Unconventional Pretreatment of Lignocellulose with Low-Temperature Plasma

Jens Vanneste, Thijs Ennaert, Annick Vanhulsel and Bert Sels

ChemSusChem 2017, 10, 14–31

DOI: 10.1002/cssc.201601381
Lignocellulose represents a potential supply of sustainable feedstock for the production of biofuels and chemicals. There is, however, an important cost and efficiency challenge associated with the conversion of such lignocellulosics. Because its structure is complex and not prone to undergo chemical reactions very easily, chemical and mechanical pretreatments are usually necessary to be able to refine them into the compositional building blocks (carbohydrates and lignin) from which value-added platform molecules, such as glucose, ethylene glycol, 5-hydroxymethylfurfural, and levulinic acid, and biofuels, such as bioderived naphtha, kerosene, and diesel fractions, will be produced. Conventional (wet) methods are usually polluting, aggressive, and highly energy consuming, so any alternative activation procedure of the lignocellulose is highly recommended and anticipated in recent and future biomass research. Lignocellulosic plasma activation has emerged as an interesting (dry) treatment technique. In the long run, in particular, in times of fairly accessible renewable electricity, plasma may be considered as an alternative to conventional pretreatment methods, but current knowledge is too little and examples too few to guarantee that statement. This review therefore highlights recent knowledge, advancements, and shortcomings in the field of plasma treatment of cellulose and lignocellulose with regard to the (structural and chemical) effects and impact on the future of pretreatment methods.

1. Introduction

Interest in alternative resources for energy and chemicals has increased in recent decades due to population growth and environmental issues associated with industrialization. Petroleum is the main carbon source to produce chemicals and fuels today, but with increasing demand and diminishing reserves of cheap and clean fossil oil, there is an urgency to substitute the traditional carbon supply. Apart from the alternative use of gas and coal, recent research also pinpoints lignocellulosic biomass as a possible viable alternative due to its abundance, sustainable character, and extensive functionalized chemical structure, which make this feedstock suitable for the production of new generations of biofuels and, in particular, chemicals. Lignocellulose has a complex structure, consisting of three basic polymers, cellulose (≈40–60%), hemicellulose (≈20–30%), and lignin (≈15–25%; Figure 1), and some minor constituents, such as proteins, lipids, tannins, pectins, waxes, and inorganic materials. The distribution of these polymers can vary with the source of biomass. Lignin may be considered as a three-dimensional phenolic polymer, whereas the two other components are composed of sugar units: glucose in cellulose and a mixture of hexose and pentose sugars in hemicellulose. In lignocellulose, cellulose strands are bundled together into semicrystalline microfibrils, covered by hemicellulose in a lignin matrix, forming macrofibrils and fibers through various intra- and intermolecular bonds. This complex three-dimensional structure gives the plant its rigidity and creates a more

![Figure 1. General structure of lignocellulose in plant cell walls. Adapted from Ref. [6d].](image-url)
resistant cellulose, but the downside is the low susceptibility of the structure to chemical and biological attack.

A pretreatment of lignocellulose is therefore necessary to activate the structure and allow for better refining, for instance, by selectively removing the inhibitory lignin residue that can neutralize, contaminate, or poison chemical catalysts or enzymes. The pretreatment may also improve the accessibility of glycoside bonds in cellulose to facilitate the conversion of rigid cellulose into value-added chemicals because this destruction or depolymerization is usually rate limiting in the overall processing of (ligno)cellulose. Important parameters that define the reactivity of cellulose are the purity, accessibility (porosity), surface area (particle size), the degree of carbohydrate polymerization, and crystallinity.[1b, 4]

Interestingly, cellulose, in contrast to common sugars (e.g., sucrose) and starch, is an inedible plant residue, and therefore, not directly in conflict with the nutritional needs of the global population.[5] It consists of glucose units, which are linked by β-1,4-glycoside bonds, starting with a nonreducing end and ending with a reducing end.[6] The glucose units are forced into a chair formation and they are rotated by 180° relative to each other. Consequently, the hydroxyl and hydroxymethyl groups are situated in equatorial positions, leading to a reduced flexibility of the glycoside bonds (Figure 2). Each anhydroglucose unit forms two intramolecular hydrogen bonds with adjacent units, adding to the remarkable rigidity of cellulose. The linear nature of the glucose chains allows for a dense stacking of ordered sheets. The equatorial conformation of the hydroxyl and hydroxymethyl groups enables extensive formation of hydrogen bonds between the cellulose chains in a sheet. Apart from some hydrophobic interactions, hydrogen bonds formed between different cellulose sheets also provide

Jens Vanneste obtained his M.Sc. in Catalytic Technology (Bioscience Engineering) at KU Leuven (Belgium) in 2012. He worked on his master’s thesis at the Center for Surface Chemistry and Catalysis, where he explored the possibility of plasma-assisted pretreatment of cellulose material. He is currently studying for a Ph.D. at VITO (Belgium), also under the guidance of Prof. Bert F. Sels and Dr. Annick Vanhulsel. His research focuses on the pretreatment of cellulose with plasma to enhance chemical conversion into value-added chemicals.

Thijs Ennaert obtained his PhD at the Centre for Surface Chemistry and Catalysis (KU Leuven) in 2016 under the guidance of Prof. Bert F. Sels, where he explored the activity and stability of USY zeolites for biomass conversion in hot liquid water. He is currently working at the same university in the context of a post-doctoral stay. His general interest focuses on the synthesis and application of active and stable hierarchical zeolite catalysts for the transformation of fossil resources and biomass to value-added chemicals and fuels.

Annick Vanhulsel is project manager in the Sustainable Materials unit at VITO (Flemish Institute for Technological Research, Belgium). She graduated from the University of Leuven as M.Sc. in Materials Engineering and obtained her Ph.D. in 2002 in the field of diamondlike carbon coatings. At VITO, she is working in the Plasma Technology Group on the development of atmospheric plasma processes for surface functionalization of materials both in the framework of international R&D projects, as well as in industrial collaborations. Her current research activities focus on the atmospheric plasma engineering of fibers and powders for application in, for example, composites.

Bert F. Sels (1972), currently Full Professor at KU Leuven, obtained his Ph.D. in 2000 in the field of heterogeneous oxidation catalysis under guidance of Prof. Pierre Jacobs. He was awarded the DSM Chemistry Award in 2000, the Incentive Award by the Belgian Chemical Society in 2005, and the Green Chemistry Award in 2015. He is currently Director of the Centre for Surface Chemistry and Catalysis (COK), and active in designing heterogeneous catalysts for future challenges in industrial organic and environmental catalysis. His expertise includes heterogeneous catalysis in biorefineries, design of hierarchical zeolites and carbons, and the spectroscopic and kinetic study of active sites for small-molecule activation. He is co-chair of the Catalysis Commission of the International Zeolite Association (IZA) and co-founder of European Research Institute of Catalysis (ERIC). He is also member of the European Academy of Sciences and Arts, board member of the international advisory board of ChemSusChem (Wiley), and Associate Editor of ACS Sustainable Chemistry and Engineering.
additional stabilization. As a result of this plurality of hydrogen bonds, cellulose is an ordered, semicrystalline material, which is difficult to penetrate by enzymes, solvent molecules, and catalysts. The amount of glucose units connected to each other is quantified by the degree of polymerization (DP), which determines the water solubility. Cello-oligomers with a DP ranging from 2 to 6 are soluble in water at room temperature, whereas oligomers with a DP from 7 to 13 are merely partially soluble in hot water. At higher DP, the cello-oligomer, including cellulose, is only slightly soluble in certain conventional solvent and solvent mixtures—whereas it is highly soluble in some ionic liquids, which can disrupt the crystalline structure and swell or partially dissolve the cellulose by breaking inter- and intracellulosic hydrogen bonds.

Pretreatment of lignocellulose can be divided into three main categories: physical, chemical, and biological pretreatment. Physical pretreatment of lignocellulose usually involves a milling process that causes a reduction in particle size, depolymerization, and a reduction in crystallinity. These structural and chemical changes result in a higher reactivity of cellulose for further (bio)chemical conversion. Chemical treatment of lignocellulose facilitates its conversion by contact with chemicals, such as adding a solvent or a mixture of chemicals, which may either impact on the structure of lignocellulose physically (through solvolysis, disturbing hydrogen bonding) or chemically (through chemical reactions).

