

DEPARTMENT OF CHEMISTRY

KJ2900 - BACHELOR PROJECT

**Analysis of Pretreatment Methods for
Bio-based Plastic Production Based on
Enzymatic Hydrolysis and Microbial
Fermentation**

Author:

Arne Onsrud

Advisor:

Alexandros Asimakopoulos

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Abstract

Lignocellulosic biomass can be found in substantial amounts on our planet, and it represents a viable alternative to fossil sources for production of carbon-based fuel and high-valued chemicals such as bio-based plastic. Unfortunately, lignocellulosic biomass has a high recalcitrance to conversion via enzymatic and microbial hydrolysis. This makes it necessary to add a pretreatment step in order to make production of high-value chemicals economically viable. There are a multitude of different pretreatment methods available for implementation. However, they all come with their own advantages and disadvantages. This paper presents the major categories within pretreatment of lignocellulosic biomass and compares their ability to reduce the inherent recalcitrance of lignocellulose.

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1 Introduction

With a predominantly steady increase in the awareness of the continued introduction of fossil-based carbon in the atmosphere and in turn its negative effect on the environment, the importance of "green", sustainable alternatives is at an all-time high. This increased societal focus has also been reflected in political rhetoric as well as in scientific journals.^[1] In our close geopolitical sphere, the EU, through its "new circular economy strategy" has pushed for a major refocus on circular economy and the sustainable aspect of material production as well as recycling.^{[2][3]} Their goal is a continued reduction of greenhouse gas emission leading to a net neutral economy for the European mainland in 2050.^[4] Cellulose being the most common organic polymer representing approximately 1.5×10^{12} tonnes annually biomass production has been seen as a viable replacement to fossil-based products.^[5] In some sectors, e.g. transportation fuels, such as jet fuel and diesel, bio-mass derived alternatives are even seen as the only viable renewable option.^{[6][7]} In fact, there are two sources for renewable carbon: plants and the air. The estimated cost per tonnes of extracted CO_2 has for atmospheric carbon capture been reduced substantially, but the current technological solutions are not yet viable as a source of large scale replacement of fossil-based products.^[8]

Plant-based carbon can in turn be differentiated into three divergent production strategies. Firstly, direct use such as the conversion of timber into wood and planks. Natural rubber, starch, sugars, and cotton also fall into this category. The second category is the refining of biomass into high-value chemicals (e.g. plastics, foams, coatings, and adhesives) through hydrolysis reactions such as fermentation or alkaline/acid catalysis. It is predicted that bio-refining technology will play a major role in replacing fuels and chemicals currently based on fossil oil.^[9] The third category is the in-situ production of chemical building blocks and biopolymers in transgenic plants.^[10] Despite the plentiful benefits of using a lignocellulosic biomass as a replacement for fossil fuel, the solution does incur some technological hurdles. First and foremost, the highly specified high value chemical supply chains and the technical difficulties in accessing cellulose sugar within the biomass. Due to the high recalcitrance within the lignocellulosic structure a number of different pretreatment solutions has sprung up in order to reduce cost and increase efficiency in cellulose sugar extraction.^[11] Figure 1.1 shows a schematic overview of the biorefining procedure. This paper will give an overview of different pretreatment techniques applied to the bio-conversion of lignocellulosic material into bio-based chemicals, mainly enzymatic and microbial polymers. As such it will mainly focus on the first of the orange squares seen in figure 1.1.

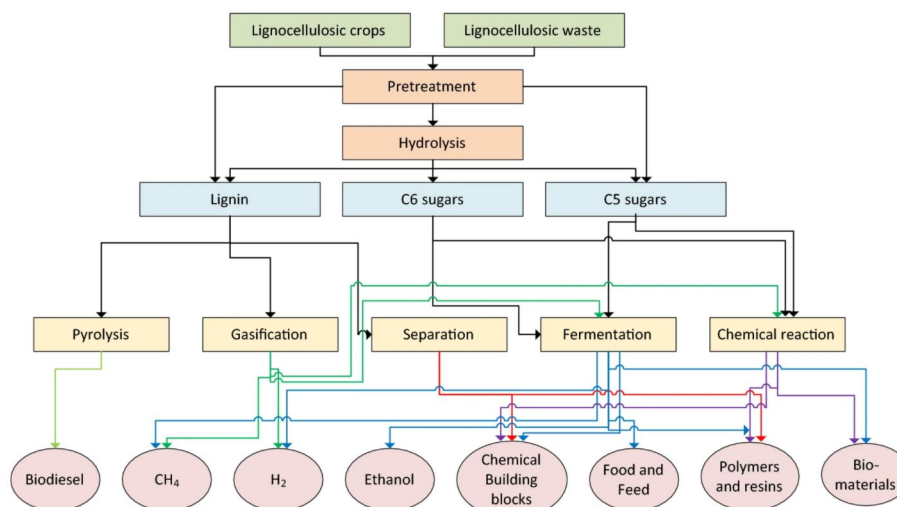


Figure 1.1: Schematic presentation of a biorefinery. Taken with permission.^[12]

2 Bio-based Plastic

Plastics are amorphous organic solid polymers. They are widely used for their merits such as light weight, easy processing and resistance to chemicals and the environment. Plastic polymers are one of the main uses of crude oil, second only to energy production. It is estimated that approximately 10 % of all plastic is incinerated, less than 10 % recycled, and the remaining 80 % stored in landfills or littered into the oceans or nature at large.^[13] The majority of plastic produced today are commodity plastic such as polyethylene (PE), polypropylene (PP), and polystyrene (PS), and the need for a sustainable biodegradable alternative has never been higher. Plastic is classified as either thermoplastic (PE, PP, PS), which can be melt-moulded, or thermosets, which polymerise and cross-link upon heating. Thermoplastic is furthermore divided into four main categories depending on their environmental impact seen below in table 2.1.

