Potential use of alkaline hydrogen peroxide in biomass pretreatment and valorization – a review

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Excessive dependence on crude oil and non-renewable energy source to drive human’s daily activities have caused noticeable changes to the world’s climate and stresses to the world’s natural reserves. Immediate actions are needed to divert the reliance on fossil fuel to relieve the stresses to our mother earth. Recently, a significant focus has been given to the valorization of biomass waste which is usually neglected and not fully utilized despite its abundance and potential to transform into bioenergy and bio-based products. However, the recalcitrance of biomass structure limits the accessibility of carbohydrates and thus, several pretreatments stages are required to facilitate the subsequent biomass processing. Conventional pretreatments for lignocellulosic biomass are costly, hindering the development of a feasible economic process to valorize such biomass. Alkaline hydrogen peroxide was first introduced in 1984. Its ability to selectively attacks carbonyl and ethylene groups is benefiting the delignification process of lignocellulosic biomass. The capabilities of alkaline hydrogen peroxide in dissociating the recalcitrant matrix of biomass highlight its potential in improving the accessibility of biomass to achieve valorization. Furthermore, alkaline hydrogen peroxide pretreatment can be performed at mild process conditions and using small amount reagent. Also, its decomposition products are oxygen and water which could be regarded as one of the promising low cost, low toxicity and environmental friendly pretreatments. In this paper, background information of alkaline hydrogen peroxide and its reaction pathways are provided. Moreover, the applications of alkaline hydrogen peroxide in biomass processing are discussed, highlighting the effects of alkaline hydrogen peroxide in process selectivity and biomass dissolution.

Introduction

Waste valorization – a phrase corresponds to any processes or activities that utilize or convert normally neglected waste to highly useful and value added products or energy sources [1]. This phrase has been widely used in recent research publications indicating the shift of global research focal point to the alternative sources for building blocks chemical and bioenergy derived from waste. Annually, hundreds billion tonnes of lignocellulosic biomass waste are produced which are usually neglected by disposing to landfill or burning [2]. These practices do not contribute to any economic benefits to the industry at the same time did not fully utilize the biomass despite its abundance in mass. The availability of lignocellulosic biomass are not limited by geographical distribution and its low carbon footprint and low cost, hence making it a promising feedstock for biofuel production and other value added products [3,4]. Furthermore, the abundance of lignocellulosic biomass from agricultural residues and could be acquire at low cost making it a better and more promising option as a raw feedstock for sugar platform biorefinery on a large scale since it does not compete with primary food productions [5,6]. To date, more and more research on the valorization of agro-industrial residual as feedstocks for biofuel production has attracted great attention, mainly due to the potential of these residues as an alternative to fossil while contribute to the positive effects from both economic and environmental aspects and long- term energy sustainability with greenhouse gas mitigation [7,8]. Therefore, valorization of lignocellulosic biomass for the value added products and biofuel productions could potentially relieve the stresses to the natural reserves and shifting the dependencies of fossil fuel to sustainable energy sources.

Lignocellulosic biomass is a complex structure of carbohydrate polymers which consist of cellulose (38 – 50%), hemicellulose (23 – 32%), and aromatic polymer lignin (15 – 25%) [9]. Cellulose is a strong molecular structure in biomass which is formed by long chains glucose monomeric units connecting via β-glycosidic linkages with reducing and non-reducing ends [10]. These cellulose microfibrils which possess both amorphous and crystalline properties are then stacks in parallel due to the present of hydrogen bonding and weak van der Waals forces. Thereafter, these microfibrils are interlaced together with hemicellulose and lignin to form macrofibrils [11]. As a results, access to cellulose by chemical or enzymatic means is difficult due to the present of complex linkages network and hydrogen bonding [12]. Hemicellulose is considered an amorphous polymer which is a cluster of heterogeneous polysaccharides with a mix of hexose and pentose monosaccharides [13-16]. When chemicals and/or heat are applied, hemicellulose is relatively easier to dissociate as compared to cellulose [17]. Typically, softwood based biomass contain mainly glucomannans and mannans, while xylan (β-(1,4)-

linked xylose homopolymer) are usually found in hardwood based biomass [10]. Therefore, cellulose and hemicellulose contribute to most of the sugar present in the biomass.