Acidic, basic, aqueous, oxidative, organosolv, and CO₂ treatment, as well as steam explosion, AFEX, and cellulose solvents, are all different chemical pretreatment methods, which affect the lignocellulosic properties. Acidic treatment and steam explosion cause a reduction in the cellulose crystallinity and DP, whereas basic and oxidative treatments more specifically focus on lignin removal. Organosolv treatment is responsible for the solubilization of hemicellulose and lignin, creating a purified cellulose. Catalytic solvent treatments, such as the catalytic reductive refinery, which is a specific type of organosolv processing that produces a low-molecular-weight lignin oil, while leaving the entire carbohydrate pulp—cellulose and hemicellulose—largely intact. Ionic liquids decrease the crystallinity, while altering the organizational structure of the lignocellulose. Biological treatments of lignocellulose are mainly used to remove the hemicellulose, lignin, or antimicrobial substances through microbial oxidation of the substrate. In most cases, a combination of different techniques is recommended to promote the refinery and conversion of biomass.

Recently, plasma treatment of lignocellulose has emerged as a new technique, which may become a viable alternative for conventional treatments. Plasma treatment is a (dry) gas-phase process that supports high chemical freedom due to the partial ionization of the gas, stimulating both chemical and physical changes in the lignocellulose structure. The main advantage of plasma is the absence of polluting and toxic chemicals because plasma uses electricity to form a highly reactive ionized gas that can be used for several applications. Electricity from renewable energy sources (solar and wind, for instance), especially during periods with an excess of renewable electricity, makes this technology an interesting future path to consider. This review focuses on the use of low-temperature plasma, at both atmospheric and low pressure, for the pretreatment of lignocellulose to break down the complex structure and, if possible, improve the convertibility into value-added chemicals. This cold plasma causes the formation of a chemically rich environment, while remaining near room temperature, which is an interesting ability exclusive for low-temperature plasma.

This review distinguishes between the effect of plasma treatment on lignocellulose (mainly with regard to purification) and the activation of cellulose (mainly with regard to facilitate subsequent chemical reactions). We begin with a short clarification, classification, and description of (low-temperature) plasma for nonexperts before reviewing knowledge about and

---

**Figure 2.** Molecular structure of cellulose. Reproduced with permission.

**Figure 3.** Overview of pretreatment methods of lignocellulose. AFEX = ammonia fiber expansion.
recent advancements in (ligno)cellulosic feedstock pretreatment.

2. Cold Plasma

Plasma is a partially ionized gas that consists of positive and negative ions, electrons, radicals, and neutral species. It is often regarded as the fourth state of matter. Actually, most visible matter in the universe is in the plasma state. Plasma produced for industrial application is often classified into two categories: equilibrium and nonequilibrium plasma. Equilibrium plasma (also called high-temperature plasma) is characterized by an equal energy level for all species (e.g., ions, electrons, and neutral species). Typically, thermal plasma sources can reach up to 15,000 K and they are usually used for applications such as cutting, welding, remelting, purification, and gasification. Nonequilibrium plasma is characterized by a higher electron temperature than that of other species (ions, atoms, molecules) and is often referred to as cold plasma. Due to the high electron temperature, inelastic electron collisions occur, which cause the formation of other active species that sustain the plasma and create a chemically rich environment. In contrast to the equilibrium plasma, cold plasma is used in applications for which temperature control is important, such as in biomedical applications.

This review focuses mainly on the use of nonequilibrium plasmas both at low and atmospheric pressure. These cold plasma discharges can be generated in different setups, such as microwave (MW), radiofrequency (RF) and dielectric barrier discharges (DBDs). The RF and MW processes are usually electrodeless, whereas DBD plasma is generated between two electrodes, which have at least one dielectric layer between them. An applied electric field can accelerate electrons in the plasma, hence transferring energy from the external electrical power into the electrons, while the overall gas temperature remains close to near-room-temperature levels, creating the nonequilibrium condition. Most of the plasma configurations discussed in this review are as follows:

1) Parallel-plate DBD reactors, which consist of two electrode plates, in which one electrode is grounded, while the other is connected to the high-voltage source. The sample is placed on one of the electrodes, while the gas between these plates is partially ionized by the discharge. The gap between these electrodes is usually quite small to attain a homogeneous plasma.

2) Remote plasma systems, in which the discharge zone is separated from the treatment zone. The plasma can be formed in coaxial or parallel-plate DBD reactors or by means of MW or RF sources. Remote plasma configurations are often used for ozonolysis.

3) RF and MW plasma processes use a power supply in the mega- and gigahertz ranges, respectively. These reactors can ionize the gas within a chamber without being limited by the gap between two electrodes. The samples are mostly placed inside the chamber, while the discharge ionizes the gas.

Overall, the effect of plasma treatment depends on the type of plasma (MW, RF, DBD, etc.), gas composition, and operating pressure.

The active chemical species in a plasma are formed by means of several excitation and dissociation processes. It is the chemical constitution of the plasma that determines the effect on the chemical structure of materials exposed to it. By controlling the gas mixture, one is able to induce the formation of a variety of highly reactive species, establishing a chemically rich environment. The operating pressure is one of the crucial parameters for a plasma process and determines the temperature of the plasma components, heat exchange intensity, degree of nonequilibrium, and even the structure of the discharge. For instance, a MW discharge appears as a nonequilibrium uniform plasma at low pressure, but it can transform into a contracted thermal form at atmospheric conditions.

Low-pressure plasmas are widely established. In particular, the application of DBD plasma has been gaining a lot of attention in different fields, especially because of the potential operation under atmospheric pressure conditions. However, mainly the investment and operational cost of vacuum processes and the handling of matter under vacuum can hinder the implementation of low-pressure plasma treatment. Although the use of atmospheric pressure in DBD plasma eliminates this need, the formation of nonequilibrium plasma at atmospheric pressure presents another challenge. The discharge can easily contract into arcs, turning it into thermal plasma. To overcome this sparking, special designs are implemented, for instance, DBD with reduced discharge gaps.

The exact mechanism of plasma–lignocellulose interaction is extremely complex and a matter of debate and controversy, since it involves both the chemistry of various active radicals and the physics (through vibrational and electronic excited states) of plasma–surface interactions. The aim of this review is therefore to clarify some issues and to pinpoint challenges and unknowns to develop a better understanding of the impact of plasma (under various circumstances and different discharges) on the chemical structure of lignocellulose.

3. Plasma Pretreatment

3.1. Pretreatment of lignocellulose

In lignocellulose, cellulose strands are bundled together into semicrystalline microfibrils, which are covered by hemicellulose in a lignin matrix, forming macrofibrils and fibers through various intra- and intermolecular hydrogen bonds. Such a complex structure of lignocellulose protects cellulose from chemical and biological attack. Low-temperature plasma pretreatment is capable of disrupting the chemical bonds or linkages within such a complex organization, ultimately leading to bleaching and delignification reactions. Such plasma treatment is thus able to advance the purity of cellulose and to make it more accessible for any catalytic or enzymatic process. The treatment of lignocellulose by plasma may be divided into different categories based on the utilized gas composition: nitrogen/air (wet and dry), argon, and ozone.
3.1.1. Nitrogen/air plasma with water

Lignocellulosic particles from corn stalk and sugarcane have been activated in a plasma of nitrogen or air (with water) with the purpose of forming value-added chemicals more efficiently. The corn stalk has, for instance, been subjected to a mixture of nitrogen and water in a nonequilibrium near-atmospheric-pressure DBD plasma (at 3.0 kV, 2 h, 0.9 bar; 1 bar = 10^5 Pa) in a parallel-plate reactor.[27] The lignocellulose of the corn stalk was converted into simple sugars, mainly disaccharides, glucose, xylose, and mannose, under plasma circumstances, yielding 76% total sugars, as analyzed by HPLC. Collection of the liquid product occurred at the end of the DBD reactor in a cold trap and by washing the grounded electrode with distilled water.