Table 2.1: Schematic classification of plastic^[14]

	Bio-based plastics (renewable)	Oil-based plastics (fossil)
Biodegradable plastics	Poly(lactic acid) (PLA) Polyhydroxyalkanoate (PHA) Polysaccharide derivatives (low DS)* Poly(amino acid)	Poly(ϵ -caprolactone) (PCL) Poly(butylene succinate/adipate) (PBS/A) Poly(butylene adipate-co-terephthalate) (PBA/T)
Non-biodegradable plastics	Polysaccharide derivatives (high DS)* Polyol-polyurethane Bio-polyethylene (bio-PE) Bio-poly(ethylene terephthalate) (bio-PET)	Polyethylene (PE) Polypropylene (PP) Polystyrene (PS) Poly(ethylene terephthalate) (PET)

* DS = Degree of substitution.

Bio-based plastic (plastic in which 100 % of the carbon is derived from renewable resources) has the benefit of not adding any extra carbon to the carbon cycle, but bio-based plastic is not necessarily good for the environment either. Its production processes may use environmentally harmful chemicals and the product itself as seen in table 2.1 can be non-biodegradable. Non-biodegradable bio-based plastic can be chemically identical to fossil-based alternatives such as PE, PP or PS. In the latter case, those products will impact the environment just as much as conventional fossil-based products when they have lost their purpose. The standard for biodegradation was standardised by SO/TC61/SC5/WG22 (ISO 472/DAM3, Amendment 3, General Terms and Terms Relating to Degradable Plastics).^[15] In summary, it states that products made with biodegradable plastic should be able to degrade by means of organic recycling in composting plants or in anaerobic digesters. The degradation rate is dependent on the desired design criteria for different products, and it may be fine-tuned by changing factors such as crystallinity, lamellar thickness, and molecular conformation. PLA is listed as biodegradable plastic. However, it does not biodegrade if littered, and it must be delivered to a specific recycling plant. On the other hand, the short chain version of PHA is biodegradable without the need for composting^[16] Bioplastic such as PLA and PHA, has, due to its advantage as a low carbon impact material and high biodegradability, rapidly emerged as a viable contender to fossil-based plastic. PHA and PLA are both biodegradable biopolymers. PLA can be polymerised through standardised fermentation, whereas PHA is a biogenic polyester produced in microbial cultures. This gives PHA a large variety of different biomass to use as a source for production, and it has the added benefit of making it possible to tailor its properties to fit specialised products as well as current fossil-based commodity plastic.^[17]

The global production of bio-based plastic amounts to just over 1 % of total plastic production. But the demand is rising, and as the technology matures and the economy of scale is put in place, the price is estimated to reduce sharply. European bioplastic interests estimate an increase in bioplastic production from current 2.11 million tonnes to approximately 2.87 million tonnes in 2025.^[18] As seen in table 2.1 above and a bit more nuanced in figure 2.1 below, bio-based plastic is now being used to produce a multitude of different types of commodity plastic.

Global production capacities of bioplastics 2020 (by material type)

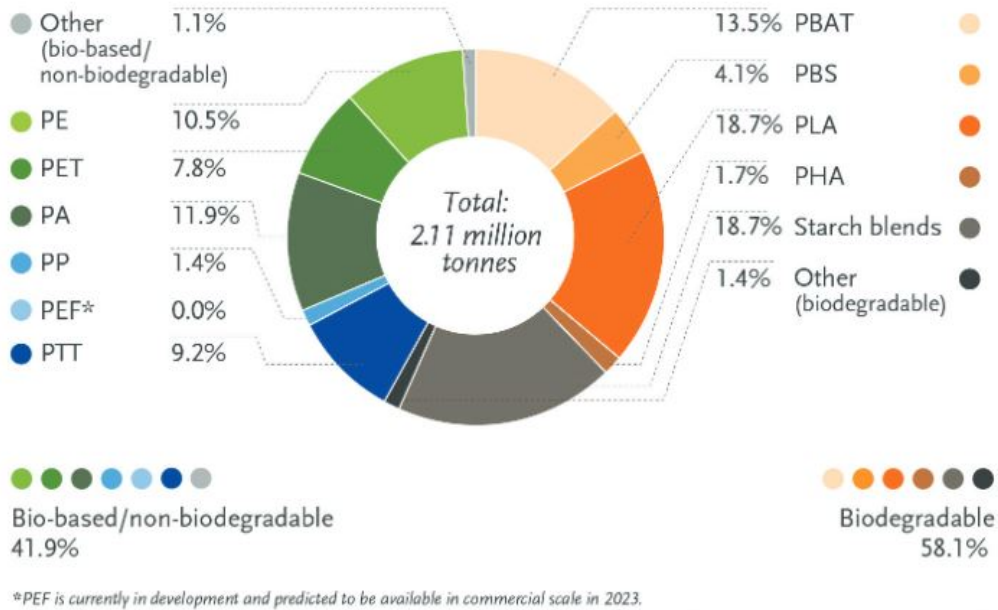


Figure 2.1: Bioplastic market in 2020 as per European Bioplastics data. Taken with permission. [18]

