignocellulose, thereby increasing the accessibility of cellulose to cellulases (Tu and Hallett, 2019). A small amount of acid (e.g., H<sub>2</sub>SO<sub>4</sub> or HCl) is often added to enhance the pre-treatment effects. Organosolv pre-treatments are usually performed at temperatures ranging between 100-250°C and the operation serves to divide biomass into at least three distinct fractions – insoluble cellulose, dry lignin and aqueous hemicelluloses (Mesa et al., 2016).

Solvents may be easily recovered from the different fractions by distillation. This facilitates recycling and leaves extracted dry materials. Alcohols are popular solvents for this process and primary alcohols are highly effective for general delignification. However, n-butyl-

alcohol-water is also efficient in removing lignin from wood. During the chemical recovery process lignin can be isolated as a solid and non-cellulosic carbohydrates can be independently separated as a syrup. However, extracted residues must also be thoroughly washed in organic solvents to prevent reprecipitation of lignin (Mesa et al., 2016).

Organosolv pre-treatments can be augmented by the addition of catalysts such as mineral acids, magnesium, calcium/barium chloride/nitrate, neutral alkali earth metals, magnesium sulphate, sodium bisulphate and sodium hydroxide (Zhao et al., 2009). The use of such catalysts can permit the process to be carried out effectively at temperatures at or below 180°C. For example, an Organosolv process was applied to poplar wood at 180°C for 60 minutes, with 1.25% sulphuric acid and 60% ethanol (Pan et al., 2006; Zhu and Pan, 2010). As a result, almost 75% of the lignin was removed and recovered as a high purity by product. Approximately 70-80% of the xylan was also removed with a monomeric xylose recovery of over 50% in the soluble stream. Of the original glucan, 88% was retained and most was enzymatically hydrolysed within 24 hours.

### *3.7.1.3 Advantages and disadvantages*

One of the key advantages of the Organosolv process, particularly when compared with the Kraft process, is the ease with which solvents can be separated from the extracted moieties, recovered and recycled. Recovery can generally be achieved using traditional distillation processes although the cellulose-rich product will require extensive washing with water prior to enzymatic hydrolysis and fermentation if the solvents used are inhibitory to those processes. Another potential advantage is that a primary size reduction treatment is not always required prior to Organosolv processing because the material breaks down during the pre-treatment (Zhu and Pan, 2010). There are, however, several drawbacks associated with the process. For example, organic solvents are expensive and even though they can be recovered, the methodology will lead to increased energy expenditure on an industrial scale plant. As part of such recycling, every stage of this process must be air-tight and thoroughly maintained, since organic solvents are highly volatile and flammable and the risk of

fire/explosion is inherent. Zhao, et al. (Zhao et al., 2009) stated that costs of chemicals and specialist high pressure equipment made the process uneconomic for the pre-treatment of biomass at the time of writing (2008). Losses of some solvents, particularly alcohols, can be mitigated in several ways. For example, ethanol retained in the cellulose-rich residue may be recovered during downstream processing if the biorefinery focuses on fermenting the sugars released by saccharification into ethanol. This would not be the case if it were producing different products such as citric acid. Methanol losses may be further compensated for by demethylating lignin through high-temperature cooking (Zhao et al., 2009). Overall, the system has unique and promising qualities and future success is likely to depend on creating high-value co-products from lignin and hemicellulose (Zhu and Pan, 2010). Despite the danger and high costs associated with the process, published patents and papers have been increasing consistently since 2004 (Mesa et al., 2016), suggesting that interest in the process continues to grow.

### *3.7.2 Ionic liquid pre-treatment*

#### *3.7.2.1 Introduction*

Ionic liquids are generally described as liquids that are composed of ions (i.e. salts) whose melting points are below 100°C (Amarasekara, 2016; Yoo et al., 2017). Because of the strong electrostatic forces between the component ions, ionic liquids are not very volatile. The cations of ionic liquids are organic, whilst the anions may be either organic or inorganic. The interest in ionic liquids as pre-treatments for lignocellulose relates to the ability of some to solubilise plant cell walls and facilitate the controllable fractionation of the main cellulosic, hemicellulosic and lignin components (Da Costa Lopes and Bogel-Lukasik, 2015). The selection and combination of anions and cations in conjunction with the control of other

processing parameters can be used to “tune” the properties of the ionic liquids in extracting cell wall components.

### *3.7.2.2 Effects of ionic liquid pre-treatment on lignocellulose*

To date, much academic research has been carried out on exploring the potential of ionic liquids to pre-treat a wide range of biomass feedstocks. Ionic liquids are thought to be able to solubilise cell wall components by disrupting hydrogen bonds and can even solubilise cellulose (Bhatia et al., 2020). The permutations in approach are broad. Ionic liquid pre-treatments often involve complete solubilisation of the lignocellulose followed by precipitation of the cellulose. This has been demonstrated with milled switchgrass (Li et al., 2010). The switchgrass was dissolved in 1-ethyl-3-methylimidazolium acetate (3% w/w) by stirring for 3h at 160°C after which deionized water (“anti-solvent”) was slowly added to form a precipitate. After washing exhaustively with water, the residue was found to be highly enriched in cellulose with a large (69.2%) reduction in lignin and a moderate reduction in hemicelluloses. Enzymatic digestibility was increased from less than 3% in untreated switchgrass to 96% in the ionic liquid-pre-treated residue. This compared with 48% digestibility for hot, dilute-acid-pre-treated switchgrass in which less lignin, but more hemicellulose had been removed. This total solubilisation approach can result in a lower level of cellulose crystallinity (Brandt et al., 2013). In other manifestations, the cellulose can be purified through selective dissolution of the lignin in the ionic liquid. Such an approach has been demonstrated for miscanthus pre-treatment using 1-butyl-3-methylimidazolium methyl sulfate and 1-butyl-3-methylimidazolium hydrogen sulfate water mixtures at 120°C for up to 22h (Brandt et al., 2011). The IL extract contained most of the lignin and hemicellulose, and the cellulose-rich residue was highly digestible (circa 90% yield). The degree of lignin and hemicellulose solubilisation is influenced by the nature of the lignocellulose, the type of ionic liquid(s), temperature and residence time. For example, partial lignin removal was achieved for oak hardwood and pine softwood after treatment in 1-ethyl-3-methylimidazolium

acetate at 110°C for 16 hours (Sun et al., 2009). In contrast, 93% lignin was removed from sugarcane plant waste using 1-ethyl-3-methylimidazolium alkylbenzenesulphonate at 190°C for only 2 hours (Tan et al., 2009). However, these studies did not determine the impacts on saccharification.

Although most of research on Ionic liquid pre-treatment appears to focus on selective extraction and fractionation of cell wall polymers, ionic liquids with Brönsted acidic properties may be potentially used for the acidic hydrolysis of cellulose (Amarasekara and Owereh, 2009). This involves initial solubilisation of the cellulose by the anhydrous, acidic, ionic liquid, followed by addition of water to aid the hydrolysis step. Similar approaches may be used for hemicellulose hydrolysis which can be extended to produce furans as breakdown products (Da Costa Lopes and Bogel-Lukasik, 2015).

### *3.7.2.3 Advantages and disadvantages of ionic liquid pre-treatments*

Ionic liquids have often been described as “green solvents” (Bhatia et al., 2020) due to their interesting solvation characteristics and the potential for recycling. They are able to effectively and substantially enhance the enzymatic saccharification of cellulose (see above). In addition, unlike other pretreatments, such as acid-catalysed hydrothermal pretreatments, ionic liquid pre-treatments are notable for producing lower yields of breakdown products which are inhibitory to enzymolysis and fermentation, unless used directly for the production of such products (Da Costa Lopes and Bogel-Lukasik, 2015). However, their employment within a whole biorefinery context still presents many challenges. For example, they are usually expensive (Arumugam et al., 2020) and, to minimise pollution, their re-use is almost mandatory. However, quantitative recycling of ionic liquids is made difficult by the costs and considerable complexity of the necessary processing (Bhatia et al., 2020). It is further complicated by the requirement to recover and purify ionic liquids from all the different fractions produced, which may contain a range of different contaminants. This is made even

more difficult by issues related to pH effects. Furthermore, there may be a number of unknown risks associated with these relatively new extractants, for example there is apparently little information about the toxicity and environmental impacts of many ionic liquids (Da Costa Lopes and Bogel-Lukasik, 2015).

To try and reduce ionic liquid costs, researchers have tried to identify low-cost chemicals. For example, a more recent Ionic liquid study (Gschwend et al., 2019), reported the use of three protic, low-cost ionic liquids (1-butylimidazolium hydrogen sulfate, triethylammonium hydrogen sulfate and low cost N,N-dimethylbutylammonium hydrogen sulfate) to fractionate pine biomass over a range of biomass/ionic liquid ratios and processing conditions. The most successful conditions reportedly facilitated quantitative enzymatic release of glucose. However, exploitation of dissolved hemicelluloses requires further research. A scaled up, 200 L version of this promising 1 L process has been carried out by the researchers, with a report expected to be released soon.

For economic viability to be favourable, the quantitative recovery and recycling of ILs is also critical and requires further research and development (Da Costa Lopes and Bogel-Lukasik, 2015). The methods of recovery also need to be appropriate for, and integrated with, the downstream processing of lignocellulosic fractions. Focus is needed on removing residual IL from insoluble or precipitated cellulose and other (high purity) fractions. Extraction approaches including liquid-liquid extraction and the use of supercritical fluids have been considered for low molecular weight products. Elaborate processes including the integration of IL extraction with saccharification followed by membrane separation techniques in conjunction with electrodialysis have been proposed (Abels et al., 2013).

