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A critical review on water overconsumption in lignocellulosic biomass pretreatment for ethanol production through enzymic hydrolysis and fermentation

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Abstract: Global demand for renewable and sustainable energy fostered the considerable development of biomass-to-ethanol valorization strategies. Thermochemical pretreatment methods have been proposed to render biomass more amenable to enzymatic and microbial digestion. However, the efforts have not led to its industrial-scale worldwide realization. One of the obstacles to commercialization could be related to water overconsumption, as excessive water washing of the pretreated slurry is often performed to remove inhibitory compounds and residual chemicals after biomass pretreatment. Only increasing solid loading for biomass pretreatment results in ineffective pretreatment performance, more inhibitors formation, and high viscosity, which in turn necessitates the water washing step. A number of physicochemical and biological methods are applied to detoxify the acid-pretreated liquid fraction for enzymatic hydrolysis and fermentation. Among them, alkaline neutralization and liquid-liquid extraction are preferred because of their simple operation and low cost. Seemingly, recycling black liquor for alkali pretreatment offers a pathway to reduce water and chemical consumption, but alkali replenishment and inhibitor accumulation significantly weaken this technology. Interestingly, quite a few studies have removed the water washing and even solid-liquid separation steps after (liquid hot water, Tween 40, and CaO) pretreatment. Whereas there is still a huge room for future studies to render biomass pretreatment more feasible in terms of economic and environmental points of view. This review provides a deep understanding of wastewater generation during biomass upgrading and discusses the solutions to reduce water consumption critically.

Keywords: Biomass; pretreatment; detoxification; wastewater generation; ethanol production

1. Introduction

Global demand for coal and petroleum based energy sources resulted in the potential depletion of non-renewable fossil fuels.[1–3] Exploring alternative bioresources with renewable and sustainable characteristics is an essential worldwide task to alleviate overdependence on fossil fuels and collateral environmental issues.[4–6] The share of coal in the total U.S. energy consumption reduced in favor of emerging energy sources such as natural gas and nuclear for the past century.[7] Strikingly, in the last two decades, other renewable energy sources stood out and occupied approximately 12% of U.S. primary energy consumption in 2020, as it plays an important role in reducing greenhouse gas emissions. Biomass-related resources approximately account for 39% of renewable energy.[7] Woody biomass is what we often recognize as lignocellulosic biomass, which is commercially used by power plants to generate electricity and steam via combustion with/without coal to reduce net CO_2 emissions. Counting its annual production, lignocellulosic biomass from agriculture (corn stover, wheat straw, sugarcane bagasse, etc.) and forestry (paper mill and sawmill discards) is typically underutilized.[8,9]

Lignocellulosic biomass has been considerably recognized as a competitive candidate for ethanol production via pretreatment, enzymatic hydrolysis, and fermentation steps due to its substantial cellulose and hemicellulose components.[10–12] However, the recalcitrance of robust lignin necessitates physicochemical pretreatment to render lignocellulosic biomass more amenable to enzymatic and microbial degradation.[13–15] The mechanisms and effectiveness of pretreatment technologies can be found elsewhere.[10,11,16–19] Despite half a century of efforts, the biomass-to-ethanol commercial realization has stagnated confronting several technical and economic bottlenecks such as the difficulty in a massive collection of raw biomass and unaffordable production cost. From the production point of view, excessive water washing and anti-solvent addition after biomass pretreatment are commonly needed, since the inhibitory compounds such as derivatives [acetic acid (HOAc), formic acid, levulinic acid, furfural, 5-hydroxymethylfurfural (HMF)] from sugar degradation and phenolic compounds from lignin as well as residual chemicals (acids, alkalis, and ionic liquids) play determinant roles in constraining enzymatic and microbial activities.[20–23] Obviously, wastewater generation and subsequent discarding would result in water overconsumption.[24–26] From the perspective of an economic aspect, such routine operations are inevitably not feasible in long-term commercial production, because the process of biomass-based ethanol refinery is affected by the availability of water resources and excessive wastewater generation can inevitably increase purification treatment costs.[7,27] However, there is significant controversy among the studies regarding the technoeconomic feasibility of biomass processing plants.[28–33] Although the directly unambiguous comparison between the studies is unfeasible, this is probably related to the inconsistency between process simulation and lab-scale experimental steps.[34,35] For example, Zang et al.[30] reported that switchgrass pretreated by choline chloride:ethylene glycol (ChCl:EG) with 1% H_2SO_4 was washed with a water-acetone mixture and then hydrolyzed after adding water and enzymes directly, however, it was not in agreement with the cited reference where the citrate was used as a buffer.[30] Besides, it was highlighted that after lignin precipitation the resultant liquid fraction was subjected to evaporation at 70 °C to remove water for ChCl:EG recovery, however, during process simulation the resultant liquid fraction was directly reused for switchgrass pretreatment.[30] Acetone-water washing of pretreated switchgrass could dilute the ChCl:EG concentration, even if some of it was reused, it would result in abundant wastewater (containing ChCl:EG) generation. Therefore, reducing water consumption during lignocellulosic ethanol production is crucial for a circular economy.[36]