3 Lignocellulose

Lignocellulosic biomass (LCB) is a renewable resource from both terrestrial and aquatic plants. It is composed of the two polysaccharides; cellulose and hemicellulose, the aromatic polymer lignin, and some minor traces of ash, proteins, and pectin. [19] Lignocellulosic biomass (LCB) is a favoured alternative to fossil resources and may replace it in some fields in the coming year. LCB, mainly from second-generation sources such as non-edible feedstock and forestry feedstock, is particularly of interest since its use will not interfere with food production but aims at utilising the non-edible residue, that otherwise would have gone to waste. The main hurdle in accessing the energy stored in LCB is to overcome the materials recalcitrance (resistance to degradation), of which there are a multitude of factors divided into direct and indirect factors. The direct factor for recalcitrance refers to accessible surface area. The indirect factors include biomass structure-relevant factors, chemical composition, and cellulose structure-relevant factors. Specific examples of structure-relevant factors include pore size, volume, particle size and specific surface area. Chemical composition refers to the amount of lignin, hemicellulose, and cellulose (see table 3.1), while the structure-relevant factors for cellulose includes cellulose crystallinity and degree of polymerization. [20] The lignocellulosic composition varies depending on the type off bio-material, as seen in table 3.1 below. In general, the amount of cellulose are between 40-60 wt%, [21] hemicellulose between 20-35 wt% [22] and lignin between 15-40 wt%. [23]

LCB has a high recalcitrance to microbial and enzymatic degradation and is not naturally water soluble. This recalcitrance has evolved over time as a means to protect sugars from microbial and animal attacks. The recalcitrance is mainly due to the plants cell wall, its highly formed crystalline structure, high degree of polymerisation, pore structure and protective effect of hemicellulose and lignin. [20] Figure 3.1 below, shows a great depiction of how different components in lignocellulose interacts. The cellulose shown in blue are depicted as clusters of glucose polymers being interwoven by hemicellulose in yellow all in the presence of lignin shown in green. In the following sections the specific properties in each of the major lignocellulosic components will be explained in more detail in order to explain why pretreatment is needed to increase the efficiency of cellulose utilisation.

Table 3.1: Chemical composition of different lignocellulosic biomass (% of dry basis).

Source	Cellulose	Hemicellulose	Lignin	References
<i>Hardwood</i>				
Oak	43	22	35	Yu et al. (2017) ^[24]
Paper Birch	42	38	19	Ragauskas et al. (2006) ^[25]
<i>Softwood</i>				
Spruce	47	22	30	Yu et al. (2017) ^[24]
Pine	46	24	27	Yu et al. (2017) ^[24]
<i>Agroindustrial waste</i>				
Barley straw	31-45	27-38	14-19	Saini et al. (2015) ^[26]
Corn cobs	42-45	35-39	12-15	Rabemanolontsoa and Saka (2013) ^[27]
Corn leaves	26	13	15	Rabemanolontsoa and Saka (2013) ^[27]
Cotton stalk	31	11	30	Silverstein et al. (2007) ^[28]
Oil seed rape	27	20	14	Petersson et al. (2007) ^[29]
Sugarcane bagasse	42-48	19-25	20-42	Rabemanolontsoa and Saka (2013) ^[27]
Timothy grass	34	30	18	Kumar et al. (2016) ^[30]
Wheat Straw	33-38	26-32	17-19	Rabemanolontsoa and Saka (2013) ^[27]

3.1 Cellulose

Cellulose is a linear, polysaccharide, syndiotactic (alternating positions of its side chains) glucose polymer. It is strongly linked together via β -(1,4)-glycosidic bonds. The high number of hydroxylic groups being present, leads to the formation of hydrogen bonds. The cellulose polymers are also being interlocked due to Van der Waals forces. Both of these forces cause the cellulose structure to form stereoregularity, reactive hydroxylic groups, and crystallinity resulting in a high tensile strength. Cellulose has different orientation throughout the structure leading to two generic phases: amorphous (low crystallinity) and crystalline (high crystallinity). A higher crystallinity index will in general indicate a higher recalcitrance and a reduced ability to bio-degenerate. The cellulose molecule is furthermore insoluble in water unless in the presence of high temperature or certain catalysts. Each cellulose chain is roughly 500-1400 D-glucose units and an estimated molecular weight of 100,000.^{[32][33][34]}

3.2 Hemicellulose

Hemicellulose is an amorphous, branched, heterogenic polysaccharide that can be easily degraded to monomers such as: arabinose, xylose, galactose, fucose, mannose, dextrose, or glucuronide. Hemicellulose has short, branched chains which binds to cellulose microfibrils as well as interacts with lignin. It has a low degree of polymerisation of about 100-200 units an estimated molecular weight of less than 30,000. This low polymerisation and non-crystallinity eases degradation.^[35] Hemicellulose, in contrasts to cellulose, consists of mainly five carbon monosaccharides. There is still not a consensus on the specific effect of hemicellulose on the recalcitrance of LCB. Some studies indicate that the removal of hemicellulose in pretreatment processes improves the accessibility of cellulose, but whether or not that is caused by removal of hemicellulose or lignin is hard to tell.^{[11][36]} Some studies indicate that removal of hemicellulose is best for improving enzymatic hydrolysis,^{[37][38]} though other indicate lignin removal as the most important factor.^{[39][40]}