The authors claimed that the presence of xylose and mannose was indicative of the selective disruption of hemicellulose; the lignin composition remained largely unchanged after the plasma treatment. XRD revealed a significant drop in crystallinity of the remaining solid after the plasma treatment; this also indicated a breakdown of the cellulosic crystallinity. Notably, this observation was in contrast with later studies, in which plasma treatment of pure cellulose led to an (initial) increase in crystallinity, which was explained by the selective removal of the amorphous cellulosic parts.[28]

The authors suggested that the combination of water and nitrogen in the plasma allowed the formation of a proton-containing active layer around the cellulose, which assisted the hydrolysis of (cellulose and) hemicellulose into mono- and oligosaccharides. This observation is a common phenomenon in plasma treatment of surfaces. Electrons are accelerated by the electric field and hit neutral molecules present in the gas flow, which causes the formation of highly reactive species. Electrons gain more speed than ions, due to their small size, which causes an accumulation of negative charges at the surface (here of lignocellulose). The formation of a negative potential prevents more electrons from settling on the surface and simultaneously attracts positive ions, such as protons, which are present due to the presence and plasma activation of water. After stabilization of the electric potential between the surface and plasma zone, a space charge zone is created, which surrounds the cellulosic powder layer enriched in protons (Figure 4).[29] Such a layer acts as a catalyst, which accelerates the hydrolytic breakage of glycoside bonds. This example suggests the importance of the presence of water in processing biomass feedstock with plasma through a catalyzed hydrolysis pathway.

Another atmospheric plasma treatment of sugarcane biomass was recently reported in presence of air and water in a “tornado”-type MW plasma (Figure 5).[30] Sugarcane (about 2 g reactor load) was subjected to the late afterglow of a MW plasma source (2.45 GHz, 200–700 W) in air. In this system, the remote plasma generates a family of reactive species, namely, radicals and charged and neutral species, in the absence of a biomass feedstock. The interaction of plasma with the biomass, which is indirect, is assumed to proceed through the existence of long-lived reactive species, since the biomass (in particular, hemicellulose and lignin) is clearly degraded after plasma treatment. The authors observed a substantial thermal degradation of the biomass sample when it was placed closer to the discharge zone. This was due to the high amount of elastic collisions and excitation on rotational and vibrational levels, which heated the gas. The reactive species, which were identified as singlet delta oxygen (Δg 1O2), OH radicals, and HNO2 and NO2 molecules (formed in the plasma from N2 and O2), were capable of rapidly breaking C–C bonds, creating additional functional groups such as carbonyls in the biomass (easily ascertained by IR spectroscopy) through oxidation processes. Long interaction may lead to extensive CO formation. IR spectroscopy confirmed a significant loss of the oxidative-sensitive C–C vibrational stretch after plasma pretreatment. This removal proves that plasma may also cause the disappearance of aromatic substances such as the coniferyl/sinapyl alcohol building blocks of lignin, and therefore, the disruption of the sugar cane lignin. The presence of oxygen-containing reactive species thus seems to be of utmost importance for the breakdown of lignin.

Plasma-treated sugarcane showed a substantial improvement in its fermentative conversion into cellulosic ethanol. The higher digestibility proves that the indirect plasma treatment is indeed capable of creating a more reactive cellulose.

Maksimov and Nikiforov faced some disadvantages of low-pressure oxidizing plasma (with air, oxygen, or water vapor).[31] During bleaching of the flax with such plasma treatments, the authors also observed an unwanted oxidation of the cellulosic itself.

To better understand this overoxidation, they developed two new plasma systems to determine the chemical reactive species that took part in the delignification (bleaching) process. The first system flows dry air in a plasma zone, which is then directed over the biomass as such, or after bubbling the activated air first through an aqueous solution. The second system uses the aqueous solution as one of the gas-discharge electrodes (Figure 5). The plasma can be either generated in the plasma zone in contact with the solution or in the solution itself, which enhances the delignification process of biomass within the solution. Bleaching with the two plasma systems follows different mechanisms and depends on the reactive species present. Because analysis of molecular reactions between radicals revealed an immediate decay of radicals in the (gaseous) plasma zone, more stable oxidants such as ozone were the dominant reactive species that reacted with the biomass in the gas flow system, whereas in the plasma solution system O2 radicals are formed in close proximity to the solution, which leads to hydrogen peroxide.

![Figure 4](image-url)
formation in the bulk of the solution. According to the authors, the efficiency of chemical activation of the primary species was higher in the plasma solution system \((\text{OH})\) than that in the dry system (ozone), resulting in more reactive species in the former one. The plasma solution system should, according to this study, be the best system for delignification of biomass without the unwanted oxidation of cellulose.

3.1.2. Ozone plasma treatment

To render the cellulose more accessible to hydrolysis and further conversion into value-added chemicals, the complex structure of lignocellulose should be disrupted. Ozone, which is formed easily from molecular oxygen in plasma, is a useful oxidant for this purpose. Ozone treatment is especially efficient to degrade aromatic structures (lignin) in the biomass. As a result of selective lignin removal, ozone-treated samples, such as wheat straw and sugarcane bagasse, usually show faster degradation properties in chemical and biochemical processes, for instance, facilitating ethanol production in a fermentative process.\(^{(32)}\) One example of using a remote DBD plasma (230 W, 1–7 h, 1 bar) with air or oxygen-enriched air demonstrated a high 65% lignin degradation without compromising the structure of cellulose and hemicellulose. Others found some cellulose oxidation, especially after long treatment times, for example, 20 h contact time, causing the formation of carboxylic acid groups, which resulted in a substantial pH drop of the aqueous cellulose suspension (also called acidic ozonation).\(^{(31)}\)

The presence of water and the particle size of the feedstock were the two main parameters in such plasma processing of biomass.\(^{(34)}\) Water is believed to assist the diffusion of ozone molecules into the plant material, where it will attack the aromatic lignin structure.\(^{(35)}\) The optimum dry matter content of biomass for maximum lignin degradation of 1 mm particles was therefore reported to be about 50%. This value was later supported by the work of García-Cubero et al.\(^{(36)}\) Other detailed studies showed that a moisture content up to 30% was already sufficient to improve the transport of ozone from the gas to the solid surface. Somewhat higher contents are likely to further assist the diffusive penetration of the reactive molecule into the lignocellulosic structure, for example, due to the swelling property of water. The mechanism of the ozone/water interaction has been accepted by several authors.\(^{(36c, 37)}\) Ozone solubilizes in free water, after which it diffuses into surface-bound water. Water may therefore be regarded as a necessary reaction medium, dissolving ozone before it interacts with the biomass structure.\(^{(34)}\)

Optimization of the pretreatment protocol offered a fast (0.5–2 h contact time for 100 grams of biomass) and simple method to plasma-treat biomass with a dry matter content of 45–60%.\(^{(32)}\) The optimization process included a washing step of the treated wheat straw samples with water (230 W, coaxial DBD, \(\text{O}_2/\text{N}_2\) 40/60) to eliminate the inhibitory components, which formed during lignin degradation. These improvements together led to a glucose yield of up to 78% (from wheat straw), which was further fermented with a 52% ethanol yield, whereas only 6% ethanol yield was obtained from untreated biomass.

Figure 5. a) Scheme of an air–water tornado-type MW plasma (Reproduced with permission\(^{(30)}\)). Schematic of plasma–solution systems: b) electrolytic cathode glow discharge: 1) electrodes, 2) plasma zone, 3) electrolyte solution, 4) stirrer; and c) diaphragm discharge: 1) electrodes, 2) quartz cell with the diaphragm, 3) plasma zone, 4) electrolyte solution, 5) stirrer. Reproduced from Ref. \([31]\) with permission.
wheat straw. Despite the promising results in ethanol production, it will also be interesting to see future studies that evaluate chemical conversions of such ozone-treated lignocellulose to platform chemicals, even without the additional washing step, because such reactions are less prone to deactivation by lignin degradation products than enzymes and microorganisms.

Another interesting side effect of the ozone-plasma-assisted pretreatment (for instance, in the case of wheat straw) is the removal of waxes from the lignocellulosic material.\[39] This is especially interesting for composite panels, such as paper products, but wax removal may also be beneficial for other biomass valorization strategies. Some authors speculate that wax removal has an additional positive effect on the enzymatic degradation of the treated wheat straw. For example, enzymatic hydrolysis was much more efficient after the ozone treatment of wheat straw, showing 67% of the theoretical maximum ethanol yield relative to 21% for the untreated wheat straw.