#### *3.7.2.4 Combination pre-treatments*

Unsurprisingly, researchers have explored the potential to combine ionic liquid pre-treatment technologies with other chemical and physical pre-treatments. Complementing the use of

acid ionic liquids (see above) researchers have also explored combination pre-treatments involving alkali. Rice straw, for example, has been successfully pre-treated with an ionic liquid followed by ammonia extraction resulting in a reported synergistic benefit (Nguyen et al., 2010). Bamboo biomass has also been subjected to a sequential combination of aqueous alkali (NaOH) pre-treatment followed by extraction with an ionic liquid (Kassaye et al., 2017). This increased the extent of hot acid saccharification. The impact of combining ionic liquid pre-treatments with physical pre-treatments has been demonstrated by the use of ultrasound technology during ionic liquid pre-treatments (Yang and Fang, 2014). In this study, the rice straw feedstock was pre-treated with two ionic liquid solutions for 3h at 60°C whilst being subjected to ultrasonic pre-treatment. The study showed improved enzymatic saccharification, increasing the initial rate and slightly increasing the final yield. Another study on rice straw involved the sequential use of a screw-press pre-treatment followed by an ionic-liquid pre-treatment after which the residue was washed and subjected to enzymatic saccharification (Sriariyanun et al., 2015). The results suggested a synergy between the two pre-treatments, resulting in lower quantities of ionic liquid to achieve similar saccharification yields.

### **3.8 Oxidative pre-treatments**

#### *3.8.1 Introduction*

Oxidative pre-treatments have generated much interest because of their ability to significantly reduce the levels of lignin and hemicelluloses and thereby enhance the enzymatic saccharification of the residual cellulose. The main methods that have been evaluated are ozonolysis, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) treatment and air/oxygen – based wet oxidation processes.

### 3.8.2 Effects of oxidative pre-treatments on lignocellulose

*Ozonolysis:* this is a highly oxidative delignification pre-treatment usually carried out at ambient temperature and pressure (Arumugam et al., 2020). As an example, it has been used successfully for pre-treating wheat and rye straw (García-Cubero et al., 2009). The feedstock straws were milled, hydrated and placed in a fixed-bed column reactor (circa 250 ml) through which ozone was passed (e.g. 60 L/h, 2.7% w/w ozone, 40% w/w moisture) at ambient temperature. The treated biomass was then dried and assessed for chemical changes in enzymatic saccharification. Optimum conditions resulted in a decrease in acid insoluble lignin and hemicellulose degradation and enhanced enzymatic saccharification by about 3-fold in each straw type.

*H<sub>2</sub>O<sub>2</sub> treatment:* this has been used for pre-treating lignocellulose, usually in combination with other pH-modulating chemicals. An early example involved treating residues in a solution of alkali (Gould, 1984) and showed that delignification was most effective at pH 11.5. This reportedly resulted in almost 100% enzymatic saccharification yields. However, H<sub>2</sub>O<sub>2</sub> has also been used in conjunction with other chemicals. For example, recent studies (Luo et al., 2019) compared the pre-treatment of birch, poplar and straw lignocellulose feedstocks with four previously published peroxide cocktails: (i) concentrated phosphoric acid and H<sub>2</sub>O<sub>2</sub>, for -0.2°C for 2.9h; (ii) acetic acid and H<sub>2</sub>O<sub>2</sub>, at 80°C for 2h; (iii) H<sub>2</sub>O<sub>2</sub> at pH 11.5 (with NaOH) at 35°C for 24h and (iv) H<sub>2</sub>O<sub>2</sub> with Fe<sup>2+</sup> (Fenton chemistry) at 25°C for 120h. The pre-treatments were carried out with 10g/100g solution. They observed that the acidic H<sub>2</sub>O<sub>2</sub> pre-treatments were the most effective at delignification, with H<sub>2</sub>O<sub>2</sub> – phosphoric acid pre-treatment resulting in the best enzymatic yield of glucose.

*Wet oxidation:* this pre-treatment essentially involves carrying out a liquid hot-water extraction (at temperatures around 190°C) with elevated levels of oxygen (e.g. 12 bar), often with small amounts of added alkali such as sodium carbonate (Martín and Thomsen, 2007; Schmidt and Thomsen, 1998) and could be viewed as a combination process. Depending on the conditions, the pre-treatment will solubilise much of the hemicellulose and lignin and

increase cellulose enzymolysis. However, the level of cellulose conversion (i.e. glucose yield as a percentage of available cellulose) is often in the region of 50-60% theoretical (Martín and Thomsen, 2007).

### *3.8.3 Advantages and disadvantages of oxidative pre-treatments*

Ozonolysis pre-treatment has the great advantage of being able to degrade lignin over and above carbohydrates, creating carboxylic acids that are readily removed by washing (Travaini et al., 2016). Hemicelluloses may also be removed, but with very low levels of breakdown products such as furfurals (Chen et al., 2017) and the process has been considered to be environmentally friendly (Arumugam et al., 2020). However, it is generally reported that the method is extremely expensive to implement at industrial scale due to the costs of ozone production and its corrosive properties (Arumugam et al., 2020; Chen et al., 2017). Nevertheless, costs of the technology and process are apparently continuing to decrease; viability could be helped by effective valorisation of other lignocellulosic components even as heat (Travaini et al., 2016). As in the case of alkali-based pre-treatments discussed earlier, pre-treatment using H<sub>2</sub>O<sub>2</sub> and wet oxidation methods in conjunction with additional acids or alkalis will create downstream water treatment challenges as well as requiring specialist equipment. Also, breakdown products and inhibitors will require detoxification steps.

### *3.8.4 Combination processes*

Although oxidation processes are often developed as combination processes with other chemicals, they have also been combined with other physical processes as highlighted earlier in which Ozonolysis was integrated with ball milling. (Shi et al., 2015).

### **3.9 Microwave pre-treatments**

#### *3.9.1 Introduction*

Microwave technology has been used for domestic and industrial purposes for over 50 years and is widely considered to be a green and efficient heating method (Li et al., 2016) due to its direct impact on the material to be heated. Microwaves can induce movement (e.g. rotation) in molecules with a dipole such as water and lipids. The interaction between the rapidly moving molecules (and others) then generates heat. In domestic and food processing equipment the microwave frequency is tuned to 2.45 GHz which is below the resonant frequency of a water molecule, thus permitting the radiation to penetrate and heat the interior of food materials and achieve volumetric heating. However, “standing waves” within microwaves cause hot spots which means the material needs to be moved or stirred to ensure even heating.

#### *3.9.2 Effects of microwave pre-treatments on lignocellulose*

One of the earliest reports of using microwave technology in the pre-treatment of biomass was by Ooshima and colleagues (Ooshima et al., 1984). Their study involved milling rice straw and bagasse and hydrating to a water content of around 80-90%. The samples were then sealed in glass vessels which were irradiated in a microwave oven. This enabled temperatures of up to 230°C to be achieved and the pre-treatments (optimised for time and temperature) were able to enhance the enzymatic saccharification of the lignocellulose by 1.6 x for rice straw and 3.2 times for bagasse. The authors highlighted the similarity with steam pre-treatments. The benefit of the approach included short heating and cooling times, ease of control of moisture content and the cleanliness of the microwave technology. They also showed that excessive treatments actually reduced the benefits, probably due to carbonization of the substrate. Similar results were also obtained in a separate study (AZUMA et al., 1977).

Microwave pre-treatment of biomass at 100°C or below is almost ineffective (Chen et al., 2017). The Ooshima study relied on the use of sealed glass vessels in which the lignocellulose was pre-treated, allowing the temperature and pressure to rise to well above ambient. Saha and colleagues also assessed microwave pre-treatment of wheat straw at high temperatures and pressures for saccharification and fermentation (Saha et al., 2008). If the wet lignocellulose had been pre-treated at ambient pressure, then the water would have been expected to flash off at 100°C and the biomass would have dried out, possibly undergoing pyrolysis and/or burning. Indeed, microwave-induced torrefaction for decomposition of biomass is an emerging area of interest (Satpathy et al., 2014) as well as microwave pyrolysis of biomass (Durán-Jiménez et al., 2018). However, pre-treatment temperatures well above 100°C have been achieved at atmospheric pressure using microwave technology. For example, ground rice straw or sugar cane bagasse could be microwave pre-treated at temperatures of up to 200°C and at ambient pressure, when immersed in glycerine containing small quantities of water (Kitchaiya et al., 2003). The pre-treatment doubled the yield of reducing sugars after enzymatic saccharification. However, the most common use of microwave technology in the pre-treatment of lignocellulose is in combination with other pre-treatments, which are highlighted below.

### *3.9.3 Advantages and disadvantages*

Microwave technology is simple in operation, clean and direct and takes a short time to heat up the material. In addition, it is often considered to be energy efficient. (Chen et al., 2017). It will also contribute to a reduction in both particle size and cellulose crystallinity. From the general research viewpoint, domestic microwave ovens are very cheap. Hence, this technology has attracted much interest for biorefining. However, its major disadvantage is that the cost of capital is very high. Interestingly, although microwaves may interact directly with some of the biomass components, their main functionality is to heat up the water. Apart from the heating effect, there is little evidence that the microwaves have any other function (Bichot et al., 2020). Nevertheless, the impact of microwave heating on plant cell walls has

recently been extended in related research on microwave-assisted extraction of pectins (Arrutia et al., 2020; Mao et al., 2019).