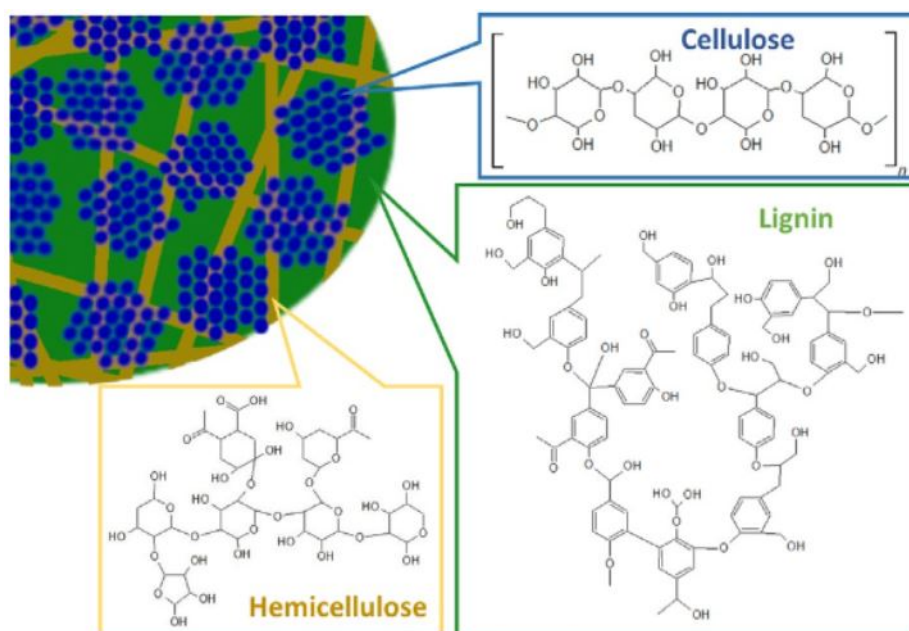


Figure 3.1: Cross section of a macrofibril of wood and its three most common components. Taken with permission.^[31]

3.3 Lignin

Lignin is a large, complex, aromatic, amorphous heteropolymer. The lignin is cross-linked with a high molecular weight and is the reason for LCBs hydrophobicity. Lignin binds hemicellulose to cellulose in the plant cell wall matrix shown in figure 3.1. Lignin consists of phenylpropane units such as coniferyl alcohol and sinapyl alcohol. These structures are formed via ether linkages or carbon-carbon bonds, and they lack any regularity of the repeating units.^[11] Lignin limits the accessibility of polysaccharides, by acting as a physical block by forming covalent bonds to cellulose and hemicellulose. This forms a rigid three-dimensional structure that has the ability to adsorb enzymes, reduce enzymatic digestibility and in turn increase its inhibitory effect on microorganisms.^{[32][41]} Lignin is the most recalcitrant component and is therefore the main reason pretreatment is required in order to utilise LCB.

4 Pretreatment

As shown in figure 1.1, the production procedure for bio plastic varies considerably. Common thermosets such as phenol formaldehyde (PF) or polyurethane (PUR) both necessitates the separation and modification of lignin in their production. Thermoplastics such as PLA or PHA are produced either enzymatically or chemically from the five or six carbon-based molecules in cellulose and hemicellulose. Starch and cellulose-based alternatives (e.g. cellulose acetate and cellophane) on the other hand are made through chemical modification, either esterification or etherification. All these processes have the first step in common, pretreatment, but the goal of this step might vary depending on the next steps in the production chain. The pretreatment methods of biomass can therefore be divided into two categories. Group 1, biochemical/chemical constraints, such as cellulose recovery, digestibility/enzymatic activity, and inhibitor formation. Group 2, limited by bioprocess costs, water usage and energy efficiency.^[42] The goal of pretreatment will naturally differ between the two groups. The goal of pretreatment is in general to improve the cost effectiveness of the production chain by improving utilisation of the major fractions in LCB, removing enzymatic inhibitors (group 1), limit the use of chemicals due to environmental and cost concerns, and increase throughput.

Older pretreatment methods, mainly focused on yield from one major fraction, e.g. the use of cellulose in bioethanol production. Newer methods seek to increase the overall yield of cellulose in addition to hemicellulose and lignin fractions. These largely efficient fractionation methods make it more difficult to define the outer borders of the pretreatment process.^{[12] [43]}

To improve utilisation of the major fractions in LCB, e.g. cellulose, a good pretreatment method should seek to improve specific structure relevant factors and chemical composition of the fractions. Examples of structure-relevant factors include pore size, volume, particle size, and specific surface area. Chemical composition refers to the amount of lignin, hemicellulose, and cellulose (see table 3.1). The structure-relevant factors for LCB include cellulose crystallinity and degree of polymerization, and degradation of hemicelluloses.^[20]

Throughput is affected by solid content during the pretreatment hydrolysis. A less diluted biomass sludge would in general be beneficial in order to reduce the time, and in turn production cost, but unfortunately this has its own drawback due to the high-solid effect. As solid concentrations increase, conversion yields decrease in an apparently linear fashion^[44]. Studies have shown that the high-solid effect is presumed to be caused by water interaction due to water constraints and diffusion into the biomass matrix.^[45]

It is also worth noting that pH plays a significant role in the pretreatment process as depicted in figure 4.1 below. At low pH hemicellulose hydrolyses into monomers and enzymatic inhibitor by-products such as furfural and hydroxymethylfurfural (HMF). At high pH on the other hand, lignin gets dissolved (e.g. pulping of wood).^[42] High yield pretreatment steam explosion with acidic impregnation (H_2SO_4) is an excellent method for glucose extraction and a good example of a low pH procedure. It has a high percentage of cellulose recovery, but it is not able to utilise the hemicellulose content in any productive way, and if the next production steps rely on fermentation or microbial processes it will hamper and, in some cases, completely inhibit any form of fermentation. And furthermore, limit the use of the technique as a viable alternative overall.^[46]

