Chapter 13
Understanding Fundamental and Applied Aspects of Oxidative Pretreatment for Lignocellulosic Biomass and Lignin Valorization

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13.1 Oxidative Pretreatment: An Overview of Processes

The application of oxidation in the processing of lignocellulosic materials has a long history, with the earliest industrial use of gaseous chlorine in bleaching dating back to the eighteenth century [1]. Oxidation of lignocellulosic materials alters the physiochemical properties of plant polymers, resulting in fractionation and modification of plant cell wall components. Oxidative bleaching of plant pulp, in particular, has been the primary industrial application of lignocellulose oxidation.

Oxidation reactions have also been studied as an approach to improving the accessibility of polysaccharides during biochemical conversion of lignocellulosic biomass, and to extracting lignins from biomass for further valorization. Since these reactions precede enzymatic saccharification of plant polysaccharides, they are colloquially referred to as oxidative pretreatment. The use of oxidants in pretreatment has been demonstrated to increase the yield of sugars from enzymatic digestion of pretreated biomass, and to result in physiochemical changes in plant cell wall components. This section reviews the processing characteristics of various oxidative pretreatments, and the following section focuses on the effects of oxidative pretreatment on plant cell wall components.

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13.1.1 Pulp Bleaching Processes

Pulp bleaching is a sequence of unit operations (also referred to as stages) where wood pulp is treated with oxidants in order to whiten the pulp. A detailed overview of the process can be found in the writings of Sjöström [2], Biermann [3], and Bajpai [4]. Most pulp bleaching sequences use oxidation reactions to remove residual lignins and to convert naturally occurring wood chromophores to products that absorb little or no light in the visible range. During these reactions, color-bearing lignins and aromatic extractives are removed from wood pulp, leaving behind bleached product that is primarily cellulose. Because of the economic impacts of cellulose depolymerization during pulp bleaching, the oxidants used in pulp bleaching.

The oxidants used in pulp bleaching has evolved from early options of chlorine [5] and hypochlorite to more environmentally friendly chlorine-free oxidants including molecular oxygen, hydrogen peroxide, and ozone [6].

13.1.2 Oxidative Pretreatment for Biochemical Conversion of Lignocellulose

Biochemical conversion of lignocellulose offers potential in delivering fuel, chemicals, and materials with reduced life-cycle of greenhouse gas emissions compared to petroleum refining [7]. Pretreatment of lignocellulose disrupts the plant cell wall structure in biomass, leading to increased yields in enzymatic saccharification and sugar bioconversion. As a result, a pretreatment stage is indispensable in any economically viable biochemical biomass conversion process.

Oxidative pretreatment is a category of pretreatment methods that involves the use of oxidants as a means of disrupting and fractionating lignocellulose. Compared to other pretreatment methods, oxidative pretreatment is unique in its ability to modify and remove lignin from lignocellulosic biomass [8]. The type and extent of chemical changes to lignin, as well as other plant cell wall components, vary depending on the type of oxidant used, operation conditions, and the characteristics of biomass being pretreated.

13.1.2.1 Wet Oxidation

Wet oxidation is a pretreatment method that employs molecular oxygen or air in a pressurized reaction loaded with biomass and an alkaline aqueous solution (e.g., Na_2CO_3, NaOH). Biomass suspended in water is treated under pressurized (5–20 MPa) air or oxygen, and elevated temperature (150–350 °C). This pretreatment method has been demonstrated to be effective on various biomass feedstock

including wheat straw [9, 10], reed [11], rice husk [12], rape straw [13], sugarcane bagasse [14], and wood pulp waste [15].

During wet oxidation of herbaceous biomass, lignin is converted to carbon dioxide (CO_2) as well as water-soluble carboxylic acids including acetic, formic, succinic, oxalic, and glutaconic acids. Hemicellulose is also solubilized during wet oxidation, with the extent of dissolution and degradation positively correlated with temperature [9]. Compared to steam explosion pretreatment, wet oxidation is advantageous due to lower processing temperature (<200 °C) and reduced generation of sugar dehydration products (e.g., furfural, 5-hydroxyformaldehyde) known to inhibit sugar-fermenting organisms [10].