Water thus has an important role in ozone plasma. The amount should be sufficient to solubilize ozone and facilitate contact with the biomass surface and penetration into the structure. The ideal water amount is dependent on the biomass types because of the moisture saturation point of the fibers.\[38, 43] Although low water contents serve insufficient contact of ozone with the biomass, too high a moisture content blocks delignification due to diffusion limitation.\[40] Bue et al., for instance, illustrated the beneficial effect of the moisture content.\[41] They reported the highest sugar recovery improvements, namely, from 13 to 63% for the untreated and ozone-treated samples, respectively, after enzymatic hydrolysis of 90% saturated wheat straw. Lower sugar recoveries were found, for instance, for wheat straw samples with 30% moisture content. The optimal moisture level of corn to achieve the highest impact of plasma ozonation on further biomass conversion was reported to be 60%.\[42] The ideal moisture content for plasma treatment of aspen wood is about 40%, which increases enzymatic hydrolysis 10 times compared with untreated wood.\[38, 43] The ozone absorption rate passes through a maximum at 40% relative humidity of wood. After this threshold, diffusion limitation exceeds wood swelling, which decreases the uptake of ozone in the substrate; thus reducing the ozonolysis efficiency. Both dry wood and aqueous suspended wood were unable to absorb sufficient quantities of ozone to increase its reactivity.

Other cellulosic-containing waste has also been subjected to ozone plasma treatment. Ozonation of waste newspaper and magazine paper increases the extent of enzymatic hydrolysis from 37 to 52%. Key in these experiments was the purifying effect of ozone by selective oxidation (and subsequent solubilization) of paper containing additives (such as binders, fillers, coatings, strengtheners), rather than a chemically disrupting effect of the cellulose structure.\[44] The laboratory-scale experiments, treating 10 g of wet paper samples, showed an optimal moisture content of 70%. Apart from additive removal, detailed characterization of the remaining pulp showed an increase in specific surface area and total pore volume after plasma treatment, in addition to the decreased lignin content. Such changes clearly improve the accessibility of the inner structure and chemical bonds of the cellulose structures for enzymes and other chemicals.

Ozonolysis has also been combined with other biomass pretreatment methods, such as mechanical ball milling. One study shows a facilitation of the enzymatic hydrolysis of corn straw into glucose and xylose, as a result of delignification (a plasma effect) and cellulose amorphization (a ball-milling effect).\[45] Combining both techniques substantially improved the enzymatic conversion of corn straw. Ozonolysis (for 90 min) and subsequent ball milling (for 8 min), for example, led to 40% glucose yield (and 10% xylose yield), compared with 15 (8) and 26% (10%) for separate ozonolysis and ball-milling treatments, respectively. Hydrolysis of the untreated corn straw samples gave only 6% and 2% glucose and xylose yields, respectively. Importantly, the order of execution of the two techniques has a clear impact. Ball-milling (1 min) corn straw prior to ozonolysis (90 min) led to a higher glucose yield, but appeared to be less efficient for delignification when the milling time was increased to 8 min. Supposedly, decreased delignification was due to the agglomeration of finer particles, caused by milling, which resulted in a smaller reaction area for ozone.

Pretreatment of hydrated biomass with ozone plasma, combined or not with other treatments, shows beneficiary effects, and therefore, may have a direct impact on the cost of biomass valorization. One paper, for instance, reported that the higher reactivity of the cellulose fraction reduced the required amount of costly cellulase (Celluclast 1.5 L, €2 mL\(^{-1}\)) in the hydrolysis step; a tenfold reduction of cellulase load was demonstrated, namely, from 15 filter paper unit (FPU) Celluclast to 1.5 FPU per gram straw, by pretreatment of the straw with ozone first.\[45] Clearly, the plasma pretreatment itself also comes with certain process and equipment costs, but an overall evaluation of the economic and sustainability parameters is unfortunately lacking in the literature.

Apart from straw, the combined impact of ozonation and other mechanical treatments (milling and extrusion) of different biomass types (switchgrass, big bluestem, and Japanese cedar) on the enzymatic conversion was also reported. The studies generally agree that ozonation is responsible for delignification, although mechanical manipulation is likely to affect structural deformation of the lignocellulosic structure.\[40, 46] The moisture content is again highlighted, for instance, 25% is found to be optimal for switchgrass. The ozonation time is plant-type dependent. A contact time of 2.5 min at a low ozone flow rate (37 mg h\(^{-1}\)) was sufficient for the switchgrass samples, but a higher ozone flow rate (365 mg h\(^{-1}\)) was required to efficiently delignify big bluestem. Processing of biomass through extrusion and subsequent ozonolysis improved the glucose recovery upon enzymatic hydrolysis to 3.4 (66.4%) and 4.5 times (90.8%) for switchgrass and big bluestem, respectively.

The effect of ozone plasma treatment of lignocellulosics has been demonstrated several times, but only a few reports attempt a deeper chemical understanding of the delignification process under ozone plasma conditions.\[46] Souza-Courrêa et al.
distinguished three stages in the delignification process with ozone by monitoring the gaseous products in the plasma by mass spectrometry.\[47] From these mass spectra, the so-called “control” spectrum (1 h measurement in an empty plasma reactor without biomass) was subtracted, leaving the reactive oxygen species distribution, such as that presented in Figure 6. Upon treating 20 g of sugarcane bagasse in a DBD setup, in the first stage they analyzed mainly CO (likely to result from the easiest side-group decomposition in the lignin structure), whereas the second stage was predominantly characterized by the presence of intermediate oxygenates, such as O\(_2\), CH\(_3\)OH, CO\(_2\), and H\(_2\)O, in the plasma, which they concluded to result from the reaction between ozone and carbon double bonds of the aromatic lignin backbone. Reactions of ozone with aromatic lignin compounds can result in the formation of CO, OH, and a catechol, which in the final stage can be overoxidized, resulting in low-weight fragments, such as HCOOH, CO\(_2\), and H\(_3\)O\(_2\). These results are necessary to evaluate the reaction mechanism of ozone–biomass interactions and also support the mechanism described by Criegee.\[49] The latter mechanism describes the formation of ozonides (1,2,3-trioxolanes) from the reaction of ozone with alkenes. Such an ozone decomposes into a carbonyl oxide intermediate and a carbonyl group. The carbonyl oxide is a zwitterion, which can react with a carbonyl or alcohol group or polymerize into hydroperoxide intermediates, diperoxides, polymeric peroxides, or a more stable ozone. The ozone–lignin and ozone–hexenuronic acid interactions also give the possibility of cellulose degradation due to the formation of hydroxyl radicals.\[50] These radicals can induce depolymerization and oxidation of the cellulose structure.\[51] Other research found that the occurrence of a superoxide radical formed the initial step of delignification.\[52] The reaction of ozone with the aromatic structure would form a trioxide intermediate that could decompose into a superoxide radical. Superoxide radicals are easily converted into hydroxyl radicals, and vice versa, which makes it difficult to decide which of these radicals are initially formed.