Lignin is a hydrophobic polymer composed of several cross-linked polymer of phenolic substances [18] bounded to the hemicellulose and cellulose in a complex polymer matrix which acts as a physical barrier against microbial attack as well as provides structural support to the plant cell [19,14-16]. Lignin content a number of functional groups, such as hydroxyl, carbonyl, and methoxyl in the lignin matrix which contribute to the high polarity of lignin macromolecule [20]. These recalcitrance properties of plant biomass prevent enzymes from accessing the carbohydrate substrate [5]. In general, pretreatment to overcome lignocellulosic recalcitrance are costly and sometimes involve inhibitors to the biofuels conversion [21], hence hindering the development of an economically viable process to convert biomass to biofuels or other valuable products [22]. Many research have revealed the negative effect of lignin in enzymatic digestion of biomass, therefore effective lignin extraction or disruption could significantly alter the structural matrix and crystallinity of cellulose and ultimately enhance biomass saccharification in fermentation [19,14-16].

A typical biorefinery processing of lignocellulosic biomass involves pretreatment step, subsequent enzymatic hydrolysis to disintegrate polysaccharides, and fermentation of released sugar solution to produce biofuels and biochemical [7,8]. In order to facilitate downstream biorefinery of lignocellulosic biomass, a variety of pretreatment stage have been proposed by various researcher to overcome recalcitrant structure of biomass by disrupting carbohydrate matrix and disintegrate high molecular structures like lignin, hemicellulose, and cellulose into smaller compounds, such as monosaccharides, organic acids, and phenolic compounds [23,22]. Thermochemical pretreatment technologies of lignocellulose biomass, i.e., gasification, pyrolysis, combustion, and others, have extensively studied over the past decades. On top of that, a wide range of biomass fractionation approaches have been studied, such as chemical (alkali, dilute acid, organic solvent), physical (grinding, milling, irradiation), physiochemical (wet oxidation, auto-hydrolysis), and biochemical [11].

To date, disruption of the complex polymer matrix to release monosaccharides remained as the main issue for effective utilization of lignocellulosic biomass. Thus, methods that improve the biomass digestibility for subsequent enzymatic or microbial attack have gain much attention in the field of research [24]. Recent research discovered that alkaline peroxide are widely used in biomass saccharification process due to effective generation of radicals and its extraordinary performance in delignification in most crop residue with little sugar degradation and furan derivatives formed as compared to other pretreatment approaches [13,25-27,15,28,16]. Hydrogen peroxide (H_2O_2) is commonly used in pulping and bleaching industries. By tuning the peroxide solution to alkaline using hydroxide ions (OH^-), radical species (e.g., HO• and HOO•) and molecular oxygen are formed as a result of H_2O_2 decomposition, which in turn selectively react with lignin in several reaction pathways, resulting in complicated total oxidation reaction mechanism and leading to high delignification [5,29]. Many research have confirmed, the rate and extent of enzymatic hydrolysis or microbial digestion are inversely proportional to lignin content in the biomass [5,26]. The excellent delignification of alkaline peroxide is not the only reason why researcher are paying more attention to in the past decades. Unlike acid or alkaline pretreatment, alkaline peroxide pretreatment can be perform at relatively mild conditions (concentration, temperature) and atmospheric pressure, while effectively delignified various agricultural residues, thus does not require any special reaction chamber [24]. Furthermore, alkaline peroxide is a relatively “green” reagent with low environmental impact as it can be easily decompose to yield water and oxygen.

To our knowledge, although alkaline peroxide have received much attention and been applied in various research field, in depth literature reviews specifically targeting on the applications of alkaline peroxide in the biomass processing field is limited. Thus, this review paper serves to compile, highlight, and discuss the vast potential and role of alkaline peroxide in biomass valorization.

Background Information of Alkaline Hydrogen Peroxide (AHP)

There is often solutions available in the nature to tackle most of the research problem, which offer the potential approaches that mimic the natural processes in the biosphere as an effective strategies. According to Li et al. [8], oxidative deconstruction of plant cell wall by aerobic saprophytes are commonly seen in the nature which is potentially important to offer a direct effective pretreatment strategies, however the understanding to the carbon and energy propagation pathways is still incomplete. Particularly, brown rot fungi which is proposed to uses a non-enzymatic mechanism to selectively alter lignin and cellulose by using hydroxyl radicals generated through Fenton chemistry [30] from hydrogen peroxide yielded from plant cell wall polymer oxidation [8].

Potential to enhance conversion process and induced synergism with the biological conversion as a result from pretreatment approaches that implement effective trait of biological deconstruction routes of biomass have brought attention to oxidative pretreatment technologies [8]. Over the past decades, various oxidizing agents specifically used in pretreatments are developed, such as peracids [31], organic peroxides [32], oxygen

[33], ozone [34], and hydrogen peroxide [35]. Recent research have shown inclination towards the uses of AHP in biomass pretreatments due to its excellent delignification effect in lignocellulosic biomass and retaining carbohydrates that are highly susceptible to enzymatic hydrolysis and fermentation [36]. Under normal circumstances, hydrogen peroxide can only react with aliphatic part of lignin, while no changes and degradation of phenolic compounds are observed [37]. However, hydrogen peroxide is able to attack the phenolic compounds when it is under alkaline conditions and heated to a relatively high temperature which will expose the phenolic ring and causes carboxylic groups to be added to the macromolecular structure [5,38]. Such alteration of biomass structure by AHP pretreatment has leads to effective lignin and xylan removal, while decrease cellulose crystallinity as well as swelling of biomass due to the insertion of polar groups into the molecule [39].