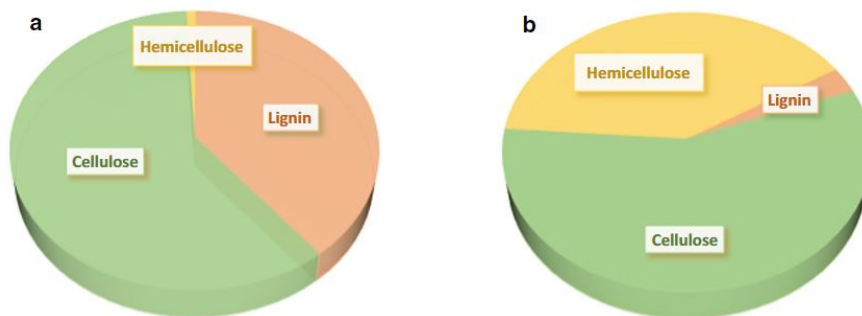


Figure 4.1: The impact of pH on lignocellulosic materials: A is at low pH and B is at high pH. Taken with permission.^[12]

Pretreatment methods could be categorised by their pH level, but the far more common way is to separate them into more generic groups: physical (e.g. milling and grinding), chemical (e.g. alkali, acid, and ionic liquid), thermochemical (steam explosion with or without catalysts) or biological. A select few examples from each different category will be featured below, and although combining different categories is possible, it will not be highlighted in this paper.

4.1 Physical

4.1.1 Milling

In this process LCB will first be chipped or ground before commonly milled either by ball-milling, wet disk milling, hammer milling or colloid milling. After the milling, the particle size of the material is usually around 0.2-2 mm, but the benefit of reducing the particle size to less than 0.4 mm for enzymatic hydrolysis has been shown to be limited.^[47] The final particle size is determined by method of milling, type of biomass, and time. Milling also has the added benefit of not generating and inhibiting molecules in its process and is therefore a viable option for enzymatic hydrolysis or fermentation, although it does not remove any lignin from the biomass. Milling increases the materials porosity and specific surface area and reduces the materials particle size, polymerization degree and cellulose crystallinity.^[48] Unfortunately, milling does suffer from capital cost of equipment, high operating cost due to energy requirements, equipment maintenance and depreciation. These cost disadvantages when combined, does make the method less economically feasible.^[49]

4.2 Biological

Biological pretreatment methods can be divided into three branches: microbial, fungal, and bacterial, though the branches do overlap to some degrees. LCB's high degree of polymerisation, crystallinity and general recalcitrance have made it challenging to create a cost-effective biological pretreatment method. Fungi have gained some popularity as some species secrete enzymes that works well on breaking the plant cell wall. White rot fungi have shown good results for breaking down lignin, while brown rot works great for hemicelluloses and lastly soft rot has to some degree shown affinity for cellulose.^[12]^[21] Fungi depolymerises the LCB in order to generate monomers which are more accessible in the next step of the production chain, enzymatic hydrolysis. Lignin degrading enzymes such as peroxidases and laccases from white rot fungi, have the capability to selectively target low molecular weight lignin and hemicellulose all while only affecting minor amounts of cellulose.^[50]^[51] Studies have also been done on bacterial degradation of LCB. One test was done on a strain of laccase producing bacteria, *Pseudomonas*, on a miscanthus biomass sample. This test showed that bacteria can reduce lignin content by 50 % and double the glucose yield after enzymatic hydrolysis.^[52] Natural combinations of different bacteria and fungi are also possible in order to further increase total outcome outside the selective properties of either.

Biological pretreatment is a great environmentally friendly alternative. It does not need any harmful chemicals, nor resource intensive machinery. It is a cost-effective alternative. However, the drawbacks have made it, albeit interesting and of late popular idea, challenging to implement in large scale production. The main disadvantage is the slow rate of degradation, from a week to nearly a month in some cases.^[50] It also requires a large space in order to scale up production and the bacteria and fungi require quite precise growth conditions. A further disadvantage is that some amounts of biomass are consumed in the process by the microorganisms, leading to minor reduction in cellulose content.

4.3 Chemical

4.3.1 Alkaline Hydrolysis

Alkaline pretreatment is very common in the industry today. It uses high pH, (see figure 4.1) in order to increase the utilisation of cellulose. Common types of alkali chemicals are sodium, potassium, calcium, and ammonium hydroxides. Among these, sodium hydroxides are found to be the most effective.^[53] The use of sodium hydroxide pretreatment of rice straw has achieved an increase in glucan content by 85.6 % and substantial delignification of the straw at 76.9 %.^[54] Alkaline pretreatment technologies are rather similar to the Kraft paper pulping technology used in 90 % of the global production of paper pulp. The Kraft technology is mainly used to remove lignin from LCB by the use of high temperature and added sodium

hydroxide (NaOH) and sodium sulfide (Na₂S). The Kraft process have the added benefit of enabling recovery of chemicals used in the process.^[55] Although the Kraft technology mainly uses forestry biomass, it has been found that agroindustrial biomass are more affected by alkaline pretreatment methods, and thus has a higher efficiency.^[51] Alkaline pretreatment methods have been found to be more effective than acid or hydrothermal methods, in solubilisation of lignin and making cellulose more digestible.^[42]

Alkaline pretreatment interacts with the LCB in two ways, firstly the swelling of cellulose and secondly the removal of lignin and hemicellulose. When biomass gets in contact with sodium hydroxide a swelling occurs which increases the internal surface area of the biomass. A further decrease in degree of polymerisation and cellulose crystallinity leads to a saponification reaction where inter molecular ester bonds between hemicellulose and lignin breaks. This solubilises the lignin and hemicellulose fragments. This process additionally increases the porosity of the cellulose.^[56] The removal of in particular acetyl and uronic acid substitutions in hemicelluloses, increase the accessibility of enzymes in remaining cellulose and hemicellulose fragments for further processing.^[43] The alkaline pretreatment can be used in ambient temperatures, even as low as -15 °C.^[57] Although the alkaline pretreatment in general takes shorter time with an increase in temperature, studies have shown an increase in efficiency at lower temperatures (-15 °C).^[58]