#### *3.9.4 Combination processes*

This commonly used approach is usually described as microwave-assisted pre-treatment and often involves the use of microwave heating in conjunction with the use of solvents, acids, or alkalis. As an example, Zhu and colleagues investigated three combinations of microwave-chemical pre-treatments on rice straw: alkali, acid/alkali and acid/alkali/H<sub>2</sub>O<sub>2</sub> (Zhu et al., 2006). The treatments involved immersing rice straw (1-2cm length pieces) in an aqueous solution of acid or alkali and thermally treated by microwave heating. A subsequent treatment (non-heating) in H<sub>2</sub>O<sub>2</sub> was also evaluated. The most effective conditions involved all three sequential steps and resulted in the solubilisation of most material and the highest enzymatic hydrolysis yields, although significant levels of undegraded cellulose remained. In another study, microwave-assisted acid pre-treatment of wheat and rye stillage residues achieved 75% yields of glucose from enzymatic hydrolysis of cellulose (Mikulski and Kłosowski, 2020). Other researchers have used higher concentrations of chemicals and pressurised vessels to achieve higher temperatures and yields (Hu and Wen, 2008). As an example of microwave-enhanced solvent extraction, effective delignification of several feedstock types was achieved by the use of microwave heating in deep eutectic solvents (Kohli et al., 2020). Microwave processing has also been integrated with physical pre-treatments, for example ball milling (Peng et al., 2013)

### **3.10 Biological pre-treatments**

#### *3.10.1 Introduction*

Biological pre-treatments utilise naturally occurring microbial (bacterial and/or fungal) activities to effect partial degradation of lignocellulosic components (particularly lignin),

thereby increasing the propensity for enzymatic saccharification of the cell wall polysaccharides. Much like previous processes, biological pre-treatments are affected by many factors, such as temperature, time, particle size, pH and the bacterial or fungal strain. This approach has become a popular area of research due to its perceived green credentials.

### 3.10.2 Effect of biological pre-treatments on lignocellulose

In this approach, the roles of Fungi are most understood in the context of lignocellulosic decay. Ascomycetes (e.g. *Aspergillus sp.*, *Penicillium sp.*, *Trichoderma reesei*), basidiomycetes including white-rot fungi (e.g. *Schizophyllum sp.*, *P. chrysosporium*), brown-rot fungi (e.g. *Fomitopsis palustris*) and a few anaerobes (e.g. *Orpinomyces sp.*) have all been shown to produce lignolytic enzymes. These include lignin peroxidases, manganese peroxidases and laccases (Sharma et al., 2019). A soil fungus known as *Trichoderma longibrachiatum* is considered one of the more promising species as it produces three different types of cellulase that seem to synergise during hydrolysis. The use of defined mixtures of micro-organisms has been reviewed recently (Sharma et al., 2019) and termed “Consolidated Bioprocessing” (CBP).

As an example of biological pre-treatment, a white-rot fungus was allowed to colonise corn stover under non-sterile conditions (Song et al., 2013). After 6 weeks, the lignin had been degraded by over 43% and efficiency of enzymatic saccharification was 7-fold greater (circa 65%) than for non-pre-treated corn stover. Yields could be enhanced by about 10% if sterile conditions were used. This may be due to a reduction in the degradation of cellulose by contaminating microflora. Microbial lignin degradation via bacteria has received far less attention than fungal methods, but there are a number of cultures which have shown promise: peroxidases, laccases and  $\beta$ -etherases have been identified in various bacterial species (Sharma et al., 2019).

### *3.10.3 Advantages and disadvantages*

Biological pre-treatment methods have the perceived benefits of being very environmentally friendly and require relatively low amounts of energy. They are often believed to be comparatively cost effective. They also tend not to inhibit digestion and fermentation processes downstream. However, they do require much longer residence times (weeks) compared with other pre-treatments, which means that significant capital investment is required in the pre-treatment facility. Furthermore, there is a considerable lack of control over the process (Sharma et al., 2019). There are numerous factors that affect biological pre-treatments including time, temperature, aeration, particle size, surface area to volume, sources of nutrients such as carbon and nitrogen, pH, species of microorganism etc (Sharma et al., 2019) and the heterogeneity of the biomass may create further complications.

### *3.10.4 Combination processes*

Because of the long residence times, attempts have been made to combine the technology with other physical pre-treatments to shorten the overall period (Sindhu et al., 2016). These include combination (sequentially) with liquid hot water treatment, co-combination with mild physical and chemical pre-treatments such as ultrasound and hydrogen peroxide (reducing residence time by about two thirds) and sequential combination (in advance) of steam explosion or AFEX pre-treatments. Precise multi-culture combinations have also been explored for example using a combination of two microbes concurrently to maximise the breakdown of recalcitrant bonds. The selection of two cultures that can be used in combination is a very difficult process, however, and sometimes combinations found in natural habitats are best evaluated (Sharma et al., 2019).

## **3.11 Other emerging pre-treatment technologies**

While there are a number of other emerging technologies available for the treatment of lignocellulosic biomass (such as Ultrasound, Gamma ray, Electron beam, Pulsed-electric

field, High hydrostatic pressure and High-pressure homogenisation), very few are likely to be cost effective in terms of capital investment or operational costs at their current stages (Hassan et al., 2018) and are not considered in detail in this article.

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Sections 2 and 3 above have provided a general and comparative overview of pre-treatment technologies. The final section, below, reviews and evaluates published literature on the techno-economic aspects of biorefining and explores the relative economic impacts of the main pre-treatment technologies.

## 4.0 Review of techno-economic analysis

### 4.1 Introduction

One of the great challenges in commercialising 2G biofuel production is how to estimate the economic costs and benefits of a proposed 2G biorefinery. Such forecasting requires information at many different levels:

1. What is the size of the prospective market for biofuel and co-products? How will this be influenced by market forces and legislative drivers?
2. How much will the biorefinery plant cost to construct? This requires information on the capital expenditure (CAPEX) on land, buildings, equipment etc; is equipment readily available or does it require further development or adaptation?
3. How much will the biorefinery cost to run? What are the likely operating costs (OPEX) including energy, human resources, rents etc? OPEX and CAPEX will also be influenced by the geographical location of the plant.
4. What is the projected return on investment? This will require information on the profitability of the process (dependent on the cost of feedstock, processing and price of the final products), financing agreements (how long will it take to pay for the capital investment), taxation and so on, over a specified number of years.

All of these will be affected by the size of the plant (economies of scale) which may be limited by feedstock availability and the wider socio-political environment. This simple summary highlights the complexity and breadth of information required. Nevertheless, such forecasting is essential for financing organisations who are unlikely to invest in a commercial biorefinery project unless they are confident it will provide the necessary financial returns.

Today, the heart of such forecasting often relies on the techno-economic analysis (TEA) of a process. This starts with the development of a computer-model of the whole process incorporating chemical engineering principles, from which mass balances and energy flows,

plant and equipment may be derived and costed. Projected financial flows derived from CAPEX, OPEX (including feedstock costs etc) and likely revenues may then be used to produce a discounted cashflow (DCF) analysis. Financial assessments can be made by calculating a minimum ethanol selling price (MESP). This is often based on the ethanol sales price that results in a zero net present value (NPV) after discounting cash flows at 10% after tax (Eggeman and Elander, 2005). An MESP calculation avoids the uncertainty that may occur in an NPV because of unforeseen fluctuations in e.g. fuel prices. Such analyses can also include assessments of subsidies and other support and can be based on “*n*th plant” assumptions to avoid the unforeseen risk-related expenditure associated with pioneer plant development (Wooley et al., 1999b, 1999a).

In addition to informing the investment community, a TEA provides an invaluable tool to aid decision making by other stakeholders. For example, TEA is useful in identifying which technologies in a biorefinery process should be improved and require further research and development (Wooley et al., 1999a). At a wider level, the economic models derived can help policy makers to determine whether a sector requires Government help for example in the form of R&D funding or subsidies (Littlewood et al., 2013a). One of the most interesting and powerful capabilities of TEA is sensitivity analyses which involves varying the value of one or more specific parameters (e.g. feedstock price or enzyme loading) and evaluating the degree to which the process efficiency and overall economic picture changes.

Examples of TEA for cellulosic bioethanol technologies can be traced back to the 1980's and include studies on the economics of saccharification and feasibility studies on enzyme-based ethanol plants (Aden and Foust, 2009). However, it is likely that the most influential TEA models for 2G ethanol biorefinery processes have been created by the US National Renewable Energy Laboratory (NREL) and collaborators (Aden et al., 2002; Humbird et al., 2011; Wooley et al., 1999c). Since the mid-1990s, as part of a United States of America strategically funded research programme to develop cellulosic ethanol technology, several ground-breaking, comprehensive studies were published which have described the entire 2G

biorefining process in the form of granular chemical engineering process models. From these, researchers were able to develop economic models which have enabled the cost of ethanol production to be estimated and how it could change by altering process design, operational parameters and key assumptions (Wooley et al., 1999c). Such early models provided a sound basis on which to develop further technologies and underpin industrial and national strategic thinking in the area of renewable ethanol biofuels (Aden and Foust, 2009).