Reaction mechanism

The extent of AHP pretreatments relies on the H_2O_2 decomposition and its intermediate products which acts as an initiator for various oxidative reactions with biomass components. The decompositions of hydrogen peroxide can be further improved with the absence of stabilizer, such as $MgSO_4$ or sodium silicate and the presence of heavy-metal ions [40]. Under alkaline conditions specifically close to pH 11.5, hydrogen peroxide will dissociates to produce hydroperoxyl anion (HOO^-), which is responsible for the corresponding carbonyl and ethylene groups' oxidative reactions and initiator for radicals forming. Thus, AHP pretreatment for delignification is strongly pH-dependent with optimum pH of 11.5-11.6 which is the pKa for the H_2O_2 decomposition reaction [35]. These hydroperoxyl anion will react with hydrogen peroxide to produce highly reactive hydroxyl radical (HO•) and superoxide anion radical ($O_2^{-}\bullet$) [41], which are known to be strong oxidants that are responsible for lignin oxidization and depolymerization biomass into low molecular weight fragments [7,40]. According to Cabrera et al. [24], no significant alteration in biomass structure during lignin oxidation might be observed depending on the pH adopted, as only the aliphatic part of lignin is oxidized in low alkalinity conditions. Therefore, the effectiveness of delignification strongly depends on the pH employed during the reaction in order to promote the H_2O_2 derived radicals. **Fig. 1** shows the reactions illustration of hydrogen peroxide decomposition in alkaline conditions and the generation of highly reactive radicals as described above.

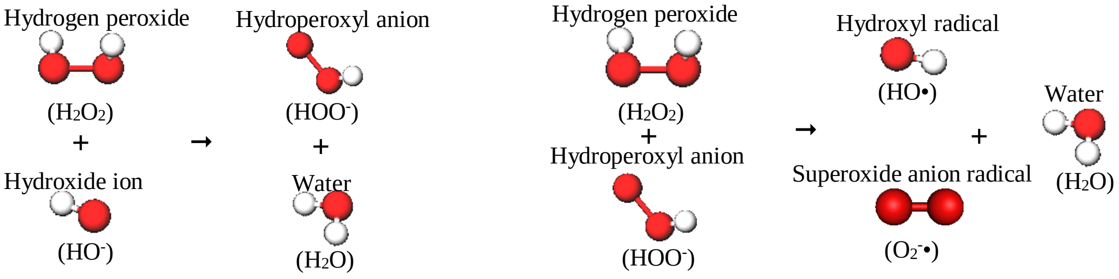

Fig. 1 Illustration of hydrogen peroxide derived radicals' generation reactions

These radicals generated from the H_2O_2 dissociation in alkaline conditions exhibit an oxidative action that initiates the depolymerization of lignin through cleaving the side chains and fragmenting lignin macrostructure into smaller compounds [24,7,40]. As mentioned by Li et al. [8], such pretreatment is effective at enhancing the dissolution of grass lignins due to the destruction of ester, ether cross-links as well as cleavage of β-O-4 bonds within lignin. On top of that, AHP pretreatment can also causes a significant amount of hemicellulose solubilisation [22]. However, undesired cellulose depolymerization also present along with delignification depending on the operational variables used. Thus, optimization of peroxide loading, reaction duration, and temperature are to be carried out for different biomass to achieve efficient biomass fractionation [40].

Recent applications of Alkaline Hydrogen Peroxide in biomass processing

Generally, one or more pretreatment stages are essential for effective hydrolysis of biomass before subsequent fermentation processes into value added products due to the recalcitrance nature of biomass to resist chemical, biological, and physical hydrolysis. Biomass without undergoing a pretreatment stage, resulting in a sugar yield of 21% [8]. However, the sugar yield can be increase up to 98% by implementing a pretreatment step prior to hydrolysis [5]. Recent literature have shown an inclined trend of AHP in biomass dissolution application and revealed the potential of AHP to be one of the economically viable pretreatment process. An effective pretreatment stage is achieved through the disintegration of lignin in the biomass structure which is the major protective barrier in biomass, which leads to increase in accessibility of polysaccharides during hydrolysis. AHP is particularly effective in enhancing the glucose yield in the subsequent hydrolysis due to its ability to solubilise lignin and hemicellulose, which are the two protective barrier to cellulose [22,8]. **Table 1** summarizes several available literature entries regarding the application of AHP in biomass delignification and sugar recovery.