13.1.2.2 Alkaline Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) has a higher standard oxidation potential (Table 13.1) than molecular oxygen, and demonstrates effectiveness in biomass pretreatment under ambient conditions. Under alkaline conditions (pH 11–12), hydrogen peroxide dissociates and evolves into potently oxidative radicals (•OH, •OOH). These radicals oxidize lignins, resulting in change in lignin dissolution and removal from the plant cell wall matrix. The presence of alkali also promotes solubilization of hemicellulose, which further increases the accessibility of cellulose to hydrolytic enzymes.

As a pretreatment method, alkaline hydrogen peroxide (AHP) pretreatment is effective in removing both hemicellulose and lignins from the plant cell wall matrix, and thus increasing the accessibility of cellulose to hydrolytic enzymes [16, 17]. AHP has been demonstrated as an effective standalone pretreatment for a number of herbaceous feedstocks including wheat straw [18], rice hulls [19], corn stover [20, 21], bamboo [22], and agave bagasse [23], while the pretreatment is not effective on cactus pear [24] and gooseweed [25]. The efficacy of AHP pretreatment under elevated temperature (150–180 °C) on woody biomass has also been reported for Douglas fir [26, 27] and hybrid poplar [21].

AHP pretreatment has also been reported as a secondary post-treatment used in tandem with other pretreatment methods including wet oxidation [12], alkali pretreatment [28, 29], steam explosion [30], and microwave [31–33]. As part of a multi-stage pretreatment process, the delignifying AHP step reduces the cell wall recalcitrance synergistically with other pretreatments that are effective in hemicellulose removal [34]. The amount of hydrogen peroxide used in AHP post-treatment is reduced as

Table 13.1 Standard oxidation potential of oxidants used in oxidative pretreatment of biomass

Oxidant	Standard oxidation potential (V)
Hydroxyl radical (•OH)	2.86
Ozone (O_3)	2.07
Hydrogen peroxide (H_2O_2)	1.77
Oxygen (O_2)	1.23

compared to using AHP as a standalone pretreatment process, and may lead to an improvement in the economic feasibility of the entire pretreatment process [29].

13.1.2.3 Catalytic Hydrogen Peroxide Pretreatment

The application of metal catalysts in AHP pretreatment has been reported as an approach to improving both pretreatment efficacy and reducing oxidant use. Examples of effective catalysts include manganese acetate [35], copper-diimine complexes [36], magnetite nanoparticles [37], and titanium dioxide [38]. The effects of catalysts include accelerated oxidation reactions, reduced time needed for effective pretreatment, and improved efficacy of recalcitrance reduction. In presence of metal catalysts, oxidation reactions between hydrogen peroxide and plant cell wall components can be accelerated, or go through pathways different from those under catalyst-free conditions. Although the specific reaction mechanisms of catalytic biomass oxidation by hydrogen peroxide is not fully understood, a number of possible reaction schemes have been proposed. Transitional metal catalysts including manganese, copper, and iron can catalyze Fenton reactions that generate hydroxyl (•OH) and superoxide ($O_2^{\bullet-}$) radicals, which in turn oxidizes lignins and other aromatic structures in biomass [39].

13.1.2.4 Ozone

Ozone is an environmentally friendly oxidant that generates hydroxyl radicals while dissolved in water and thus has potential applications in lignin oxidation and biomass pretreatment. Recent developments in ozone pretreatment of biomass have been detailed in a recent review by Travaini and coauthors [40]. The efficacy of ozone pretreatment is associated with the moisture content of biomass during pretreatment process, as dissolved ozone is more effective in lignin oxidation compared to gaseous ozone [41]. Currently, the high energy and financial cost of ozone generation has limited the use of ozone in biomass pretreatment as part of cellulosic biofuel production. To overcome these limitations, ozone pretreatment with low severity may be used in tandem with other synergistic pretreatment methods [42].