The NREL process models were founded on substantial research and development and included all the stages of biorefining. They started with feedstock cost, handling and pre-processing; hot, dilute acid pre-treatment and partial hydrolysis; neutralisation and removal of fermentation inhibitors; simultaneous saccharification and co-fermentation; product recovery (distillation); water recovery and waste treatment including combined heat and power generation. The models were published along with highly detailed process flow diagrams (PFDs), references to data on equipment and plant construction, along with economic evaluation. The PFDs were modelled using ASPEN Plus™ software (Aspentech, 2020) to develop mass and energy balances. The PFDs were then used to design equipment and derive purchase and installation costs, and then plant investment and operating costs. This information was used to inform discounted cash flow (DCF) analyses from which the cost of ethanol production (MESP) could be calculated (Wooley et al., 1999c).

Although the NREL lignocellulose-to-ethanol models were very comprehensive, they were highly focused, being designed for exploiting strategic feedstocks such as yellow poplar, corn stover and or switchgrass and using a specific set of unit processes (Aden et al., 2002; Humbird et al., 2011; Wooley et al., 1999c). However, the immense detail and supplementary information supplied with these models has provided opportunities for many other researchers to develop further models for different feedstocks and processes.

TEAs thus provide the opportunity to evaluate many parameters *in-silico*, exploiting novel experimental, as well as published information and data for the component processing steps.

They provide a low-cost and low-risk route to assessing the potential of a process and variations therein and help to inform decisions on development of pilot and larger scale facilities. At the current time there are probably in the order of 70-100 or more published TEAs concerning lignocellulosic ethanol processes portraying a wide range of aims and objectives. Many of the TEAs model a single process scheme of interest, for example a specific feedstock and pre-treatment, with the purpose of assessing potential economic viability and to perform sensitivity analyses to identify areas of economic risk (much like several of the milestone NREL models). Others make broader comparisons, such as between different feedstocks. Examples of these are discussed in section 4.2. Finally, and most importantly for this article, a select number of TEAs have also been carried out specifically to investigate and compare the economic impacts of different pre-treatment technologies. These are described in section 4.3.

## **4.2 Techno-economic analyses of process schemes.**

### *4.2.1 TEA of individual pre-treatments*

It is appropriate to emphasise the influence of the NREL and their collaborative studies on conversion of yellow poplar, corn stover and switchgrass lignocellulose to ethanol. From the outset (Aden et al., 2002; Aden and Foust, 2009; Wooley et al., 1999c) the process focused on the use of hot, dilute acid pre-treatments (0.5% sulfuric acid) which hydrolysed the hemicelluloses and a small amount of cellulose to soluble sugars (mostly xylose, arabinose and galactose), releasing acetic acid and creating degradation products such as furfurals. A large proportion of the inhibitory components could be removed by vaporisation. After neutralisation with lime, the insoluble cellulose was subjected to simultaneous enzymatic saccharification and co-fermentation (SSCoF) along with the already soluble xylose. This was achieved using a specialist fermenting bacterium *Zymomonas mobilis* which can

anaerobically ferment both glucose and xylose to ethanol. The ethanol was subsequently recovered by distillation. Waste solubles were concentrated to a syrup and used to produce methane via anaerobic digestion. The methane and waste solids (lignin and underutilised insoluble polysaccharides) were burnt to create steam energy and electricity, some of which was in excess and sold. Further research and development resulted in additional improvements to the process (focusing on corn stover), including the use of ammonia for neutralisation rather than lime to avoid a solid-liquid separation requirement (Humbird et al., 2011). In the latter configuration, a TEA of building and operating an “*n*th-plant” (this assumes that many plants had already been built, thus avoiding the costs of unforeseen development problems and early stage development costs) handling 2,205 tonnes dry matter per day, and producing ethanol to 76% theoretical level, predicted an ethanol selling price of \$2.15/gal at 2007 prices. Interestingly, the largest single component contributing to the minimum ethanol selling price (MESP) was the cost of feedstock and its handling (see Fig. 17 in (Humbird et al., 2011)). This was followed by the costs of cellulase enzymes and then wastewater treatment and pre-treatment and conditioning. In a limited sensitivity analysis, (excluding feedstock), the most influential criteria comprised variations in total capital investment, enzyme loading and efficiency of hydrolysis.

There followed numerous TEA studies, often building on and utilising data from the NREL published work, to individually assess a range of other pre-treatments, usually with other feedstocks reflecting differences in lignocellulose availability across the world.

**[NOTE:** although many of the studies produced estimates of MESP, they were spread across several decades. It is thus difficult to make comparisons between MESP from different studies without mathematically normalising the data to a given year (as done by NREL (Humbird et al., 2011)). However, comparisons within a study and sensitivity analyses are still helpful.]

For example, several TEAs have been performed on solvent-based pre-treatment processes. Shafiei and colleagues (Shafiei et al., 2011) performed a TEA on a hypothetical

ASPEN-plus modelled production of ethanol and biogas from spruce wood. Pretreatment involved solubilising wood cellulose in concentrated N-methylmorpholine-N-oxide (NMMO) followed by reprecipitation and SSF to produce ethanol. Non fermented sugars, including hemicellulosic moieties, were sent to AD to produce biogas. Sensitivity analysis indicated that the most influential variables were enzyme price and feedstock price, whilst the main costs contributors to ethanol production were the pretreatment stage, hydrolysis and fermentation, and feedstock costs. Other researchers, building on NREL data and using ASPEN Plus™ modelling, performed a comprehensive TEA on a model formiline pre-treatment biorefinery to convert wheat straw into ethanol and valuable by-products such as furfurals and lignin (Zhao and Liu, 2019). For a 200,000-dry tonne per year processing facility, ethanol production costs were estimated to be as low as \$196/tonne. Sensitivity analysis highlighted the importance of feedstock price which, if too high, could negate even the benefits of co-product value. By assessing available data the authors proposed that the variability in feedstock price was principally dependent on logistics for harvesting, storage and transport of this low-density substrate. In a more limited scenario, researchers in Germany developed a TEA on a beechwood-to-glucose (not ethanol) pilot-scale biorefinery involving ethanol/water Organosolv pre-treatment (Laure et al., 2014). With a hypothetical 400,000 tonnes dry wood per annum, the study highlighted the importance of selling C5 sugars and lignin co-products and avoiding waste streams on viability and were able to compare variations in the pulping process.

Other TEAs of specific processes and individual feedstocks include evaluation of AFEX, LHW and phosphoric acid pre-treatments. Bals et al (Bals et al., 2011) developed a TEA to investigate the impact of AFEX pretreatment conditions on ethanol costs using an Aspen Plus™ model. This limited study highlighted the wide variation in MESP which arose when varying processing conditions; it also demonstrated the effects of varying ammonia loading and residence time on the economics of ethanol production, the importance of plant size and production of other products. In a study considering LHW as the pre-treatment of choice, a

TEA of a potential bamboo lignocellulose-to-ethanol biorefinery derived from the 2011 corn stover NREL model (Humbird et al., 2011) was made using Aspen Plus™ software to assess the potential of such an industry in China (Littlewood et al., 2013b). The study was used to evaluate the potential competitiveness of a bioethanol supply chain, analysing the main contributory costs to MESP. The research highlighted the primary importance and sensitivity of feedstock costs and handling (the authors highlighted this as a common trend for biomass-to-ethanol TEAs), followed by the costs of saccharification and fermentation, then pre-treatment and wastewater treatments. The study also highlighted the benefits of CHP exploitation of wastes in keeping with the earlier NREL study (Humbird et al., 2011). Finally, in an assessment of dilute phosphoric acid instead of sulfuric acid for dilute acid steam explosion pre-treatment, Gubicza and colleagues carried out a TEA of ethanol production from sugarcane bagasse using SCoF of hexoses and pentoses (Gubicza et al., 2016). Their analysis suggested that the main contributors to the cost of ethanol production were feedstock costs and CAPEX, although in a sensitivity analysis, the ethanol yield had the largest effect on MESP. For a 300,000 dry tonne pa. plant, they estimated a best case MESP of \$0.5038 per litre.

#### *4.2.2 Use of TEA in the comparison of feedstocks*

TEA provides an ideal opportunity to compare, virtually, the impact of different feedstocks on a process under similar operating conditions. Several such studies have been carried out over the last 20 years. In one of the earliest examples, ASPEN Plus™ software, was used in a TEA to compare four different feedstocks: aspen, switchgrass, corn stover and hybrid poplar, by exploiting published data on their biorefining properties and compositions (Huang et al., 2009). The process model was based on an early NREL cellulosic ethanol process model (Aden et al., 2002) and other data (Wooley and Putsche, 1996) using similar operating conditions, including those for dilute acid pre-treatment and downstream enzymatic saccharification and co-fermentation. The TEA focused on the role of feedstock

type and composition on the production of ethanol and electricity from a plant using 2000 tonnes dry matter feedstock per day. Their results suggested that the production of ethanol was generally in line with the amount of holocellulose (cellulose + hemicellulose) provided, regardless of the feedstock. Aspen wood had the highest holocellulose content and consequently gave the largest ethanol production yield per tonne of dry matter. Interestingly, the amount of electricity generated was not linearly proportional to the amount of non-holocellulose combustible material. At the time of modelling, corn stover gave the lowest minimum ethanol selling price. Ethanol production costs were shown to decrease with increasing plant size, although this also led to increased transportation costs because of the wider area from which feedstock had to be sourced. They also reported that “as expected”, the costs of ethanol production were dependent on the feedstock price on delivery.