The treatment of lignocellulose by Souza-Courrèa et al. resulted in 65% delignification in the case of sugarcane after 4 h for 0.5 mm particles (and 50% dry matter).\[57] Other research also stated that delignification of wheat straw, caused by ozonation, established a clear decrease of the aromatic ring structure, as well as the simultaneous increase of carbonyl functional groups, according to FTIR spectroscopy.\[53]

Water is an important ingredient in the plasma ozonation of biomass, but research also showed a clear effect of the biomass particle size on the ozonation delignification efficiency.\[53] A particle size below 0.5 mm gives a higher degree of lignin degradation—values ranging from 75 to 82% are measured—but these small particles also show an unexpected (usually unwanted) loss of cellulose, up to 15% for particles smaller than 0.08 mm. Overall, reducing the particle size causes small improvements in delignification, but an even greater beneficial effect is observed in the release of sugars during the subsequent enzymatic hydrolysis process. Garcia-Cubero et al. thus observed an increase in enzymatic hydrolysis of the polysaccharide fraction into glucose from 29 and 16% to 89 and 57% after plasma-induced ozonolysis on wheat and rye straw, respectively.\[36a] They explained this drastic result due to lignin and, to a small extent, hemicellulose removal. Moisture was again a reaction rate controlling parameter for values below 30%. Control of the ozonation contact time is essential for small particles to avoid sugar degradation through overoxidation; in their setup, ozonation for 120 min of 50–55 g cereal straw, after setting the humidity to 40%, with a gas flow rate of 60 L h\(^{-1}\), and an ozone concentration of 2.7% w/w, showed the most favorable delignification circumstances, resulting in a very hydrolyzable (ligno–)cellulose.\[54]

Apart from the removal of lignin, the ozonation of aspen wood revealed several structural changes, such as a decrease
in particle size and an increase in specific surface area; both properties are responsible for better (bio)chemical digestion of the treated biomass.\[38\] The crystallinity index of cellulose initially remained unchanged at low doses of ozone; however, with longer treatment, the removal of lignin and hemicellulose disturbed the structural integrity, causing oxidation and depolymerization of cellulose, as well as an amorphization of the cellulose structure. This change in crystallinity reverted after processing (washing, drying), probably due to partial recrystallization.

Ozonation has not only been investigated for its effect on enzymatic hydrolysis and subsequent sugar-based fermentative ethanol production. Also, the an aerobic conversion to methane was studied in the presence of ozone-plasma-treated wheat straw. Ozone was generated in a DBD by using 12 standard liter per minute (slm) of a mixture of \(O_2/N_2\) (40/60) at 230 W and at atmospheric pressure and ambient temperature.\[39\] The most dominant reaction in this type of discharge (alternating current (AC) voltage pulse, 54 \(\mu\)s) involves the formation of oxygen atoms that recombine with \(O_2\) to form ozone. Such pretreatment led to a decreased in lignin content from 20 to 16% and an increase in available sugars (measured by HPLC), from 7 to 14.5%, ultimately leading to 112 L methane per kg straw in the fermentation process. Interestingly, the extra energy demand of the plasma process was completely cancelled out by energy benefits in the fermentation process. Because plasma ozonation of biomass is in its early stage of development, improvements to the system, such as recycling exhaust gas and efficient ozone feed, may in the future result in a positive energy budget and, as a result, plasma-assisted pretreatment will be a feasible technique for improved methane production from lignocellulose resources.

Technical advancements are essential to improve the control of the delignification process during the ozonation process. Instead of classical ozonation equipment, Souza-Correa et al. also used an atmospheric pressure microplasma jet (144 MHz, 20 W, 3 h) with 10 slm argon combined with 2% molecular oxygen for the treatment of sugarcane bagasse that had already been hydrothermally pretreated.\[40\] They demonstrated that this setup allowed a more controlled delignification process, while preserving both the hemicellulose and cellulose. The authors reported a degradation of lignin without removal of the lignin fragments (e.g., into gaseous compounds such as CO, CH\(_2\)OH, and CO\(_2\)) after the plasma treatment. By subjecting these samples to additional extraction steps, it may be interesting to investigate the identity of residual lignin fragments and clarify complex ozonation chemistry in the biomass. Such lignin fragments may be interesting for further valorization into chemicals.\[41\]

### 3.1.3. Argon plasma

Argon plasma usually establishes a high amount of radicals, which are rapidly formed due to the low activation energy of argon atoms. These reactive radicals can subsequently interact with the surface of the substrate, such as cellulosic materials. The effect of argon plasma on lignocellulose material, such as jute, luffa, and Kraft fibers, as well as chemo- and thermomechanical pulp (CTMP), was studied. Argon plasma appears to have a strong effect on the aromatic structure of lignin by forming radicals and disrupting chemical bonds. The use of low-pressure argon RF plasma (13.56 MHz, 0.13 mbar, 200 W, 20 min) on jute fibers, for instance, showed an increased intensity of the C=O vibration in FTIR spectroscopy (at \(\nu = 1730\) cm\(^{-1}\)), which the authors reported to be indicative of improved crystallinity of the cellulose.\[57\] Because the absorption band near \(\nu = 1650\) cm\(^{-1}\) (measured at \(\nu = 1643\) cm\(^{-1}\)), associated with the bending of water, was significantly reduced, it was suggested that argon plasma reduced the water content due to a temperature effect and radical bond cleavage. This, together with other minor changes in the IR spectra of lignocellulose, provides strong evidence that argon plasma treatment is able to cause severe changes to the chemical and microstructure of lignocellulose fibers.

In contrast to ozone plasma, argon plasma initially contains no reactive oxygen species (unless indirectly formed upon contact with the material or water), and therefore, its oxidative power is expected to be less. This is indeed supported by a report on the crystallinity and surface images of jute before and after plasma treatment. The X-ray reflections of crystalline cellulose in jute are presented in Figure 7. A comparison of the powder diffractograms reveals two reflections, one at \(2\theta = 16^\circ\) and the other at \(22.6^\circ\). According to the authors, the first peak is assigned to both indexed (110) and (110) reflections, which are indicative of the presence of amorphous materials, such as lignin, hemicellulose, and amorphous cellulose, whereas the latter peak can be indexed to the (002) reflection, corresponding to the crystalline cellulose phase. A shift of the (002) indexed cellulose reflection to lower angles was observed, corresponding to an increase in d spacing. The reduced intensity of the amorphous peak at \(2\theta = 16^\circ\) suggests an increase in crystallinity after argon plasma treatment and is likely to be a consequence of the first removal of amorphous constituents and recrystallization of cellulose. Although rearrangement of the

![Figure 7. X-ray diffractograms showing part of the reflection pattern of raw jute (due to crystalline cellulose fibers) before and after both Ar and O\(_2\) plasma treatments. Reproduced from Ref. [57] with permission.](image-url)
crystalline phase towards denser cellulose explains the shift, the reduced intensity indicates loss of material. Selective removal of amorphous cellulose after plasma treatment is likely. The full-width at half-maximum (FWHM), crystal size, d spacing, and strain, as calculated by the XRD method, also suggest an increase in the particle size by 1.5% after argon plasma treatment. This shows that the argon plasma treatment causes strain in the fiber, resulting in increased d spacing, and subsequently, might lead to rupture of cellulose particles and a weakened fiber. The argon-treated fiber showed a smooth, compact surface in an optical microscope with a magnification of 500×, whereas the oxygen plasma was more aggressive, locally destroying the surface through interactions with energetic reactive oxygen species.

Although mild on cellulose, cold argon plasma (RF, 0.05–1 mbar, 75 W) is able to form high amounts of phenoxy radicals in the lignin structure of CTMP and kraft fibers of unbleached softwood, as ascertained by EPR spectroscopy.\[56, 58\] Although the authors found a significant amount of radicals after argon plasma treatment in both fiber samples, quenching of the radicals was faster in the CTMP fibers due to substantial formation of new intermonomeric bonds, mainly β-β bonds and 5,5′-O-4 linkages (Figure 8), as measured by \(^{13}\)C NMR spectroscopy. Radical formation occurs during the first few minutes of the treatment (RF, 13.56 MHz, 20–100 W, 30–300 s) and their presence decays after several minutes.\[59\]

**Figure 8.** Intermonomeric bond formation within the lignin structure. Adapted Ref. [58].

Atmospheric argon plasma pretreatment of luffa fibers was investigated by Wang and Shen,\[60\] who suggested the treatment as a valid alternative for chemical pretreatments. The removal of lignin was demonstrated by the removal of a waxy and gummy substance at the luffa fibers, as imaged by SEM. They found a recovery of 85% of cellulose after the plasma pretreatment (10 s, 110 W), which was substantially higher than that for the classic NaOH biomass treatment (80%) and untreated luffa fiber (66%). The authors noticed almost no loss of structural integrity of cellulose because the vibrations of most characteristic cellulose functional groups remained apparent and unaltered in the IR spectrum. Although the surface of the fibers clearly showed (in scanning electron micrographs) indentations, which they explained to be due to the removal of lignin and hemicellulose, the fiber morphology was left intact. The authors claimed that Ar plasma was very promising, compared with classic wet NaOH treatment, since the plasma pretreatment is shorter, solvent free, and results in a feedstock with a higher (more pure) cellulose content. An economic cost comparison was, however, not addressed in this study.