Alkaline pretreatment methods using lime (Ca(OH)₂) are widely used as well. Lime pretreatment has in general lower operating costs and is better for the environment compared with other alkaline solutions. The reason it is better for the environment is due to ease of chemical recovery by reacting with CO₂.^[59] As a consequence of limes low solubility at high temperatures, the reaction temperature is comparatively lower than for sodium hydroxide pretreatments. This increases the total reaction time.^[60]

Alkaline pretreatment does have some minor drawbacks, including recovery alkali chemicals, relatively high operating cost as a result of chemicals, and harsh operating conditions. In some cases, the process also generates minor amounts of inhibitors.^[53]

4.3.2 Acid Hydrolysis

Acid hydrolysis is an unpopular, although well studied pretreatment method.^[61] Acids such as HCl, H₂SO₄, HNO₃ and H₃PO₄ have all been used to some degree, but sulfuric acid is favoured.^[59] Its main advantage is the effect low pH (see figure 4.1) has on LCB. Acid solubilises the hemicellulose from lignin and degrades it, this in turn makes the cellulose more accessible for further enzymatic treatments. Its main benefits are high reaction rates and high relatively high yield. A study done on olive tree biomass reached a 76.5 % hydrolysis yield with 1.4 % H₂SO₄ at 210 °C. The study also backs up the notion that higher acid concentrations increasingly solubilise hemicellulose, xylan in particular.^[62] Concentrated acid pretreatment is highly discouraged because its harsh environment causes corrosion problems to the equipment, generates large amounts of enzymatic inhibitors and causes severe degradation of cellulose. Concentrated acids do also have higher operating costs and require the recovery of chemicals.^[63] Diluted acid, on the other hand limits the disadvantages of concentrated acid pretreatments. A study comparing alkaline hydrolysis and diluted acid hydrolysis on pineapple peel prior to anaerobic digestion found that alkali pretreatment had a 67 % higher biogas production than diluted acid pretreatment. alkali pretreatment also required less time.^[64]

Acid treatment is corrosive to the LCB as well as the equipment, and in conjunction with the cost of the chemicals makes chemical recovery crucial. The formation of enzymatic inhibiting products such as furfural and HMF from hemicellulose degradation, further impacts the process. The treatment itself require high temperatures, large amounts of water and may even require the use of alkaline substances in order to neutralise the hydrolysate.^[61]

4.3.3 Ionic Liquids

Ionic liquid pretreatment of LCB is a relatively new idea and although the amount and rate of new studies are encouraging, the state of the technology right now is not developed enough to be implemented large scale. Ionic liquids are salts that are composed of a small inorganic anion and a larger organic cation. The salts prefer to stay in liquid state. The cation is often either sterically hindered or shielded. It incorporates an organic core of mainly imidazonium salts, although other salts (e.g. imidazolium, phosphonium, pyrrolidinium, cholonium etc) could also be used for LCB.^[65] The large variety of different anions and cations are a huge advantage with ionic liquids as it allows a higher specialisation and fine-tuning of the solvent in the pretreatment process.^[12] Ionic liquids are considered to be nonvolatile, thermostable, non-derivatising, with a high polarity and a low melting point (less than 100 °C), in addition to being environmentally friendly. Ionic liquids do not generate any toxic or explosive gases. However, they are toxic to microorganisms and enzymes and therefore have to be recovered or removed in order for the LCB to be enzymatically hydrolysed. This complicates the procedure, but the solvent has to be recovered anyhow due to its high cost.^{[59][66]} With the aforementioned recovery of ionic liquids, the hydrolysis rate of the regenerated LCB can be massively increased.^[67]

It is estimated that the reason for ionic liquids great performance in fractioning of LCB is due to a competitive advantage in creating hydrogen bonds and thus disrupt the bonding between cellulose, hemicellulose, and lignin.^[61] Ionic liquids induce a lower crystallinity, and higher accessibility to enzymes in cellulose, and enables the extraction of lignin.^[68] Multiple studies have shown the efficacy of ionic liquids on cellulose.^{[67][68]} LCB on the other hand require more severe conditions. Nevertheless, ionic liquids have been used on some LCB such as straw and wood.^[69] The use of ionic liquids in pretreatment of LCB is interesting, but at the current state it is too early to say if the method would be economically viable. The efficient fractioning of LCB, ease of recovery of solvents, and cellulose fraction, makes the method promising.

4.3.4 Organic Solvent

Organic solvent or organosolv, is primarily a delignification pretreatment method. It can use both protic and polar aprotic organic solvents at high temperatures (100-250 °C). It may use solvents with both low (e.g. methanol and ethanol) and high (e.g. ethylene glycol, glycerol and tetrahydrofurfuryl) boiling point, and additives such as ethers, ketones, or pheols. Organic solvents, (and in particular ethanol), have shown great results when it comes to delignification, significant increase in cellulose conversion, and use in other value-added production methods.^{[70][71]} Organic solvent pretreatment proceed via a cleavage of both α - and β -aryl ether linkages in lignin. This produces low molecular weight lignin fragments, some phenols, and lignin dissolution. Organosolv also causes swelling and a depolymerisation of the cellulose, and minor dissolution and degradation of hemicellulose. A study done on *populus* showed that organic solvent pretreatment gave the highest glucan content (about 85-86 %) with significant removal of lignin and xylan compared to diluted acid, hydrothermal, and alkaline pretreatment methods. The study also showed a nearly 95 % lignin removal.^[72] Organic solvent can also benefit from using acid-based catalysts or organic salts catalyst (e.g. FeCl_3). These catalysts will reduce the operational temperature and reaction time, due to an increased polymerisation of cellulose.^[73]