A further in-depth TEA to compare feedstock types again used an Aspen Plus™ to model NREL processes, this time for thermochemical mixed alcohol production and biochemical conversion pathways (Daystar et al., 2015). Financial and environmental assessments were performed to evaluate and compare the use of switchgrass, sweet sorghum, eucalyptus, unmanaged hardwood and pine feedstocks. Switchgrass and pine with high ash and lignin contents respectively, had lower conversion yields and this resulted in higher environmental impacts and lower financial performance for both thermochemical and biochemical processing. Also, they reported that the thermochemical conversion route gave lower GHG emissions in relation to biofuel produced.

A more recent TEA involved evaluating the impact of feedstock type in relation to ionic liquid pre-treatment and saccharification relevant to biofuel production (Baral and Shah, 2016a). The study, modelled in SuperPro Designer® software (Intelligen), reportedly involved assessing a  $113 \times 10^6$  litre/year cellulosic ethanol biorefinery and used 1-ethyl-3-methylimidazolium acetate ionic liquid. However, the model stopped at sugar production and did not include the fermentation and product recovery stages. Three feedstocks were

compared: corn stover, switchgrass and poplar, and the estimated sugar production costs were similar (between 2.7 and 3 \$/Kg). Interestingly, sensitivity analysis showed that for each feedstock, sugar production costs were most sensitive to the recycling of the IL and IL costs. The latter was also the most expensive cost component.

#### *4.2.3 TEA of conversion of lignocellulose to products other than ethanol*

Numerous studies have used TEA to evaluate the conversion of lignocellulose not just to ethanol but to other products. Several TEAs have been carried out on the production of lactic acid which can serve both food and non-food (e.g. plastics) industries. Liu and colleagues (Liu et al., 2015) built on the NREL bioethanol process (Humbird et al., 2011) and developed a lignocellulose to lactic acid process in Aspen Plus™ software using corn stover as the feedstock and using an engineered strain *P. acidilactici* TY112. The process, which also sought to minimise waste-water production, included a high level of detoxification of the dilute acid pre-treated stover. The minimum lactic acid selling price (MLSP) was \$0.523 per Kg, close to that of commercial lactic acid made from starch at the time, even though the process did not exploit pentose sugars. The main contributors to selling price was feedstock cost (nearly half) and enzyme costs. Researchers at Stellenbosch University have also carried out TEAs to evaluate the conversion of sugarcane lignocellulose to lactic acid. One of these explored scenarios to add value to sugar cane bagasse and leaves by converting both C5 and C6 sugars to LA (Gezae Daful and Görgens, 2017). They developed 6 processing scenarios for exploiting separately the hemicellulosic-rich liquid fraction and cellulose-rich solids fraction from steam exploded bagasse and leaves. These included investigating the effects of neutralisation or use of an acid-tolerant thermophilic lactic acid bacterium. Aspen Plus™ software was used to model the processes and produce mass and energy balance data and information on equipment scales from which to carry out economic analyses and environmental impact assessments. The most economically and environmentally beneficial scenarios involved use of  $Mg(OH)_2$  for neutralising the steam

exploded effluents, followed by its recycling using triethylamine. No sensitivity analyses were reported. A similar TEA study was also carried out in the same research group in which steam exploded sugarcane lignocellulose streams were respectively used for a combination of ethanol and lactic acid production, along with electricity generation from residual materials (Mandegari et al., 2017). They also used similar approaches to evaluate the conversion of sugarcane lignocellulose to xylitol, citrate and glutamate along with electricity generation (Özüdoğru et al., 2019) and butadiene (Farzad et al., 2017).

NREL extended their R&D to produce other chemicals from lignocellulose feedstocks resulting in the publication of several TEAs. In a significant departure from bioethanol production, researchers evaluated the economic potential for producing hydrocarbon fuels from lignocellulose (Bidy et al., 2016). The research focused on conversion of (generic) lignocellulose to biodiesel via pretreatments, saccharification followed by microbial production of lipids and their conversion to renewable diesel blendstock (RDB). In a scenario that modelled the production of RDB alone, the MFSP was very high (circa \$9.55 / gasoline gallon-equivalent). However, this could be lowered significantly (to \$5.28/gal) by a modelled co-production of succinic acid from the hemicellulose stream released by pre-treatment. Although uneconomic at the time, the study exemplified the way that TEAs may be used to help to investigate routes to address economic hurdles and determine strategic R&D priorities whilst avoiding expensive pilot plant construction. NREL also carried out TEA studies on the gasification of lignocellulose to produce syngas from which hydrocarbons including ethanol may be produced (Dutta et al., 2012; Humbird et al., 2011). Whilst no pre-treatment or saccharification was involved, it is worthy of consideration because it demonstrates the potential to use TEA to compare completely different processing approaches. It is also worthy of note that the highest cost contribution came from feedstock costs. TEA has also been employed for analysing the economics of producing butanol from lignocellulose. Using an acetone-butanol-ethanol fermentation process modelled in SuperPro Designer® software, Baral and Shah comprehensively evaluated the economics of

converting corn stover (Baral and Shah, 2016b). As well as estimating butanol production costs of between \$1.5 and \$1.8 /L, the study involved careful sensitivity analyses. Whilst the largest contributor to overall cost of butanol production was the feedstock cost, the most sensitive parameter was that of butanol recovery. Optimizing the most sensitive parameters allowed a butanol production cost of \$0.6/L to be proposed.

#### *4.2.4 Use of TEA to improve the economic efficiency of the biorefinery process*

The development of in-silico process models from experimental results and previously published data has enabled researchers to assess a range of processing permutations from economic and sometimes environmental perspectives. This has often been augmented by the implementation of sensitivity and Monte Carlo analyses. An important aspect of the cellulose-to-ethanol biorefining process is the level of solids loading (Elliston et al., 2013) and an early TEA on converting softwood to ethanol indicated an economic benefit of increasing the concentration of insoluble solids during saccharification (Wingren et al., 2003). In that study, a biorefining process based on hot-dilute acid pre-treatment followed by SSF was modelled in Aspen Plus™ software from which a TEA was derived. The authors predicted that increasing the insoluble solids from 5% to 8% during SSF could decrease production costs by 19% and this could be augmented by various other process improvements such as steam recovery. In addition, they compared SSF and SHF approaches for enzymolysis and fermentation and concluded that SSF gave higher ethanol yields and lower capital costs compared with SHF. However, SSF had its own challenges, particularly the recirculation of yeasts. More in-depth experimentation followed by TEAs have been carried out on conversion of corn stover and wheat straw to bioethanol at high solids loading (Kadhum et al., 2017). Based on the NREL process (Humbird et al., 2011), new experimental data and modelled in SuperPro Designer®, the studies indicated that at a solids loading of 45% a final ethanol concentration of nearly 116 g/l could be achieved after fermentation for 60h. This not only predicted a significant economic benefit, but also a much-

reduced environmental impact. Sensitivity analysis indicated that the most sensitive variables were ethanol selling price, followed by plant capacity and then cost of feedstock. A further NREL-derived (Humbird et al., 2011) ASPEN Plus™- based processing model and TEA study has been recently performed (Larnaudie et al., 2019). The study assessed the conversion of switchgrass to bioethanol involving LHW pre-treatment and production of high value chemical co-products such as furfural and formic acid. The outputs highlighted the importance of solids and enzyme loadings and fermentation efficiency on MESP and the influence of feedstock composition. Recently (as mentioned in Section 2) research at NREL on deacetylation and mechanical refining (DMR) processing has permitted high sugar concentrations of up to 230 g/L to be achieved, enabling ethanol concentrations of up to 10.9% (v/v) (Chen et al., 2019, 2016). A TEA on the process suggested the DMR approach has considerable promise (Chen et al., 2015). The importance of solids loading has also been highlighted in a recent metabolic modelling study which explored yeast cell and substrate loading within and SSF process which led to an increase in ethanol production of up to 65g/l (Unrean et al., 2016). As metabolic modelling becomes more advanced, so too will the complexity and scope of TEA studies.

Surfactants, across a range of concentrations, have been found to improve the enzymatic hydrolysis of lignocellulose and subsequent fermentation yields. This is thought to be due to a combination of factors including the reduction in the adsorption of cellulases to lignin, the protection of enzymes from denaturation, and generally enhancing their accessibility to cellulose (Börjesson et al., 2007; Li et al., 2012; Qing et al., 2010). The potential economic and environmental impact of surfactants in biorefining have recently been considered in a TEA based on a SuperPro Designer® model of NREL processes and incorporating experimental data from saccharification and subsequent fermentation of hot acid pre-treated banagrass (Kadhun et al., 2018). The research involved assessing the effects of a range of polyethylene glycol (PEG) surfactants and Tween80 at between 0 and 8% (w/w) on ethanol production. The subsequent TEA showed that PEG6000 at an addition of 2% (w/w) provided

the best return on investment (ROI) at 3.29% and reduced global warming potential but was less effective at above or below this level.

Additional attempts to increase the efficiency of cellulose-to-ethanol biorefining processes include the intensification of the process by minimising the number of steps. Whilst a TEA has yet to be performed on such modifications, the concept of a “1-pot” design in conjunction with production of HMF has been suggested (Sadula et al., 2017).