Other research studies report the use of argon atmospheric plasma (MW torch, 2.45 GHz, 100 W) to improve the hydrolysis of sugarcane bagasse.\[61\] After 3 h of treatment under the plasma conditions, a clear wash out of short lignin fragments from the lignocellulosic structure with 1% NaOH was established, as observed by UV/Vis spectroscopy. The interaction of low-energy electrons of the plasma with the substrate was proposed to explain the mechanism of the successful pretreatment. The authors claimed that a sort of “temporary electronic resonance”, which was the attachment of plasma electrons to either α* and π* orbitals to form long- and short-lived anions, respectively, was at the origin of the activation process. Similar effects have been discovered in DNA, in which low-energy electrons can induce single- and double-strand breaks through such a resonance mechanism.\[62\]

A recent investigation in RF atmospheric argon microplasma also concluded a direct interaction of “hot” electrons in argon plasma to be responsible for bond activation and rupture. They found that such direct interaction was only possible for electrons with an energy above 0.55 eV.\[63\] The authors monitored the chemical destruction of lignin (in pellets) by diffuse reflectance IR Fourier transform spectroscopy (DRIFTS). Because both C=C and C=O stretching vibrations were affected, the electrons in the microplasma were reported to be capable of inducing a breakage in aliphatic chains, as well as in the aromatic rings of the lignin structure. Since remote plasma had no effect on the lignin, (long-lived) radicals were excluded to play a key role in such argon-plasma-promoted lignin degradation.

### 3.2. Pretreatment of cellulose

The previous section showed promising results with various plasma technologies with regard to the selective removal of lignin and hemicellulose from lignocellulose. As a result, a purified and more accessible cellulose, which more easily converts into value-added molecules, such as glucose, is obtained. Additionally, plasma technology may also be useful to activate pre-purified cellulose to further enhance its reactivity towards chemical and enzymatic hydrolysis or to increase its water solubility.\[64\] Moreover, the use of pure cellulose in such studies may allow a deeper fundamental understanding of the different structural and chemical aspects of plasma treatments and their underlying processes. The plasma processes applied in these studies range from low pressure to atmospheric pressure, with MW, RF, or DBD to attain a stable plasma.\[65\] Different gas compositions produce different active species in the plasma, resulting in different interactions with the cellulose structure. In this segment, four types of plasma processes, which have been used to treat pure cellulose, are discussed: atmospheric air plasma, atmospheric argon plasma, low-pressure air/oxygen plasma, and low-pressure argon plasma.
3.2.1. Atmospheric air plasma

Treatment of pure cellulose powder and a film of cellobiose (the glucose dimer in cellulose) was recently reported in a parallel-plate reactor with atmospheric air plasma. The purpose of this study was to evaluate the ability of plasma to improve the acid-catalyzed hydrolysis of cellulose. The active species present in atmospheric pressure air plasma include NO, (which is formed from reactions between O₂ and N₂ in plasma), hydroxyl radicals, oxygen radicals, and ozone. The experiments showed that the acid-catalyzed hydrolytic cleavage of cellulose was substantially improved, in both the presence of Brønsted acids and enzymes, after plasma treatment. Benoit et al., for instance, treated microcrystalline cellulose (MCC; 0.2 g; Avicel PH-105) statically in a parallel-plate DBD reactor (of 25 cm²) for 3 h or longer, with air as a discharge gas (26 W, 2 kHz). Their treatment led to an increase in glucose yield from <1% (without plasma treatment) to 22% for a catalytic hydrolysis in the presence of Amberlyst A35.

The hydrolysis improvement was explained by a substantial decrease in DP from 200 to 120 units. Initially, they tentatively proposed hydroxyl radicals, which were generated in the oxygen plasma, to be responsible for the partial depolymerization, while also causing a slight oxidation of the cellulose fragments. Later, they found more decisive evidence that not ‘OH, but rather NO₂ species were playing a crucial role. These NO₂ species interact with water initially contained in the polysaccharide, forming acidic species, perhaps HNO₂ and HNO₃, causing the partial depolymerization of cellulose. The oxidation was evidenced by the appearance of a weak vibration band at \( \tilde{v} \approx 1724 \text{ cm}^{-1} \), which was assigned to carboxyl/carbonyl groups in the FTIR spectrum. The authors compared plasma technology with other conventional and unconventional pretreatment methods, such as ball milling and treatment with ionic liquids (e.g., [BMIM]Cl; BMIM = 1-butyl-3-methylimidazolium). Their study showed that plasma-treated cellulose was the most reactive cellulose towards acid-catalyzed hydrolysis, with 22% glucose yields relative to only 13 and 9% achieved with ball milling and ionic liquids, respectively. Although, within this research, non-thermal atmospheric plasma (NTAP) showed a staggering increase in the conversion of cellulose to soluble glucans (i.e., glucose), it is important to compare energy consumption with conventional mechano-catalytic techniques. Jérôme et al. reported that the treatment with mechano-catalytic processes used between 3.4 and 21 kWh to convert 90% of cellulose (1 kg) to glucans. To obtain a similar conversion, NTAP appeared to use around 6.4 kWh. This shows that emerging plasma technology has the potential to compete with conventional ball-milling technology.

A decrease in DP is only partially observed, up to values of about 100, even after prolonged plasma treatments (\( > 8 \text{ h} \)). Because the crystallinity of cellulose, as measured by XRD (Figure 9), was significantly increased during this period, plasma-induced depolymerization was likely to occur largely in the amorphous phase. Perhaps the amorphous cellulose structure, with higher surface area and some porosity, allows better transport of the reactive NO, oxygen species, but this was not unambiguously investigated. If the latter were true, amorphous cellulose should be more affected by plasma than crystalline cellulose. Therefore, Benoit et al. subjected MCC to ball milling to reduce the cellulose crystallinity first and then subjected milled cellulose to plasma treatment. It was observed through both viscosimetry and ¹H NMR spectroscopy (increasing number of nonreducing ends) that DP was further reduced to 36, although ball milling itself did not significantly affect the DP, only the crystallinity. The combined milling and plasma action led to very reactive cellulose, showing an unseen glucose yield of 58% after 5 min (at 150 °C) in the presence of Amberlyst A35 catalyst. In addition, this cellulose showed improved solubility in DMSO up to 85% (corresponding to 150 mg in 30 mL DMSO; Figure 10). The long plate-plasma treatment in air resulted in a coloration of the initially white cellulose to yellow; this was likely to be due to the formation of undesirable (oxidized) side products, which were not further identified. The optimal treatment time to activate cellulose is therefore about 1 h (for the 26 W plate reactor) with an O₂ to N₂ molar ratio close to that of air.

Apart from cellulose, inulin, a natural polymer of fructose units, was also subjected to the same plasma treatment. Inulin (with a DP of 48) is less recalcitrant than cellulose. Because this carbohydrate polymer is water soluble, it is more suitable to monitor the effect on the DP, for example, with the aid of size exclusion chromatography. Different gas compositions from
neat nitrogen to nitrogen with oxygen contents varying from 20 to 70 vol.% were applied to inulin. As with the untreated sample, there was practically no depolymerization of inulin into fructo-oligosaccharides when plasma treatment was performed under a neat nitrogen atmosphere, whereas inulin hydrolysis conversion was significantly higher after oxygen was cofed into the plasma. High oxygen gas levels (> 30%) are detrimental because they seem to inhibit the ability to depolymerize the sugar polymer. These observations suggest that especially NO₂ plays a major role in the depolymerization process of polysaccharides. This was further supported by similar depolymerization effects from the plasma treatment of inulin with benchmark NO₂ and N₂O–O₂ gas mixtures.