The mechanism of AHP in biomass delignification was due to its ability to destruct ester and ether cross-links as well as β-O-4 bonds within lignin [8]. However, AHP is highly dependent on the operational variables whereby undesired cellulose depolymerization can be occur. Therefore, optimization are to be carried out to specifically target different biomass for efficient fractionation [40]. In Cabrera et al. [24] studies, AHP showed no significant effect in biomass structure alteration under low alkalinity conditions, as only aliphatic part of lignin is oxidized. The efficiency of AHP in delignification was highly dependent on the promotions of H_2O_2 derived radicals which is affected by the reaction conditions. Moreover, Li et al. [21] revealed that AHP caused delignification and increase in cellulose crystallinity in pretreated biomass are due to the capability of AHP in radicals formation under alkaline conditions to selectively target lignin. These intermediate products formed as a result of decomposition of H_2O_2 under alkaline condition (pH 11.5) enabled the disruption of complex matrix in lignocellulosic biomass, thereby exposed cellulose and enhanced its dissolution capability.

As a case in point, AHP (4.3 vol% H_2O_2) caused lignin solubilisation up to as high as 92.44% when used to pretreat cashew apple bagasse for 6 h at 35 °C [7]. When da Costa Correia et al. [7] increased the concentration of H_2O_2 from 0.645 to 4.3 vol% under similar solid loadings (10%) and conditions (35 °C, 24 h, pH 11.5), the degree of delignification drastically increased from 16.5 ± 0.9% to 80.2 ± 0.5%. On top of that, da Costa Correia et al. [7] also revealed that when a lower solid loading was employed (5%), the delignification was substantially increased to a maximum of 96.7 ± 0.7% over a duration of 24 h. Delignification efficiency of AHP in pretreating hardwood based biomass are often lower than in softwood pretreatment, thus catalyst in AHP seemed to play a vital role in hardwood pretreatment in order to achieve subsequent profitable yield. According to Li et al. [8], Cu(II)(bpy)-catalysed AHP exhibited an improvement in the lignin solubilisation from 36.6% (uncatalysed) to 50.2% which resulted in total material loss of 25.9% for *Hybrid poplar* pretreatment under similar conditions (48 h, ambient temperature, pH 11.5, 10 g H_2O_2/L). Li et al. [8] claimed that the highly ordered structure of the cell wall matrix may be limiting on the oxidative radicals which may resulted in disproportionation reactions, whereas the employment of small and diffusible homogenous catalyst such as Cu(II)(bpy) provide an alternative route to improve site-specific reactions with cell wall polymer, thus improving lignin and xylan solubilization while depolymerise the corresponding biomass. On top of that, Li et al. [21] explored the efficiency of ultrasonic (40 kHz, 500 W) assisted AHP in delignification of Jerusalem artichoke and was able to improve lignin removal from 37.5% (AHP only) to 40.3%. Despite a little increment by implementing the ultrasonic mechanism in delignification, degree of polymerization by amorphous substances (hemicellulose, lignin, and pectin) removal was significantly reduced leading to crystallinity index (CrI) of Jerusalem artichoke increased from 45 to 62.5%. Thus, drastically improve the biomass porosity and accessibility.

Nonetheless, it is important to take note that the reaction conditions are biomass specific where optimization are to be carried out for different biomass and different applications. For example, optimum conditions for corn stover in delignification are 3 h, 50 °C, and 250 mg H_2O_2/g dry biomass [40], whereas for Jerusalem artichoke are 120 min, 50 °C, and 5% H_2O_2 (w/v) [21]. In contrast, Alvarez-Vasco and Zhang [22] used AHP to pretreat *Douglas fir* at relatively high temperature of 180 °C, which resulted in delignification of 22% and exceptionally high glucomannan removal (hemicellulose) of 78%, while cause little degradation on cellulose. According to Alvarez-Vasco and Zhang [22], reducing end groups on glucomannan can occur quickly at high temperature which can facilitated organic acids formation through endwise depolymerization, accompanied with termination reactions (formation of non-reducing end groups). These series of reactions provide a potential route to convert hemicellulose to valuable organic acids such as lactic acid, glycodic acid, succinic acid and formic acid, while removing protective barrier of biomass to enhance cellulosic biofuels or biochemical productions. On the other hand, Morone et al. [42] demonstrated the use of pressurized air (6 bar, 190 °C) oxidation assisted by AHP (APWAO) aimed to reduce the H_2O_2 dosage in the pretreatment. By employing wet air oxidation on AHP pre-soaked Rice straw, the H_2O_2 concentration can be reduced to as low as 0.5 % (w/v) while achieved maximum lignin removal and cellulose recovery of 77.29 and 83.01% respectively [42]. High temperature treatment can lead to higher cellulose enrichment in the biomass and better solubilization of hemicellulose and lignin. However, high temperature environment also promote sugar and lignin degradation and formation of carboxylic acids such as acetic acid as a result of acetyl groups removal from hemicellulose. The formation of such acids remarkably change the pH of the reaction content from pH 11.9 to as low as pH 5.63 [42]. Mittal et al. [40] have further revealed that lignin extraction efficiency depends on the peroxide concentration, whereby under mild peroxide concentrations, only side chain structures of lignin were oxidized, while no significant changes on aromatic rings. At 250 mg H_2O_2/g dry biomass, both side chain structures and aryl-ether bonds are dissociated, suggesting disruptions of lignin aromatic rings and β-O-4 units. It is interesting that the AHP can be customize and adapt to different biomasses by optimizing the operating conditions to suit different applications, thus allowing AHP to be one of the promising pretreatment method in bio-refinery.