#### *4.2.5 TEA studies on the integration of 2G with 1G processing*

The economic risks of embarking on commercial 2G ethanol production are well known. Although much of the process is tailored to the exploitation of lignocellulose (pretreatment, saccharification etc), some of the process steps are very similar to those used in 1G biorefining, for example separate fermentation and distillation and recovery of ethanol. Therefore, it has long been considered that one way to reduce the investment risks in 2G biorefining is to try and integrate it with already operational 1G processing facilities. An early example of this involved an in-depth TEA of integrating the conversion of lignocellulose from sugarcane bagasse and leaves to ethanol with a sugar-based ethanol biorefining process (MacRelli et al., 2012). The 2G component involved hot (steam) dilute acid pre-treatment followed by separate enzymatic hydrolysis and fermentation. Exploitation of sugarcane leaves as well as the bagasse, along with integration with the 1G process resulted in a “1G+2G” ethanol process that was much less expensive (in terms of MESP) than 2G biorefining alone, but more expensive than the reference 1G ethanol scenario. In total, 15 scenarios were evaluated and sensitivity analysis highlighted the importance of enzyme costs and electricity selling price.

Another TEA of 1G-2G process integration concerned the exploitation of wheat bran as the lignocellulose substrate. The proposed process involved combining the outputs of 1G grain liquefaction and fermentation with those of 2G bran pre-treatment, saccharification and

fermentation for distillation and ethanol recovery. The combined solid and liquid stillage would then be used to grow filamentous fungi so as to increase their value as animal feed (Rajendran et al., 2016). The process was based on an operational reference grain-ethanol plant and was modelled in Aspen Plus™ software. The TEA suggested that whilst the integrated process required a greater capital investment, the NPV was considerably higher.

#### *4.2.6 TEA and Supply chain modelling*

TEA has also played an important role in elucidating the influence and optimisation of supply chains for both woody biomass and agricultural residues. As an example an assessment of Scottish woody biomass supply chains has been carried out in relation to the production of high quality lignin, hemicellulose and cellulose using an Organosolv and ultrasound pre-treatment (Bussemaker et al., 2017). The study highlighted the importance of moisture content and drying options. It also revealed that whilst sawmill chips were normally the best feedstock, sawn logs became “competitive” if they could dry passively to a moisture content of 30% or less. The analyses suggested that supply chain options could thus be used to help to address key bottlenecks in the biorefining process. A comprehensive study on wheat straw supply chains for biorefining and energy production was also carried out in Germany (Petig et al., 2019). The study highlighted the often-missed trade-off between the benefits of economies of scale and the increasing costs of biomass supply (as distance was increased). The study also stressed the complexities in supply resulting from farm specialisation and regional differences and the impacts of high feedstock prices. Hence, in keeping with the frequent identification of feedstock prices as a key component of MESP, and as a very significant source of MESP sensitivity mentioned many times above, the two studies on supply chain economics demonstrate why it is critical to consider the whole supply (and product) chains as well as location in relation to the economic viability of prospective 2G biorefining activities. Complementing these studies, and turning this approach around, TEA can also be used to evaluate which, of several processes would be the most economically

beneficial to exploit biomass sources in a specific location. This has been carried out for a modelled biorefinery on Maui Island Hawaii. The study compared the utilisation of two lignocellulosic feedstocks (banagrass and energycane) using six technology pathways including biochemical and thermochemical routes (Rajendran and Murthy, 2017). Prices for the different products (ethanol, ethyl acetate, dodecane, ethylene and electricity) were compared economically and in relation to environmental impact. The most profitable scenario involved producing ethyl acetate from banagrass utilising both hexoses and pentoses. The authors drew attention to a range of influences including oil prices and moisture content of biomass. A sensitivity analysis also highlighted the importance of feedstock and product prices.

#### **4.3 Techno-economic analysis to compare the relative merits of different pre-treatments**

In the conversion of lignocellulose to ethanol, pre-treatment has often been referred to as the most expensive processing and capital investment step (Cheah et al., 2020; Tao et al., 2011a). However, this will depend on where the boundaries of pre-treatment are set and whether different pre-treatments incur higher or lower costs elsewhere in the process. Hence, there has been a great deal of interest in evaluating the impact of different pre-treatments on the economic viability of the whole process and several TEAs have been carried out accordingly. In these TEAs, the general aim has been to compare several pre-treatment technologies within the same “*in-silico*” model of a biorefinery. By keeping as many other criteria as equal as possible, including the methods used in calculating the OPEX and CAPEX, mass and energy flows, DCF and IRR, “*n*th” plant assumptions, plant life and methods for calculating a comparative NPV or MESP for a specific time (date), reasonably effective comparisons might be made (Eggeman and Elander, 2005; Tao et al., 2011a). It would also ensure that any assumptions and unidentified errors in non-pre-treatment processes along with sensitivity analyses and other scenario mapping would be the same in each pre-treatment test case. Of course, there are likely to be challenges due to

differences in the current states of development of different pre-treatment process and this may require additional financial modelling (Eggeman and Elander, 2005). Eleven published TEAs are considered in this section and aspects of the pre-treatment comparisons are summarised in Table 1.

One of the earliest studies compared the economics of one and two step steam pre-treatments in the production of bioethanol from spruce (Wingren et al., 2004). One step steam pre-treatment resulted in the production of considerable quantities of inhibitory breakdown products such as furfurals, levulinic and formic acids from hemicelluloses, HMF from glucose and phenolic moieties from lignin. A two-step pre-treatment was therefore designed with an initial low-severity, acid-catalysed steam treatment to maximise hemicellulose recovery (mostly mannan from spruce) whilst minimising breakdown followed by a second acid catalysed steam treatment to optimise enzymatic saccharification of residual cellulose. Building on methodology and data sources used in a previous TEA (Wingren et al., 2003), the processes were simulated for a 200,000 ton/year facility using Aspen Plus™ software. After careful evaluation, the authors found that the two-step process showed some promise and benefited from a lower enzyme requirement, used less water and resulted in a higher ethanol yield during the SSF process. It also provided opportunities for further optimisation which might lower the ethanol costs further. Unfortunately, however, the two-step process had more process steps (such as two steam pre-treatments and a washing step to recover hemicelluloses etc in-between) and this resulted in higher CAPEX and energy use. As a result, the overall cost of ethanol production was the same for both processes (circa \$0.551 /L). Further research was proposed to enhance the two-step process. In both scenarios the cost of CAPEX was the main contributor to the cost of ethanol, closely followed by the cost of the feedstock.

Soon afterwards, and building on the extensive cellulosic ethanol research programme and early process modelling (Aden et al., 2002; Wooley et al., 1999c; Wooley and Putsche, 1996), an Aspen Plus™ simulation of a full bioethanol facility based on an NREL base case

Table 1: Summary of TEA comparisons of pre-treatments

STUDY	PRE-TREATMENTS COMPARED												TECHNICAL ASPECTS				SELECTED CONCLUSIONS					
	HDA	DHDA	STEX	SO <sub>2</sub> ST	LHW	AFEX	WO	ORG	Lime	SAA	ARP	Alk	BIO	CBP	Feedstock	Product	Plant Size	NREL base	soft-ware	Best Pre-treatment (relative order of MESP or equivalent, lowest first)	Most significant cost	Most sensitive cost components
Wingren et al (2004)	Y	Y													Spruce	Ethanol	200,000 t/y.	N	A	Both similar	CAPEX; feedstock	Ethanol yield
Eggeman and Elander (2005)	Y		Y	Y	Y			Y							Corn stover	Ethanol	2000 t/d	Y	A	All similar on basis of all C5 and C6 utilisation. Otherwise, HDA is best. Feedstock	Fixed costs and depreciation;	
Laser et al (2009)	Y				Y							Y			Switch grass	Ethanol	4500 t/d	Y	A	AFEX<DA (assumes more advanced technology)	Feedstock	
Kazi et al (2010)	Y	Y		Y	Y										Corn Stover	Ethanol	2000 t/d	Y	A	Similar; HDA<AFEX<DHDA<LHW; only LHW and DHDA are higher price.	Feedstock; enzymes	Feedstock; enzymes
Tao et al (2011)	Y	Y	Y	Y	Y			Y	Y						Switch-grass	Ethanol	2000 t/d	Y	A	Similar on basis that all C5 and C6 utilisation, except for SAA with lower sugar and ethanol yield	Feedstock; CAPEX	Feedstock
Humbird et al (2011)	Y														Corn stover	Ethanol	2000 t/d	Y	A	N/A	Feedstock; Enzymes	CAPEX; enzyme loading
Kumar and Murthy (2011)	Y		Y		Y					Y					Tall fescue	Ethanol	250,000 t/y	Y	SP	Generally similar	Feedstock; enzymes	Feedstock; pentose
Littlewood et al (2013)	Y	Y	Y	Y	Y										Wheat Straw	Ethanol	2000 t/d	Y	A	Generally similar (except cheaper for WO if current costs used)	Feedstock; Enzymes	Feedstock; enzymes
Da Silva et al (2016)	Y				Y	Y									Corn stover	Ethanol	88.5 t/h	Y	A	LHW = AFEX << HDA but assumptions different to NREL		
Baral and Sah (2017)	Y	Y	Y	Y	Y						Y				Corn Stover (for b'ol)	Sugars	189 mL/y	Y	SP	HDA=STEX<AFEX<<<BioI	Feedstock; Enzymes	Feedstock; saccharification yields
Da Silva et al (2019)	Y	Y	Y	Y	Y			Y							Corn stover	Ethanol	97 t/h	Y	A	HDA is best. No proper comparisons of others.		
Kapanji et al (2019)	Y	Y													Sugar-cane	Glutarate +sorbitol				HDA<STEX (no fermentation step)		

Key: A:Aspen Plus™; SP: SuperPro Designer®; see main text for other abbreviations

(Aden et al., 2002) was developed to carry out a comparative TEA of five pretreatments (dilute acid, hot water, AFEX, ARP and lime) (Eggeman and Elander, 2005). The model assumed the processing of 2000 tonnes (dry) per day of corn stover. Projected financial flows derived from CAPEX, OPEX and likely revenues were integrated into a DCF comprising 3 years pre operation and 20 years of commercial operation. Financial comparisons between the different pre-treatment scenarios were made using MESP.