More details of the plasma-activation process of cellulose were studied in a DBD plasma reactor in pulse mode (by using a pulse generator providing 18 kV pulses with a pulse width of 20 μs and a repetition rate of 13 kHz) in the presence of synthetic air. Rather than cellulose alone, cellobiose and glucose were treated for short times (seconds) to understand the chemical changes induced by plasma.[64] A cellobiose film, 1 mm thick, was prepared by deposition/solvent evacuation. X-ray photoelectron spectroscopy (XPS), ultraviolet photoelectron spectroscopy (UPS), and metastable impact electron spectroscopy showed that oxidation of the hydroxyl to carbonyl groups (at the surface) was the major air plasma effect, whereas the ether bonds were less affected. This observation is in contrast to the aforementioned depolymerization effect, which might be explained by the shorter duration of the plasma treatment in these experiments (2 s compared with 1 h). In argon plasma, the cellulose surface is not oxidized, but reduced, in accordance with the lower oxygen to carbon ratios and the formation of surface carbon unsaturation.

3.2.2. Atmospheric argon and helium plasma

Atmospheric-pressure argon plasma treatment of purified cellulose (e.g., α-cellulose) was mainly studied for its water solubility effect.[65] Because plasma is supposed to disrupt the van der Waals forces (e.g., hydrophobic and hydrogen interactions), it may loosen the dense structure of cellulose and improve its water dissolution ability. Jun et al. investigated α-cellulose cotton fiber in an argon plasma afterglow, formed in a DBD plasma gun.[65] During such a process, argon gas is activated in the plasma zone prior to contact with cellulose; this avoids direct contact with the short-lived reactive plasma species. For example, afterglow argon plasma treatment for 30 s at 120 W led to complete cellulose dissolution in a dilute alkaline solution after 2 h, whereas only 20% of untreated samples dissolved. There is an upper limit of the power, since powers above 150 W cause carbonization of the cellulose structure, and therefore, diminished water solubility. A summary of the effects of treatment time and power is illustrated in Figure 11. Close to complete dissolution is achieved when samples are treated at 100 W for at least 1–2 min.

The authors also studied the samples by FTIR spectroscopy, and they observed a narrowing of the IR absorption at $\tilde{\nu} \approx 3650–3200$ cm$^{-1}$, which they explained as a result of the diminished strength of the hydrogen-bonding character. The remote Ar plasma treatment also caused an increase of the O to C ratio at the surface (as measured by XPS), in contrast to the usual reduction in O to C ratio in direct Ar plasma. This difference is due to the removal of carbon content on the surface, as seen from the decrease of the C 1s core binding energy (BE) peak on XPS. The intensity of the O 1s BE peak in the XPS spectrum increased after remote Ar plasma treatment, and also shifted to a higher energy. This may mean that more isolated (noninteracting) functional groups, such as –C–O–H groups, are formed on the surface of cellulose. However, the increased oxygen level may also be the result of contact with ambient air after treatment of the samples. Evidence for this suggestion was given in other studies executed in a low-pressure argon atmosphere (see below).

The efficiency of a DBD Ar plasma gun was compared with that of a parallel-plate DBD system. Whereas only 25% of untreated α-cellulose can be dissolved in a dilute alkaline solution, this increases up to 80% after parallel-plate DBD treatment and even up to 100% after treatment in a DBD plasma gun.[65] The difference was explained by the production of a high-energy plasma in the latter case. Other researchers argued that a cylindrical DBD configuration was more efficient for the treatment of fibers than a parallel-plate DBD because of the formation of a more uniform plasma.[66] The cylindrical DBD configuration is likely to have a better scalability potential for future industrial integration.
Another noble gas used for plasma activation of cellulose is helium. Helium plasma has a higher electron temperature and a lower electron density than that of argon plasma due to the low molecular weight of the atom and higher excitation potential of the first excited state.\textsuperscript{[67]} Recent research reports the use of nonthermal atmospheric plasma, which is generated by a parallel-plate DBD (0.2 g cellulose, 5 kV, 50 mA) for the activation of pure cellulose. Remarkably, this helium plasma has been proposed as a new method for cellulose valorization without the need for solvents.\textsuperscript{[26]} The release of 8% glucose after 10–30 min of plasma treatment was measured by using a mixture of helium and water in the plasma. This example shows that atmospheric plasma treatments have the potential to hydrolyze cellulose in the absence of solvents and catalysts such as enzymes, although the use of catalysis currently provides higher glucose yields.

3.2.3. Low-pressure argon and other plasmas

Previous examples of plasma treatment were carried out at atmospheric pressure. The effect of low-pressure argon plasma on the chemical structure of cellulose fibers has also been studied extensively. Similar to the afterglow argon treatment,\textsuperscript{[65]} the use of a low-pressure parallel-plate RF plasma with argon at various powers (100–200 W) shows an increase in the surface oxygen to carbon ratio of cellulose,\textsuperscript{[68]} as opposed to treatment in a pulsed argon DBD reactor under atmospheric conditions.\textsuperscript{[64]} The higher oxygen content (surface oxidation) is likely to be induced by plasma-created radicals upon contact in open air.\textsuperscript{[68]} This hypothesis was confirmed by Koláröva et al.,\textsuperscript{[69]} who subjected cellulose to a direct (glow diode) low-pressure argon plasma treatment (5 W, 10 mbar, 2–300 s, 48 cm\textsuperscript{3}). They noticed a similar increase in the oxygen to carbon ratio from 0.33 to 0.44, but the ratio decreased to 0.36 after 24 h. One hypothesis for this decrease is the simultaneous reorientation of the polar groups into the bulk and hydrophobic groups with C–C bonds to the surface. A general mechanism of cellulose activation under argon plasma was proposed, starting with the homolytic cleavage of the β-glucopyranose, followed by oxidation upon contact with air outside the reactor (Figure 12a).\textsuperscript{[68]} Such oxidations may lead to the formation of carbonates and carboxylic functional groups, which are observed in the FTIR spectra of the plasma-treated samples. Cellulose samples treated with an argon low-pressure glow diode thus react with oxygen in air upon opening the reactor. Evidence for such incorporation of nitrogen due to contact with air was also reported.\textsuperscript{[69]} Also, Ward et al. found an increase of oxygen in the cellulose surface under low-pressure argon RF plasma treatment and, as analyzed by XPS, in particular, the presence of aldehydes, next to long-lived radicals, as observed by EPR spectroscopy.\textsuperscript{[70]} The strong EPR signal was similar to that of a carbon free radical. They also concluded that nitrogen was not incorporated into the cellulose structure, but that it was present as nitrogen gas in the pore voids. Only when low-temperature nitrogen plasma was applied did they observed clear evidence of structural nitrogen in the activated cotton cellulose. The incorporation of nitrogen occurs gradually, with higher contents after prolonged contact time in the nitrogen plasma.\textsuperscript{[71]} Homolytic scission of C–C bonds in cellulose forms reactive chain ends available for interactions with active nitrogen-containing species in the plasma, leading to chemical functionalities with nitrogen.

Petrov et al. reported the ability of a high-frequency (HF) low-temperature argon plasma (0.6 A, 0.1 mbar, 60–70 °C) to cause bond ruptures, and therefore, result in decreasing DP in cellulose fibers.\textsuperscript{[72]} Longer contact times also created additional porosity and internal surface area in the cellulose fibers, as ascertained by electron microphotographs. Others observed considerable roughening of the surface to pronounced fiber degradation for long (>10 min) contact times during low-pressure argon treatment (20 W, 0.1 mbar).\textsuperscript{[66]} Low-pressure argon plasma reduces the water content in the fibers, as reflected by a decrease in the bending vibration of water at $\nu \approx 1650$ cm\textsuperscript{-1}. 

![Figure 12. Proposed oxidation mechanism for a) argon\textsuperscript{[64]} and b) oxygen plasma. Reproduced from Ref. [72] with permission.](image-url)
in the FTIR spectra. The authors attributed this water removal to the cleavage of bonds to form free radicals. The structure of cellulose also changed upon plasma treatment. Its degradation temperature, as measured by differential scanning calorimetry (DSC), lowered from 365 to 351 °C after 15 min of treatment. Some authors observed an increase in the crystallinity and particle size, which was attributed to swelling of the cellulose due to the bombardment of high-energy ions onto the fiber surface, whereas Petrov et al. observed no significant changes in the cellulose crystallinity.[73] as clearly illustrated in Figure 13.

![Figure 13](Image 66x356 to 272x641)

**Figure 13.** XRD spectra (top) before (1) and after (2) HF low-pressure Ar plasma treatment, and (bottom) before and after low-pressure Ar plasma treatment.[66] Reproduced from Ref. [73] with permission.