Sugar recovery of biomass are predominately affect by the lignin and hemicellulose removal which ultimately alter cellulose features. According to Li et al. [21], biomass after undergoing AHP pretreatment have found noticeable change in colour to transparent and reduced in particle size, yielding an abundance of fine particles. Surface morphology analysis displayed disorder fibrils and formation of tiny holes on the pretreated biomass structure, caused by removal of non-cellulosic polymers, subsequently disrupted polymers matrix and resulted in serious collapse supramolecular structure, suggesting a substantial increase in enzyme accessible surface area. The initially ordered and intact structure observed in untreated rice straw (**Fig. 2a**) became loosened with individualized fiber bundles together with cracks and cavities formed (**Fig. 2b** arrows), along with some debris observed which is suspected to be fragmentation of hemicellulose and lignin after undergoing

Table 1 Applications and performance of AHP in delignification and/or sugar recovery from lignocellulosic biomass

Biomass	Initial pretreatment	Subsequent pretreatment	Key findings	Ref.
Rice husk	*Alkaline hydrogen peroxide* Solid loading = 3:50 (w/v) T = 90 °C Time = 1 h pH = 11.5 H_2O_2 concentration = 7.5% (w/v)	*Enzymatic hydrolysis* 29.1 mL of citrate buffer (0.05M, pH 5), 0.3 mL of sodium azide (2%), 0.3 mL of NS22086 enzyme mixture, and 0.06 mL of each of the five Novozyme enzyme mixtures (NS22083, NS22118, NS22119, NS22002, NS22035) T = 50 °C Time = 72 h Mixing speed = 150 rpm	*Pretreatment with AHP* Solubilized compounds = 1.58 ± 0.07 g Sugar yield = 81.60 ± 2.63 %	[5]
Rice husk	*High pressure alkaline hydrogen peroxide* Solid loading = 3:50 (w/v) T = 90 °C Time = 30 min pH = 11.5 P = 30 bar H_2O_2 concentration = 3.0% (w/v)	*Enzymatic hydrolysis* 29.1 mL of citrate buffer (0.05M, pH 5), 0.3 mL of sodium azide (2%), 0.3 mL of NS22086 enzyme mixture, and 0.06 mL of each of the five Novozyme enzyme mixtures (NS22083, NS22118, NS22119, NS22002, NS22035) T = 50 °C Time = 72 h Mixing speed = 150 rpm	*With initial pretreatment* Solubilized compounds = 1.55 ± 0.18 g Sugar yield = 98.47 ± 6.35 %	[5]
Cashew apple bagasse	*Alkaline hydrogen peroxide* Solid loading = 5% (w/v) T = 35 °C Time = 6 h pH = 11.5 H_2O_2 concentration = 4.3% (v/v) Mixing speed = 150 rpm	*Enzymatic hydrolysis* Cellulase complex (Novozymes NS 22074, 108.12 FPU/mL), 0.3 g cellulose equivalent biomass, 15 mL of 0.1M citrate buffer (pH 4.8) and 120 µL of 10 mg/mL tetracycline in 70% v/v ethanol. Final cellulose activity at 60 FPU/g cellulose with volume of 30 mL T = 45 °C Time = 72 h Mixing speed = 150 rpm	*With initial pretreatment* Lignin removal = 92.44% Enzymatic disgestibility = 87% Glucose yield = 161 mg/g untreated biomass	[7]