13.1.2.5 Enzymatic Pretreatment

Oxidoreductases have been associated with both the plant biosynthesis and fungal biodegradation of lignins. Laccase and peroxidases have been shown to affect lignin synthesis and accumulation in *Arabidopsis* [43–45], and their use in biomass pretreatment has also been reported. Deng et al. independently reported a *Trametes versicolor* laccase pretreatment mediated with 1-hydroxybenzotriazole that reduces non-productive enzymatic binding on lignin during wheat straw hydrolysis, and the pretreatment also results in a 26% increase in the yield of reducing sugars during

enzymatic saccharification [46]. Heap et al. used *T. versicolor* laccase pretreatment in tandem with alkaline peroxide pretreatment to further improve saccharification yield from wheat straw [47]. Enhanced saccharification yield was also observed in Eucalyptus [48] and bamboo [49] pretreated with a laccase-mediator system.

13.2 Effects of Oxidative Pretreatment Processes on Plant Cell Wall Components

Physical and chemical changes to plant cell wall components, as well as an increase in enzymatic digestibility, have been associated with oxidation reactions during oxidative pretreatment. The extent of these changes is associated with the type of the pretreatment, as well as pretreatment process parameters including pH temperature, oxygen partial pressure, residence time, and oxidant–biomass stoichiometry. Some examples of pretreatment effects on enzymatic conversion are summarized in Table 13.2.

Via cleavage of covalent intramolecular linkages [39] and oxidation of lignin side chains [50], oxidative pretreatment is effective in hemicellulose solubilization and lignin removal. Redistribution and removal of recalcitrant cell wall poly-

Table 13.2 Effect of oxidative pretreatment on enzymatic digestibility of biomass

Feedstock	Pretreatment conditions	Enzyme loading	Cellulose conversion (of theoretical maximum)	References
Common reed (*Phragmites australis*)	12 bar O_2, 2 g/L Na_2CO_3, 200 °C, 12 min	25 FPU per g dry matter	82.4% after 48 h	[56]
Norway spruce (*Picea abies*)	12 bar O_2, initial pH = 7, 200 °C, 10 min	30 FPU per g dry matter	50% after 24 h, 79% after 72 h	[57]
Sugarcane bagasse	12 bar O_2, 2 g/L Na_2CO_3, 190 °C, 15 min	25 FPU per g dry matter	57.4% after 24 h	[58]
Wood pulp waste	12 bar O_2, pH = 10, 195 °C, 15 min	35 FPU per g dry matter	42.9% after 48 h	[15]
Corn stover	500 mg H_2O_2/g dry biomass, pH = 11.5, 30 °C, 24 h	20 FPU per g dry matter	88.2% after 48 h	[16]
Cashew apple bagasse	4.3% v/v H_2O_2, biomass loading at 2% w/v, pH 11.5, 35 °C, 24 h	60 FPU per g cellulose	50% after 48 h	[59]
Douglas fir (*Pseudotsuga menziesii*)	0.1 g H_2O_2/g biomass, pH 11.9, 180 °C, 30 min	20 FPU per g cellulose	95% after 96 h	[27]
Hybrid poplar (*Populus nigra* var. *charkoviensis* × *caudina* cv. NE-19)	Alkaline extraction (120 °C, 1 h) followed by AHP catalyzed by Cu-2,2′-bipyridyl (30 °C, 23 h)	30 mg protein per g dry matter	94% after 72 h	[60]

mer is associated with improved accessibility of hydrolytic enzymes. Wu et al. reported the reduced binding affinity of oxidized lignins to cellulases, which leads to improved enzymatic hydrolysis rate of cellulose [51]. The surface morphology of plant cell wall matrix is also affected by oxidative pretreatment, with an increase in surface roughness [52], pore volume [16], and pore surface area [22, 53]. Oxidative pretreatment also increases the water retention value [54], which is an indicator of enzymatic digestibility of pretreated biomass [55].

13.3 Knowledge Gaps

Oxidative pretreatment has demonstrated potential in reducing plant cell recalcitrance to biochemical conversion, although additional research is still needed in this area to better understand the physiological changes in biomass during oxidative pretreatment. Specifically, the oxidation chemistry during depolymerization and repolymerization of lignin polymers is not yet fully understood, and the effect of catalysts during oxidative pretreatment has not been well studied. The role of naturally occurring, cell wall–associated transition metals in biomass has been reported [61], which reveals the potential of breeding biomass crops with metal profiles suitable to oxidative pretreatment. In addition, more research is warranted in identifying strategies in reducing costs related to pretreatment chemical consumption and product separations during oxidative pretreatment [29].

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