The main advantage of organic solvent is the separate fractioning of dry lignin, aqueous hemicellulose, and a relatively pure cellulose fraction. This separation will reduce the complications and therefore cost of future processing of these fractions into value added products. It might also add higher value to products that would otherwise be seen as waste. Organic solvent can additionally boast of an easy recovery of solvents by distillation to reduce chemical consumption, as well as being able to utilise a high variety of biomass particle sizes. This will reduce cost, making the process less energy intensive. However, organic solvent pretreatment does come with some noticeable disadvantages. The chemical and catalyst costs are relatively high

(although cheap compared with ionic liquids). This makes an energy intensive recovery process unavoidable. In addition to the cost, it also uses high volatility organic liquids at a high temperature, which necessitates the use of containment vessels. The volatility of the liquids furthermore increases the equipment and maintenance requirements (and therefore cost) due to fire, explosion hazard, and safety concerns.^[61]

4.4 Thermochemical

4.4.1 Steam Explosion

Steam explosion is a widely studied and used method and has been used for a long time through the Masonite process. Steam explosion is a rather misleading term since no actual explosion occurs. LCB is subjected to high-pressure saturated steam (0.7-4.8 MPa) at a temperature of 160-240 °C, in order to let the water molecule penetrate the substrate before the pressure is suddenly dropped to let the water molecules escape explosively.^[61] The high pressure and temperature mediate the hydrolysis of hemicellulose and the generation of acetic acid from acetyl groups within the hemicellulose. This generated acid in turn increases the hydrolysis of hemicellulose (autohydrolysis) making the process more effective. Steam explosion is found to increase porosity in the wood fibres, but the process generates inhibitors for further enzymatic hydrolysis.^{[74][75]} Studies have concluded that less severe conditions may be beneficial for further enzymatic hydrolysis.^[76] A method to improve upon the mild steam explosion process includes the addition of SO₂ to the water. This has been found to be an improve enzymatic digestibility.

Steam explosion is a cheap pretreatment alternative that is easy to apply since it has mild restrictions when it comes to type of LCB and size. That being said, it is generally better at hardwood than on softwood (where SO₂ has been found to work well). It uses limited amount of chemicals and does not result in excessive loss of sugars. Although it uses high temperature and pressure. It still manages to be an energy efficient process due to lack of recycling steps for added chemicals. This of course changes for SO₂ steam explosion.^[77] The disadvantage of steam explosion is the generation of enzyme inhibiting molecules, the loss of some sugars (hemicellulose degradation) and an incomplete destruction of lignin. The remaining lignin may cause condensation and precipitation of soluble lignin components which may further decrease enzyme digestibility.^[61]

4.4.2 Ammonia Fibre/Freeze Expansion

Ammonia is used in at least three different ways: ammonia fibre/freeze expansion (AFEX), ammonia recycle percolation (ARP) and soaking aqueous ammonia (SAA). This overview will focus on AFEX since ARP and SAA are simply variations of AFEX with minor changes in times, temperature etc. AFEX uses liquid ammonia at a high pressure and a temperature of about 100°C to combine the benefits from alkaline and steam explosion pretreatment. AFEX has been found to have optimal conditions on switchgrass at 100 °C, biomass moisture content of 80 % and a ratio of 1:1 kg ammonia to biomass during pretreatment. Under these conditions, enzymatic glucose conversion may be increased 6-fold and xylan 23-fold compared with untreated switchgrass.^[78] Mechanistically AFEX works by causing a swelling of the cellulose, this increases the accessibility, decrystallises and leads to a phase change in cellulose (cellulose I to cellulose III). The process removes minor amounts of hemicellulose and reduces lignin content.^[43] Ammonia is regarded as a cheap chemical, and it is possible to recover nearly all the ammonia used in the process. The process does not generate any inhibiting molecules, making it interesting for further enzymatic hydrolysis or fermentation. The effect on hardwood and softwood are unfortunately a bit lower which is a drawback.^[79] The high cost of equipment due to the added safety concerns and corrosiveness of ammonia adds another hurdle to the process. AFEX is not used in any large-scale production with LCB.

4.5 Pretreatment Comparison

Table 4.1: Effect of various pretreatment methods on composition and structural factors of LCB. [43] [59] [63]

	Increase in surface area	Reduction in cellulose crystallinity	Removes hemicellulose	Removes lignin	Generation of inhibitors
Milling	■	■	■	■	■
Biological	■	■	■	■	■ ¹
Alkaline	■	■	■	■	■
Acid	■	■	■	■	■
Ionic liquid	■	■	■	■	■
Organic solvent	■	■	■	■	■
Steam explosion	■	■	■	■	■
AFEX	■	■	■	■	■

■: Major effect

■: Minor effect

■: No effect

¹ Dependant on the microorganism used.

5 Discussion

Bioplastics are a viable competitor to fossil-based plastics when it comes to properties. They can be specialised for a select property and even be synthesised to be structurally identical to fossil-based alternatives (see table 2.1). Bioplastics (especially if biodegradable) are seen as an environmentally friendly alternative to fossil-based high-value chemicals since it does not add stored carbon emissions back into the atmosphere, in addition to their biodegradability. However, the production processes of bio-based alternatives are not as refined as fossil-based chemicals, adding extra cost for equipment, chemicals, and energy requirement. It is also worth noting that the increase in demand for land area in order to produce biomass, affects the environmental impact from bio-based plastics. One promising way to reduce costs of biomass and land usage is to utilise agroindustrial and forestry waste in the production of bioplastic. It would also be beneficial to make use of the cheapest and most available biomass alternative at any given time. However, these advantages do add flexibility concerns regarding the production chain in general and the pretreatment methods specifically.