Table 2 continued

Biomass	Initial pretreatment	Subsequent pretreatment	Key findings	Ref.
Hybrid poplar	*Cu^{II}(bpy)-catalyzed alkaline hydrogen peroxide* Solid loading = 1:10 (w/v) T = Ambient temperature Time = 48 h pH = 11.5 H_2O_2 concentration = 10 g/L	*Enzymatic hydrolysis* 1M (pH 5.0) Na-citrate buffer (500 µL), 10mM tetracycline (40 µL), mixed enzyme of Novozyme Cellic C-Tec (227 FPU/mL) and H-Tec (1090 FXU/mL) T = 50 °C Time = 72 hours	*Without pretreatment* Lignin solubilisation = None Hemicellulose solubilisation= None Glucose yield = 21% *Uncatalysed pretreatment* Lignin solubilisation = 36.6% Hemicellulose solubilisation = 14.9% Glucose yield = 27% Material loss = 14.1% *Catalysed pretreatment* Lignin solubilisation = 50.2% Hemicellulose solubilisation = 32.7% Glucose yield = 50.0% Material loss = 25.9%	[8]
Jerusalem artichoke	*Alkaline hydrogen peroxide* Solid loading = 1:20 (w/v) T = 50 °C Time = 120 min NaOH concentration = 2% (w/v) H_2O_2 concentration = 5% (w/v)	*Enzymatic hydrolysis* Pretreated residue (1g), mixed-cellulase reaction buffer (0.2M acetic acid – sodium acetate, pH 4.8) supplemented with mixed cellulose (β-glucanase $\geqslant 3.6 \times 10^4$ U, cellulose $\geqslant$ 360 U, and xylanase $\geqslant 6 \times 10^4$ U) to 40 mL T = 50 °C Time = 48 h Mixing speed = 150 rpm	*Without initial pretreatment* Cellulose content = 37.7 ± 0.6 % Lignin Removal = None Degree of polymerization = 2199.2 Cellulose crystallinity ≈ 45 % Total sugar yield = 4.1 g/L *With initial pretreatment* Cellulose content = 43.8 ± 0.8 % Lignin Removal = 37.5 % Degree of polymerization = 1292.5 Cellulose crystallinity ≈ 58.8 % Total sugar yield = 8.4 g/L	[21]
Jerusalem artichoke	*Ultrasonic assisted alkaline hydrogen peroxide* Ultrasonic frequency = 40kHz Ultrasonic power = 500 W Solid loading = 1:20 (w/v) T = 50 °C Time = 120 min NaOH concentration = 2% (w/v) H_2O_2 concentration = 5% (w/v)	*Enzymatic hydrolysis* Pretreated residue (1g), mixed-cellulase reaction buffer (0.2M acetic acid – sodium acetate, pH 4.8) supplemented with mixed cellulose (β-glucanase $\geqslant 3.6 \times 10^4$ U, cellulose $\geqslant$ 360 U, and xylanase $\geqslant 6 \times 10^4$ U) to 40 mL T = 50 °C Time = 48 h Mixing speed = 150 rpm	*Without initial pretreatment* Cellulose content = 37.7 ± 0.6 % Lignin Removal = None Degree of polymerization = 2199.2 Cellulose crystallinity ≈ 45 % Total sugar yield = 4.1 g/L *With initial pretreatment* Cellulose content = 44.3 ± 0.5 % Lignin Removal = 40.3 % Degree of polymerization = 785.3 Crystallinity = 62.5 % Total sugar yield = 10.4 g/L	[21]
Douglas fir	*Alkaline hydrogen peroxide* Solid loading = 1:10 (w/v) T = 180 °C Time = 60 min pH = 11.6 H_2O_2 concentration = 4% (w/w)	None	*Pretreatment with AHP* Delignification = 22% Glucomannan removal = 78%	[22]

Table 3 continued

Biomass	Initial pretreatment	Subsequent pretreatment	Key findings	Ref.
Corn stover	*Alkaline hydrogen peroxide* Solid loading: 1:10 (w/v) T = 50 °C Time = 3 h pH = 11.5 H_2O_2 concentration = 250 mg H_2O_2 / g dry biomass	*Enzymatic Hydrolysis* 1% solid loading, CTec3 enzyme (16mg protein per gram of glucan) with 50mM citrate buffer (pH 4.8), supplemented with HTec3 (4mg protein per gram of glucan) T = 50 °C Time = 120 h Mixing speed = 130 rpm	*Without initial pretreatment* Lignin removal = None Glucose yield = 23 % Xylose yield = 17 % *With initial pretreatment* Lignin removal = 80 % Glucose yield = 90% Xylose yield = 80%	[40]
Rice straw	*Alkaline hydrogen peroxide-assisted wet air oxidation* Soaking in alkaline hydrogen peroxide Time = 14 h pH = 11.9 H_2O_2 concentration = 0.5 % (w/v) Pressurized with 6 bar air at 190 °C for 20 min with mixing at 200 rpm	*Enzyme hydrolysis* Cellulase from *Trichoderma reesei* (60 FPU/mL) and β-glucosidase from almonds (10CBU/mg), solid loading at 60 g/L in soldium citrate buffer (0.1M, pH 4.8) with 2% sodium azide. T = 50 °C Time = 72 h	*Without initial pretreatment* Lignin removal = None Glucose yield = 83.25 ± 7.34 g/kg untreated rice sraw *With initial pretreatment* Lignin removal = 77.29% Cellulose recovery = 83.01% Glucose yield = 200.34 g/kg untreated rice straw	[42]