The results showed that CAPEX for DA, AFEX, ARP and Lime pre-treatments were generally similar at between 22 and \$28M, although these were distributed between different equipment items in each case. For example, dilute acid hydrolysis pre-treatment CAPEX was dominated by the cost of the reactor. CAPEX for HW pre-treatment was much less expensive at about 1/5th of these values. However, when considering total fixed capital in terms of \$/gal annual capacity, they all fell between 3.3 and 4.5 \$/gal which in comparison to 1G corn ethanol plants (circa \$1.00-1.5/gal of annual capacity), was very capital intensive. Interestingly, the ideal pretreatment case (no cost) still gave a high total fixed capital \$/gal annual capacity and the "no pre-treatment" case, due to poor yields, was over 5 times the cost of the main pretreatments at over 22 \$/gal.

On the assumption that the enzymatically released C5 and C6 sugars would be effectively fermented to ethanol, **the TEA showed that there was very little difference between the modelled economic performances of the different pre-treatment processing options.** Although some pre-treatment reactors were cheaper than others, lower costs for pre-treatments were generally offset by higher costs elsewhere in the process. These included pre-treatment catalyst recovery, or greater expense in the recovery of the ethanol product (Eggeman and Elander, 2005). Nevertheless, the authors suggested that differentiation might be enhanced by further optimisation activities e.g. improved enzymes. In keeping with many other studies, the main contributing costs to MESP were the cost of feedstock and fixed costs (including depreciation).

In a further collaborative study involving NREL, and building on the earlier base case (Aden et al., 2002) and other NREL data, a comprehensive Aspen Plus™ modelled processing system was produced from which to develop comparative TEAs of four pre-treatment processes — dilute acid, two-stage dilute acid, hot water and AFEX (Kazi et al., 2010). These were evaluated in conjunction with several downstream processes — pervaporation recovery of ethanol, separated C5 and C6 sugar fermentation and on-site production of enzymes. By assuming “*n*th plant” construction (see above) the product value (PV: ethanol production costs incorporating a 10% return on investment) was compared for the different scenarios and several sensitivity analyses were performed. In addition, current experimental data on yields were used rather than projected data. The most cost-effective combination was the standard dilute acid pre-treatment, due mainly to the high sugar and therefore ethanol yields. However, its PV (\$1.36/LGE [Litre gasoline equivalent]) was close to that of dilute acid with high solids (\$1.44/LGE) and AFEX (\$1.47/LGE). Only hot water pre-treatment and two-stage dilute acid pre-treatments had moderately higher PVs (\$1.77 and \$1.75/LGE). Other processing permutations also made little difference. However, sensitivity analyses revealed that the most important parameters affecting PV were feedstock costs, enzyme costs and overall costs of installed equipment. For the dilute-acid pre-treatment, the CAPEX was dominated by the costs of the combustor, boiler and generator facilities. As in other studies, the relative similarity of ethanol costs for different pre-treatments belied the fact that the CAPEX values differed considerably. However, low CAPEX for some pre-treatments (e.g. LHW) was counteracted by higher costs elsewhere in the process. The authors also considered the more expensive costs of a pioneer plant, which was estimated to increase ethanol production costs considerably compared with an “*n*th plant”.

The most recent collaborative NREL TEA for the comparison of cellulosic-ethanol pre-treatment technologies focused on switchgrass and evaluated six pre-treatment processes — hot, dilute acid, LHW, AFEX, lime, soaking in aqueous ammonia (SAA) and SO<sub>2</sub>-impregnated steam explosion (Tao et al., 2011b). The TEA involved some of the most

highly experienced biorefinery research groups in the US and key industrial organisations. The overall process model derived from the earlier “*n*th plant” study (Aden et al., 2002) was adapted for the different pre-treatments and simulated in Aspen Plus™ software to provide material and energy balances. Along with data from numerous sources, OPEX, CAPEX and DCF analyses were performed. The DCF assumed a 3-year pre-operation period followed by a plant life of 20 years. Comparisons were made based on MESP (at 2007 prices) after ensuring a 10% IRR. The different pre-treatments were considered in detail to interpret similarities or differences in the MESPs. In comparing CAPEX and steam and power consumption by the different pre-treatments, it was found that there were considerable differences in the CAPEX associated with each pre-treatment, ranging from \$179 million for LHW through to \$212 million for lime. Within these totals, there were wide variations in the reactor costs as % pre-treatment CAPEX, ranging from 18% for LHW to 76% for dilute acid. Large differences were also found in steam use, ranging from 16.5 Kg steam/gal fuel after AFEX pre-treatment through to 61.7 Kg/gal for LHW pre-treatment. These were accompanied by large differences in yields of monomer sugars and oligomer sugars. However, when all the data for costs of feedstock, variable and fixed costs, depreciations, tax and returns on investment were fed into the MESP calculations and assuming that the oligomer sugars could be fermented to ethanol (or saccharified at no extra cost prior to fermentation), **the MESPs were all generally similar**, ranging from \$2.32 to \$2.94 per gallon. Thus, as highlighted in earlier studies, less expensive pre-treatments resulted in higher expenditure in other operations or capital, resulting in limited differentiation between projected costs for ethanol production for most processes, except for those that had lower monomer sugar and ethanol yields such as the SAA process. Interestingly, cost analysis showed that the largest single contributory cost to MESP was the cost of feedstock (45% - 53% MESP across the 6 cases).

In the same year as this study was published, NREL published an updated process design and economic assessment for converting lignocellulose (corn stover) to ethanol (Humbird et

al., 2011). As described earlier, the process incorporated a hot, dilute acid pre-treatment and proposed an MESP of \$2.15 /gal (in 2007 USD), significantly less than the earlier \$3.40/gal (2007) proposed by Kazi et al (Kazi et al., 2010).

In addition to the above benchmark TEAs (Eggeman and Elander, 2005; Kazi et al., 2010; Tao et al., 2011b), several other economic evaluations of pre-treatments have been carried out, often utilising data and information from the NREL-derived processes.

In a rather complex set of processing scenarios, TEA analysis of several different pre-treatments for the conversion of switchgrass to ethanol and electricity was undertaken by Laser and colleagues (Laser et al., 2009). These involved comparing a base case process involving dilute acid pre-treatment (Aden et al., 2002) along with a conventional method of power production from residual materials, with two other “mature technology” scenarios. These both used AFEX and “consolidated bioprocessing” (CBP) in which the microbial production of enzymes, polysaccharide hydrolysis, C5 and C6 fermentation occur in one step. They differed in their power generation approaches. The processes were modelled in Aspen Plus™ software and the TEAs suggested that the AFEX “mature technology” improved process efficiency and costs in relation to the base case, to the extent that they would be cost competitive with the current gasoline prices. However, the authors highlighted that (a) the modelled AFEX process was assumed to be much more advanced than technology available and would have to undergo further research and improvement if it was to perform at the levels assumed and (b) the CBP catalytic approach was hypothetical and needed to be developed. In all scenarios the feedstock costs were the largest component of the MESP.

With reference to the NREL 2002 process designs (Aden et al., 2002; Kazi et al., 2010) and other studies, a TEA was carried out to compare dilute acid, dilute alkali, hot water and steam explosion pre-treatments for use in a 250,000 tonne /year Tall Fescue grass biorefinery, modelled in SuperPro Designer® (Kumar and Murthy, 2011). As in other studies, total process CAPEX varied, being cheapest for the steam explosion process due to higher

solids loading. LHW resulted in lowest ethanol production costs, but the price of ethanol was quite similar across all pre-treatment scenarios (\$0.81 to \$0.89/Litre of ethanol). Evaluation of contributory costs showed that the biomass and enzymes together accounted for over  $\frac{3}{4}$  of the raw materials costs, which comprised the largest cost component of the MESP. Sensitivity analysis highlighted the importance of pentose fermentation efficiency and biomass price.

In a departure from evaluating US-relevant feedstocks, a TEA to compare different pre-treatments in the production of bioethanol from UK wheat straw feedstock was carried out by Littlewood and colleagues (Littlewood et al., 2013a). The process, modelled and simulated in Aspen Plus™, was based on the then recent NREL corn-stover biorefinery process (Humbird et al., 2011). The model was tailored to incorporate and compare five pre-treatment technologies — steam explosion ± acid catalysts, LHW, HDA and wet oxidation (WO) — as well as supply chain and legislative drivers and constraints. CAPEX (“*n*th-plant” assumptions) and OPEX were calculated and used in the formulation of a DCF and thence an MESP that reflected a zero NPV at a discount rate of 10%. As in other studies there were variations in the different MESP cost components (including input from electricity generation) that differed across the pre-treatment scenarios. Interestingly, the MESP for steam explosion (+/- catalyst), LHW and dilute acid pre-treatments were relatively close, in keeping with many of the other TEAs above. However, the WO pre-treatment resulted in a rather lower MESP which seemed to be due to considerably lower saccharification and fermentation costs. To date there are few, if any other TEA assessments of WO pre-treatment to provide useful corroboration of this finding. Nevertheless, all the MESP were still uncompetitive with petrol prices, but some could be lowered close to these if enzyme costs and loadings were reduced, to reflect assumed future technological advances. Under these calculations, MESP across all pre-treatments were very similar. One of the largest MESP contributory costs was that of feedstock (37-56% MESP) and sensitivity analysis showed that this fluctuation had a marked impact on MESP.