### 3.2.4. Low-pressure air and oxygen plasma

Several authors have studied the effect of low-pressure oxygen and air plasma on the chemicals structure of cellulose films and paper. It is not unexpected that such treatment causes an increase of the oxygen content in the cellulose, similar to that of low-pressure argon and nitrogen plasma, although the mechanism is completely different. There are no reports on the formation of ozone in these plasma systems.[68] Carlsson and Ström were one of the first to investigate cellulose in a low-pressure oxygen plasma (plasmaprep 100) setup.[24] They clearly observed that the oxygen content increased upon treating the cellulose sample, and the molecular weight of cellulose decreased; this was likely to be because of oxidative splitting reactions. Later, it was shown that low-pressure oxygen plasma created new $\text{O-C=O, C=O}$ and $\text{O-C-O-O}$ functional groups (as measured by electron spectroscopy for chemical analysis) at various powers (100–200 W) and pressures (0.2–0.4 mbar).[68]

It was found that oxygen plasma induced ring splitting of the glucose units, forming radical ends. Also, the use of HF low-pressure air plasma (0.6 A, 0.1 mbar, 60–70 °C) is able to split the chemical polymer structure of cellulose fibers. This effects the capillary porous structure of the cellulose fiber, while simultaneously increasing the surface roughness.[21] A mechanistic proposal for radical bond rupture is presented in Figure 12b. In contrast to Ar plasma, a C–O bond is broken, which initiates the loss of CO and the formation of new functionalities, such as aldehydes and carboxylic acids, through termination reactions.

Denes et al. claimed that the increase in surface roughness was attributed to the partial removal of (the more reactive) amorphous regions of the cellulose structure.[68] Selective removal of amorphous cellulose was also suggested by others for plasma treatments with air at atmospheric pressure.[28] In contrast, low-pressure MW plasma (0.16 mbar, 600 W, 20–640 s) did not change the degree of cellulose crystallinity.[27]

In other research, reactions of ozone with glucose and cellobiose showed clear oxidation of the molecules, resulting in the formation of acidic compounds.[37] Overoxidation due to high ozone concentrations can also give rise to the formation of CO$_2$, most likely due to decarboxylation of the acidic groups. Earlier work revealed that low concentrations of ozone in ambient air could also slightly oxidize wet cellulose.[70] Dry cellulose, however, remained unaffected, in correlation with previous results in which water is an important factor in the ozonation efficiency of lignocellulose. Recent research concluded that no oxidation occurred during the treatment of pure cellulose with ozone, nor did it cause a depolymerization reaction.[50a, b] However, in the presence of lignin and hexenuronic acids, there was clear evidence of a depolymerization reaction (see the discussion on ozone plasma treatment). This was due to the formation of hydroxyl radicals (or superoxide radicals) when ozone interacted with double bonds in the aromatic lignin backbone.[71]

### 4. Summary and Outlook

Low-temperature plasma processes are currently being developed/studied as a sustainable alternative technology for biomass pretreatment. Several chemical and physical interactions come into play when subjecting (ligno)cellulose to a reactive plasma environment. Oxygen-free plasma benefits from the presence of energetic electrons, which may collide with and induce negative charges at the fiber surface. The attraction of protons may occur to balance the charges. In particular, in the presence of water, this might generate an autocatalytic effect with improved hydrolysis activity, especially of hemicellulose and amorphous cellulose. The electrons and plasma radicals may initiate radical reactions, ultimately leading to radical C–C and C–O scissions in the (ligno)cellulosic structure. Phenoxyl radicals, for instance, have indeed been identified. Finally, electronically excited intermediates may form, which are stabilized by falling apart into different (more stable) fragments.
Oxygen-containing plasma is likely to work due to the presence of reactive radicals, such as ‘OH, at low oxygen contents, whereas ozone becomes the dominant reactive species at atmospheric pressure. Both species may selectively remove lignin, which is the most reactive component for both species due to its aromatic structure, but they may also oxidize (deliberately or not) cellulose. In particular, the amorphous part can be selectively removed in such plasmas, but crystalline cellulose can also be (surface) affected, thereby changing the surface (chemical) properties. Usually, a higher oxygen content is found at the cellulose surface after oxygen plasma treatment, generating additional functional groups, such as aldehyde, carbonate, and carboxylic acids. In a specific case, oxygen plasma in the presence of N\textsubscript{2} may generate NO\textsubscript{x} species, which, in the presence of water, is likely to form acidic species that catalyze the depolymerization of (hemi)cellulose. As a result, the DP of the cellulose fiber can be affected substantially.

This overview shows that plasma clearly affects any type of biomass; oxygen-containing plasmas are most effective. The effects are generally more pronounced when using small particles and in the presence of water (at a relatively high moisture content). This is due to a surface effect and solubilization and diffusion effects, respectively, which are especially useful if ozone and NO\textsubscript{x} are the reactive species. The major impact is likely to be explained by the removal of lignin (and hemicellulose), which produces a more accessible purified cellulose feedstock. Other beneficial effects are changes in the cellulose structure, such as a reduction of DP and the formation of polar surfaces, due to oxidation. Both changes ultimately result in cellulose samples that are more prone to undergo further chemical and biochemical reactions. Acidic and enzymatic hydrolysis, as well as subsequent fermentative processing (e.g., ethanol and methane formation), are usually used as test reactions to assess the (ligno)cellulose reactivity. In several reported cases, a beneficial effect of plasma pretreatment on the downstream processes was demonstrated, showing, for instance, higher glucose formation rates and higher ethanol and methane yields, in comparison to those of conventional (chemical) pretreatment methods. There is not one treatment that can be concluded to be better than everything else. This will depend on the future use of the product. For example, the use of ozone increases the delignification of lignocellulose, but it may also induce (unwanted) oxygen groups in the cellulose, whereas as oxidation of cellulose for other applications might be beneficial to further enhance its conversion into value-added chemicals.

An important advantage of plasma pretreatment, compared with conventional chemical processes, is the use of dry gases instead of chemicals and solvents; this eliminates the need for filtration/purification of the samples, recovery of the chemicals, and waste treatment after pretreatment. This also eliminates the added cost associated with the use and purchase of these chemicals for pretreatment, which is still increasing. Additionally, a plasma discharge is generated with the use of electricity, and may therefore be an interesting pretreatment process by using renewable energy (solar and wind, for instance), especially during periods when there is an excess of available renewable electricity. Comparison with conventional pretreatment methods is still scarce at present, but highly necessary to really assess the true sustainable character of plasma pretreatment of biomass. At least one example demonstrated that the use of plasma treatment led to an overall less-energy-intensive process for converting lignocellulose into methane through anaerobic fermentation. In other research, it was observed that the energy consumption of a parallel-plate DBD system was similar to that of conventional ball-milling techniques. Considering that plasma pretreatment of (ligno)cellulose is still in its infancy, it can be tentatively concluded that plasma pretreatment of biomass may be a viable green alternative to current pretreatment methods. Nevertheless, more research is required with regard to decent techno-economic assessments, in particular, on the energy efficiency of various plasma systems, to ensure applicability in future industries. Although the use of low-pressure plasma for diagnostics and surface treatment is known, the use of these plasmas for biomass feedstock is hampered by the need for vacuum installations. Therefore, the main focus should be on the use of atmospheric pressure plasma. Future research should go into a detailed study and understanding of the underlying plasma-assisted reaction mechanisms to determine the most efficient pretreatment, while retaining the high energy efficiency. Evaluation of the active species in the plasma and its interactions with cellulose is an important step in unraveling the mechanisms of cellulose activation.

Because most of the plasma systems studied today are static laboratory-scale reactors, which limit the size and scalability, the use of a dynamic system could improve the uniformity and homogeneity of plasma treatment on (ligno)cellulose, while also allowing a higher powder throughput to increase the potential for future industrial implementation.

Acknowledgements

J.V. thanks the Flemish Institute for Technological Research (VITO) for his doctoral fellowship and T.E. acknowledges IWT-Vlaanderen for a doctoral fellowship. The Belgian Government is acknowledged for financial support through IAP funding (Belspo).

Keywords: biomass · lignocellulose pretreatment · plasma chemistry · sustainable chemistry · synthesis design