APWAO pretreatment [42]. A similar observations was obtained from surface morphology analysis done by Mittal et al. [40], whereby biomass after undergoing AHP pretreatment resulted in fine particles (**d** arrow), transmission electron micrographs revealed less densely stained (lignin loss) and multiple delaminations (**f** arrows), and cell disjoining at the middle lamella with dimmer cell wall (**e** arrows), which is in consistent with other publications. The collective structure alteration caused by the removal of hemicellulose and lignin subsequently increased cellulose exposing area and enhanced cellulose enzymes accessibility, which significantly improved glucose recovery. A maximum of glucose yield of 90% and xylose yield of 80% was attained when AHP (250 mg H_2O_2/g dry corn stover) pretreatment was followed by enzymatic hydrolysis, in which the hydrolysate is predicted to be highly utilizable in fermentation for biofuel productions [40]. Analogous observations have been seen in Díaz et al. [5], whereby a maximum sugar yield of 98.47 and 81.6% was achieved after saccharification for high pressure AHP and AHP pretreated biomass respectively. The AHP have displayed excellent performance in pretreating corn stover, in which more than 80% delignification could be achieved after 3 h at 50 °C, while obtaining highly digestible carbohydrate-rich residue retaining more than 90% of carbohydrates initially available in corn stover [40]. In contrast, saccharification efficiency for glucose and

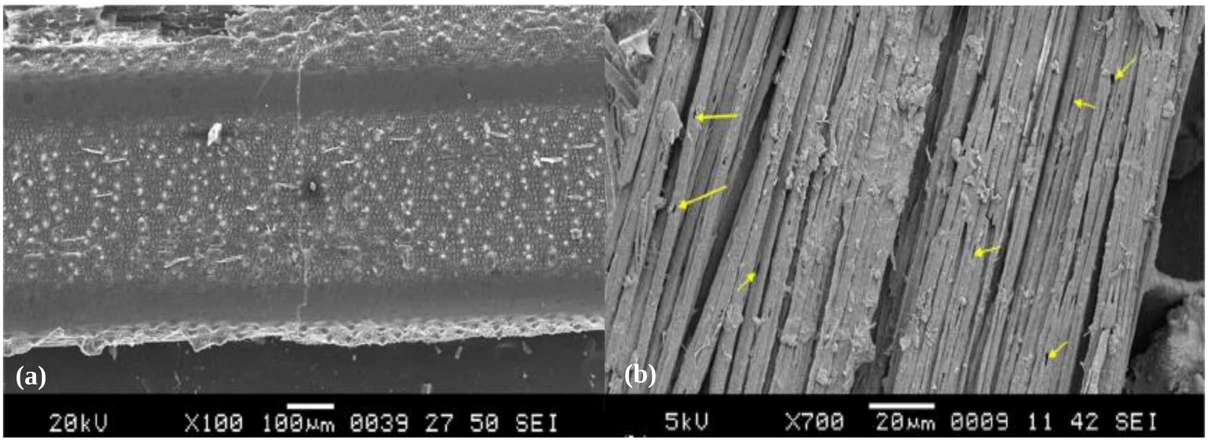

Fig. 2 Scanning electron micrograph for (a) untreated rice straw and (b) APWAO pretreated rice straw. Reprinted with permission from Morone et al. [42]. Copyright 2017 Springer Nature.