Pretreatment is a necessary step in order to increase the carbohydrate (glucose and xylose) yield from the biomass. The goal of the pretreatment process should be the disruption of LCB, in order to increase accessible surface area, depolymerisation of cellulose, and removal of lignin. Bioplastic is mainly generated through an enzymatic or microbial fermentation process, and this requires the pretreated biomass to be free of inhibiting molecules, mainly HMF and furfural. The composition of LCB varies depending on the type of biomass (seen in table 3.1). A single pretreatment facility for every type of LCB, designed based on supply availability and logistics, would be the most advantageous. This unfortunately has proven to be a great challenge due to the differences within the LCB composition and structure. Grasses and other agroindustrial waste have in general lower lignin content, and they have a macro-structure that requires less severe conditions in order to be converted. Forestry biomass, on the other hand, might be locally more accessible at a cheaper price per tonne, but would require more severe and costly pretreatment.

The variety in LCB composition has made finding the optimal pretreatment method challenging. Among the different methods highlighted in this paper, chemical and thermochemical methods are currently the most effective, but physical methods have a competitive operational cost, and are easier to implement. As seen in table 4.1, all methods have their own advantages and disadvantages. Physical methods are regarded as the oldest methods and are easy to utilise. However, they are not cost effective compared with chemical and thermochemical

alternatives if yield and by-products are included in the consideration. Utilisation of all the three major fractions in LCB will reduce the overall cost of the production chain and lead to a more cost-efficient method. The lignin or hemicellulose waste from physical and other pretreatment methods are often used to generate heat, in order to reduce the energy requirement and in turn energy cost. Depending on the quality of LCB separation, the different fractions may also be used in high-value products, e.g. vanillin production from lignin. This will greatly improve the pretreatment methods viability.

Organic solvents and ionic liquid are both relatively new pretreatment methods, with advancement in science being conducted concurrently. These methods are particularly good at separating the major fractions of LCB. This enables value added production of materials that would generally be considered as by-products, either directly or by re-selling. Ionic liquids might be somewhat undeveloped and they have yet to see large-scale implementation. Organic solvents on the other hand, albeit rather expensive due to the chemicals, have seen some implementation as a pretreatment method in biorefineries mainly through its ease of solvent recovery. Organic solvents do produce some amount of inhibitors, but the inhibitors generated do not discourage their use for subsequent enzymatic or microbial hydrolysis.

Biological pretreatment is in general a cheap and environmentally friendly alternative. The use of white rot fungi as delignification is found to be great when it comes to removing lignin, but at the cost of some minor amounts of cellulose and hemicellulose. Its main disadvantage is the exceedingly long time it takes for the fungi or bacteria to break down the LCB. This hurdle, however, might not be such a huge concern compared to the other less environmentally friendly pretreatment alternatives.

When it comes to pH-based pretreatments such as acid pretreatment the advantages are few and far between. The corrosive nature of H_2SO_4 or other acids used, drastically increases equipment costs and operation cost due to washing of the product and recovery/removal of the chemicals. Acids are great for removal of hemicellulose, but the removal of such a large fraction of otherwise useful five carbon molecules make the method inefficient. Neither is lignin filtered and separated as to be used in higher valued production. The finishing stroke to acid pretreatment comes in the high amounts of inhibiting molecules making the method rather adverse for enzymatic or microbial hydrolysis. Alkaline pretreatment is a well-tested method especially for the production of wood pulp, and has been found to have an increased efficiency on agroindustrial waste, such as grasses. A variant of alkaline pretreatment, lime pretreatment, benefits from the advantages of alkaline pretreatment, though, at a lower impact on the environment and production cost as a result of chemical recovery. Alkaline pretreatments, with lime pretreatment in particular, are thus a viable option for enzymatic or microbial hydrolysis, but the modest generation of inhibitors has to be considered.

Steam explosion is quite similar to alkaline when it comes to commercial use. It can take advantage of a large variety in LCB making it viable as a pretreatment method where the supply of LCB may vary. Steam explosion does unfortunately generate large amounts of inhibiting molecules, and it is less than optimal when implemented on forestry waste, especially softwoods. The inhibiting molecules are challenging for enzymatic or microbial hydrolysis making this method less preferable.

AFEX could be seen as an improvement to alkaline and steam explosion pretreatments in regards to generation of inhibiting molecules. It has an incredibly high cellulose yield and does not generate any inhibiting molecules making it very interesting as an alternative for bioplastic production. The pretreatment method does, however, incur the same drawbacks as alkaline pretreatment, and the added pressure used in the process also increases mechanical requirements and cost. The method is interesting for use on enzymatic or microbial hydrolysis, but the cost has to be reduced in order for it to be a feasible alternative.

6 Conclusion

The lignocellulosic recalcitrance can be overcome with the use of pretreatment methods. All the different types of methods highlighted in this paper have the ability to alter the accessibility of cellulose for enzymatic or microbial hydrolysis. Some, unfortunately, do generate inhibiting molecules to some degree, which makes it necessary to add a washing and/or filtration step to either neutralise or remove the unwanted molecules. This extra step may require higher energy usage, more equipment and chemicals, and time, increasing the total cost. This may, in some cases, make the method ineligible for practical use. The cost of bio-based plastics needs to be reduced in order for it to be competitive with fossil-based plastics. In order to achieve this, plastic production may utilise agroindustrial or forestry waste as raw material to reduce cost of biomass. However, forestry waste, while cheap, is more challenging to utilise due to its higher lignin composition compared with agroindustrial waste. All the methods listed have their own advantages and disadvantages, so a single solution or optimal pretreatment method is not possible to conclude without further research into type of lignocellulosic material and local resource availability like water, land allocation, and energy cost.

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