Also making use of the 2011 updated NREL process model and DCF and MESP calculations, (Humbird et al., 2011) and modelling in Aspen Plus™, da Silva and colleagues carried out a TEA of three different pre-treatment processes for the biorefining of corn stover in a plant with a capacity similar to that of the NREL model of nearly 90 tonnes per hour (da Silva et al., 2016). The study assessed HDA, LHW and AFEX pre-treatments. The aim was to make economic comparisons across pre-treatments that produce differing amounts of breakdown products and having different requirements for the recycling of chemicals. In addition, a range of process scenarios was considered, including differences in solids and catalyst loadings on bioethanol concentration and total energy consumption. The lowest MESP values obtained for optimised processing scenarios for the three pre-treatments were \$1.78/L for LHW, \$1.80/L for AFEX but \$2.55/L for hot, dilute acid. However, they pointed out that their modelling contained a range of different features from the NREL model, including differences in operating hours, plant financing, various variable costs such as cost of feedstock and chemicals and utilities. These contributed to different MESP values when compared with the NREL result. Interestingly, da Silva and colleagues carried out a further comprehensive Aspen Plus™-modelled study a few years later, in which both economic and environmental comparative assessments were carried out to compare HDA, LHW, steam explosion, AFEX and Organosolv pre-treatments in the biorefining of corn stover (Rodrigues Gurgel da Silva et al., 2019). Here, they concluded that the best pre-treatment process, in relation to techno-economic and environmental performance, was dilute hot acid. However, the study did not report comparative MESP values making it difficult to compare with other studies.

In one of the few economic studies to consider biological pre-treatment, Baral and Shah carried out a TEA of a SuperPro Designer® modelled biorefinery process in which it was compared with steam explosion, HDA and AFEX pre-treatments (Baral and Shah, 2017). This study also made much use of previously published NREL studies (Aden et al., 2002; Humbird et al., 2011) as well as other published literature. Although the study was

considered in relation to the commercialisation of biobutanol production via fermentation using *Chlostridium* species, the actual boundaries of the study were limited to the production of fermentable sugars from corn stover. Thus, the economic implications of including fermentation, waste treatment and other integral aspects of a biorefining process were not included within their TEA. Nevertheless, the study highlighted the disadvantages of biological pre-treatment which gave the most expensive sugar production at \$1.41/Kg. This related to the facility-dependent costs associated with the very long pre-treatment period (several weeks). Interestingly, steam explosion and hot, dilute acid pre-treatment processes resulted in the lowest sugar prices (\$0.43 and \$0.42/Kg respectively) whilst AFEX resulted in a sugar cost of \$0.65/Kg. The authors also carried out sensitivity analyses for numerous parameters; the most sensitive for steam explosion and dilute hot acid pre-treatments were feedstock costs and saccharification yields of glucose. In terms of annual operating costs, the most economically expensive parameters for steam explosion and HDA processes was the cost of corn stover. This was superseded for AFEX by costs of utilities and, for biological pre-treatment processing, by facility dependent costs.

Finally, researchers at Stellenbosch used TEA to compare steam explosion and dilute hot acid pre-treatments in the conversion of sugarcane bagasse to sorbitol or glucaric acid (Kapanji et al., 2019). Although none of the scenarios were economically viable, they reported that dilute, hot acid pre-treatment was economically more advantageous than steam explosion. However, since the post-saccharification oxidative conversion of sugars to sorbitol or glucaric acid probably have very low sensitivities to e.g. fermentation inhibitors that affect fermenting microorganisms, it is possible that this comparison is not pertinent to the comparison of pre-treatments in conventional 2G bioethanol production.

## 5.0 Conclusions

### 5.1 General considerations

The aim of this report has been to survey published literature on the wide range of pretreatment technologies for 2G biorefining of lignocellulose to ethanol and, where possible, to compare their relative costs and benefits. Section 2 described 2G biorefining as a highly complex series of processes which must contend with a range of heterogeneous feedstocks. Section 3 showed that there are numerous pre-treatments which vary considerably in their functionality and impact on different feedstocks. Some are well-established and have been used in pilot and commercial plant development (ETIP Bioenergy, 2020). Others have yet to be developed further before commercial exploitation may be considered. It is evident from the TEA assessments (Section 4) that trying to identify an “optimal” pre-treatment is difficult, particularly if the impacts of that TEA across the process (and value) chains are not considered. TEAs that compare the cost-benefits of different pre-treatment processes (Section 4.3), often indicate that the perceived benefits associated with any one pre-treatment are offset by increased costs in other parts of the process chain. Hence, the MESP values are often similar (Table 1). If one pre-treatment could be identified as the most economically beneficial, it is possibly the HDA process, but not as a clear winner. This interpretation is guarded because of the range of assumptions made in different TEA studies.

The expanding TEA literature indicates that criteria other than the costs of pre-treatment often present big challenges to the economic viability of a 2G biorefining industry. Quantification and sensitivity analysis of MESP cost components often highlight enzyme price and saccharification efficiency (Baral and Shah, 2017; Humbird et al., 2011; Kazi et al., 2010; Littlewood et al., 2013a) and occasionally the importance of C5 as well as C6 fermentation (Humbird et al., 2011; Kumar and Murthy, 2011; Littlewood et al., 2013b) (see Sections 4.2 and 4.3 ). However, the most frequently highlighted issue is **feedstock**

**pricing** (see throughout Section 4 for numerous references). Indeed there is evidence that ethanol costs are directly proportional to feedstock costs (Kazi et al., 2010). Therefore, wide variations in pricing would create considerable financial risk. Large fluctuations in UK wheat straw prices were highlighted by Littlewood et al, ranging between £29 and £45 per tonne in 2010 and this did not include transport costs of about £8 per tonne (Littlewood et al., 2013a). They asserted that the often-held view that lignocellulose feedstocks from UK agriculture are just low value by-products with little demand, is a misconception which should be discounted. Indeed, in 2019, UK big bale straw prices reached about £90 per tonne (AHDB, 2020). The authors also suggested several factors that probably determine the amount of straw available for producing biofuels. These include the need to retain straw to protect soil and water resource; the potential profit farmers might secure from selling straw; and the cost incurred in fertilizer applications to replace nutrients removed with the straw. To date, few if any TEAs have been sufficiently broad to include these important externalities, even though they would have considerable influence on feedstock supply and therefore price. As a result, although advances in technology might help to optimise the conversion of lignocellulose to ethanol, consideration of the whole supply chain is critical. In addition to feedstock price, the policy environment also has considerable bearing on economic viability. [note uk policy on biomass exploitation]. Kazi et al (Kazi et al., 2010) also pointed out further uncertainties: whilst feedstock and enzyme costs were of concern, it would be difficult to pin down the real market prices until markets were developed.

## ***5.2 Possible ways forward***

Many reviews have been published on lignocellulose pre-treatments and conclusions vary. Much is often made of the need for emerging pre-treatment technologies (Bhatia et al., 2020; Hassan et al., 2018). Some researchers promote research and development relevant to all processing steps to enhance efficiency, lower energy use and reduce costs (Ali et al., 2020; Brown et al., 2020; Cheah et al., 2020). Others have highlighted particular enhancements

such as increasing fermentation efficiency through biotechnological improvement (Avanthi et al., 2017), or enhanced exploitation of co-products by targeted valorisation (Duque et al., 2017; Ghosh et al., 2015; Ponnusamy et al., 2019; Yoo et al., 2020). There is also a long-standing interest in improving the utilisation of the lignin-enriched residues for example in their entirety as horticultural growing media (Waldron et al., 2014) or through exploitation of lignin components as chemical feedstocks (Liao et al., 2020b). The potential to make 2G residues into animal feeds have been considered through cultivation of fungal biomass (Rajendran et al., 2016). However, co-products and residues are often used in current 2G process models for production of excess steam and electricity (Humbird et al., 2011). Thus, any alternative valorisation attempts will have to add considerable extra value to these moieties above and beyond the current value as an energy source. A further source of financial uncertainty concerns the competition with mineral fuels and oil prices. Rather than concentrating on producing 2G fuels, it may be beneficial to consider the conversion of lignocellulosic sugars to more valuable platform chemicals such as succinic acid. This might address the economic difficulties seen in production of liquid fuels which compete with oil prices, although it will require considerable developments in biorefining. However, it is also possible that the higher value end products may reduce the requirement to exploit the low-cost, often impure sugar sources such as those produced by 2G biorefining.

Whatever route is followed, a TEA approach will reduce the influence of subjective or ill-informed opinion and provide a guide for strategic thinking. The potential impacts of any new or improved pre-treatment process must be considered within the context of a whole-process and whole value-chain, including feedstock characteristics, market size and profitability. Therefore, engagement between technology providers and plant designers, chemical engineers and economists is important if pre-treatment research is to have a chance of being economically beneficial.

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