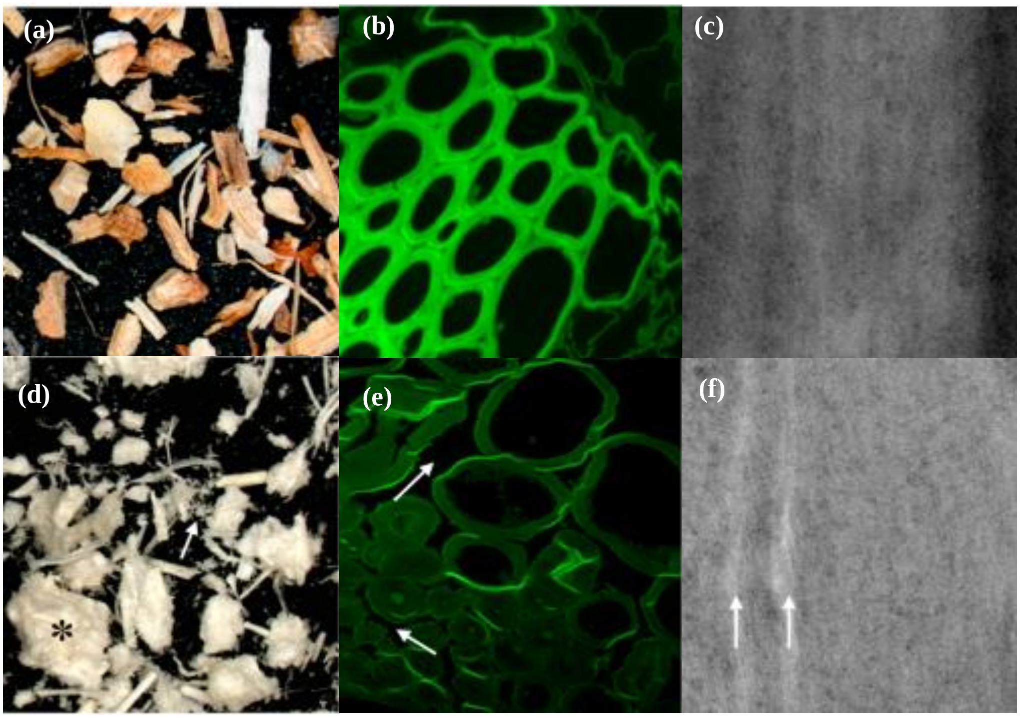

Fig. 3 (a) Stereoscope, (b) confocal scanning laser, and (c) transmission electron micrographs of untreated corn stover; (d) stereoscope, (e) confocal scanning laser, and (f) transmission electron micrographs of pretreated corn stover. Reprinted with permission from Mittal et al. [40]. Copyright 2017 American Chemical Society.

xylose achieved by Mittal et al. [40] for untreated corn stover were 23 and 17% respectively, which were comparatively lower than pretreated biomass.

Regarding inhibitor production, AHP pretreatment of lignocellulosic biomass has been known to generate small amount of acetic acid as a results of hemicellulose acetyl group removal. Most of the lignocellulosic pretreatment methods tend to generate 5-hydroxymethylfurfural (HMF) and furfural as a result of sugar degradation to different extent. Interestingly, Li et al. [21] revealed that there is no formation of HMF and furfural to a detectable level in any of the four alkali-based pretreatment (dilute sodium hydroxide, alkaline peroxide, ultrasonic assisted dilute sodium hydroxide, and ultrasonic assisted alkaline peroxide) studied. From Li et al. [21] findings, the concentration of acetic acids was significantly reduced by 38.5% to 1.15 g/L when H_2O_2 was used in the pretreatment, contrary to the negative results from ultrasonic assisted pretreatment, which suggest that H_2O_2 possess strong hydrolysate detoxification effect. Similar to that, Morone et al. [42] have reported a maximum acetic acid concentration of 1.2 g/L with absent of HMF and furfural in APWAO of rice straw. However, contradicting results were obtained by Banerjee et al. [43], whereby most studies on APWAO of different lignocellulosic biomass reported the presence of furfural and HMF which are the most potent inhibitors for subsequent bioconversion process. As explained by [42], the absence of these inhibitors suggest that there was no sugar degradation even at an elevated temperatures and sugar are mainly preserved in oligosaccharides form. Further, total phenolic content (TPC) which possess inhibitory effect to fermenting strain was found to be in the range of 0.29 to 0.36 g/L from APWAO pretreatment which is far lower than the inhibitory concentration (>2 g/L TPC) [42]. Thus, additional hydrolysate detoxification may be omitted for AHP pretreatment technologies.

Conclusion

This review has highlighted various applications of AHP, particularly for the purpose of lignocellulosic biomass processing. The advancement of biomass processing technologies is often limited by cost, environmental impact, and toxicity. AHP pretreatment have showed promising potential in the biomass valorization due to their compatibility with subsequent bioconversion, relatively safe decomposition products, and flexibility in adjusting process conditions to suit different scope of biomass fractionation. Therefore, AHP provide an alternative sustainable route to effective biomass processing for biofuels or biochemical productions. Furthermore, only limited studies have incorporate the use of ambient temperature AHP in effective biomass processing and the synergism through combinations of AHP with other existing pretreatment technologies is comparatively less explored thus far. The synergism between pretreatment strategies could potentially provide an opportunity to reduce the severity of process conditions and effectively improve process-economic. Therefore, future research on the application of stage-wise pretreatment strategies and processes scale up using AHP is essential to release its potential to the fullest as an cost effective pretreatment and contribute towards a new chapter in biomass valorization industry.

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