Maintaining high solid biomass loading from pretreatment to fermentation has been highly preferred to reduce production costs.[37–39] To a certain extent, the application of high solid loading can increase the concentration of bioethanol and reduce the cost of distillation.[40] However, with the respect to commercial promotion, it is necessary to consider the "high-solids side effect" such as the accumulation of inhibitory compounds, low cellulose accessibility, and high viscosity.[41] The undesirable outcomes might offset the advantages of operating at high solid loading.[42] In addition, physicochemical detoxification technologies for acid pretreatment and successive recycling of black liquor for alkali pretreatment have been considerably proposed. Organosolv, ionic liquid, and deep eutectic solvent pretreatments have attracted considerable attention to fractionate biomass.[17,19,43] Unfortunately, efficient and practical strategies targeting the reduction in water consumption are not available.[41] This is probably because water or anti-solvent washing is inevitable to recover these expensive reagents.[44]

The objectives of this work are to disclose the collateral challenges confronted by biomass-to-ethanol production in terms of water overconsumption and offer a consolidated source of information in connection with the latest advances from the laboratory to commercial exploration. Explicitly, following a detailed discussion of wastewater generation, the corresponding solutions including the increment of solid loading, physicochemical detoxification, and black liquor recycling are critically discussed to elucidate if these methods are effective in reducing water consumption. Additionally, several advances in response to alleviating water consumption during biomass-based ethanol production were illuminated.

2. Water overconsumption

Although physicochemical pretreatment can remove most of the hemicellulose and partially solubilize the lignin enhancing the enzymatic accessibility to cellulose, the undesired derivatives from sugar and lignin degradation can also be released during biomass pretreatment. These derivatives include furans (HMF and furfural], organic acids (HOAc, formic acid, and levulinic acid), pseudo-lignin, small lignin units, extractives, and phenolic compounds.[10,45,46] Furthermore, the residual chemical reagents used for biomass pretreatment often contribute to inhibitory influences on enzymatic and microbial activities as well. To render the pretreated biomass amenable to ethanol conversion, the solid residues after pretreatment and solid-liquid separation are commonly washed extensively with water, whereas the resultant wastewater (liquid fraction), containing residual chemicals, derivatives, and sugars, is often discarded (Table 1). For example, Nogueira et al. reported that washing (200 mL water/15g raw biomass per time) was conducted 12 times to achieve NaOH (2%, w/v) pretreated coconut fiber filtrate transparent and reach a neutral pH of this filtrate.[47] Therefore, the post-washing process inevitably results in a large amount of water consumption and chemical loss (Table 1). Herein, the inhibitory compounds that appeared in the pretreated slurry are roughly categorized into two groups to elucidate the reasons for wastewater generation and discarding.

Table 1. A summary of representative studies regarding water overconsumption under different biomass pretreatment conditions at lab and pilot scales.[1]

Biomass	Pretreatment condition	Post-treatment	Ref.
Corncob	Combining H_2SO_4 and CH_3COOH with different ratios under solid loading of 10-30% at 80-120 °C for 30-90 min	The residues are washed with distilled water and separated using a vacuum filter, and the collected residues are dried to constant weight in an oven at 105 °C	Selvakumar et al. (2022) [48]
Miscanthus	The first stage: 1% HNO_3 at 90 °C for 2 h; the second stage: 4% HNO_3 solution at 94-96 °C for 8 h	The solid residues are washed thoroughly with water until the neutral wash water is formed	Skiba et al. (2022) [49]
Chestnut shell	10% solid loading with 0-5% NaOH at 70 °C for 2 h	The slurry is neutralized with distilled water to pH 7, and the residual chestnut shell is dried in an oven at 105 °C for 48 h to completely remove the water	Lee et al. (2022) [50]
Sugarcane bagasse	Two stages ultrasonic assisted 2.0% H_2SO_4 at 6% solid loading	The solid residues are washed with distilled water until neutral pH for the filtrate, followed by drying the recovered solid overnight at 55 °C	Chen et al. (2022) [51]
Switchgrass	15% solid loading with liquid hot water at 200 °C for 5 min	The pretreated biomass is washed with 10 g of distilled water per gram of solids four times with the wastewater is discarded	Larnaudie et al. (2019) [52]
Empty palm fruit bunch	5–25% solid loading with liquid hot water at 160–210 °C for 0–60 min	The pretreated biomass is washed with an amount of water equivalent to 10 times the amount of biomass initially loaded to the reactor with the wastewater is discarded	Cardona et al. (2018) [53]

Acacia wood	5% solid loading with 0.05% H_2SO_4 at 200 °C for 5 min	The pretreated filtrate and 100 mL of washed deionized water are discarded	Lee et al. (2020) [54]
Bamboo	10% solid loading with 30% hydrogen peroxide/glacial HOAc (1:1, v/v) at 85 °C for 120 min	The pretreated biomass is washed with tap water and the wastewater is discarded	Song et al. (2020)[55]
Sugarcane biomass	2–8% solid loading with 1% (m/v) of H_2SO_4 at 121 °C for 20–60 min	The pretreated biomass is washed with distilled water until a pH close to 5.0 with the wastewater is discarded	Santos et al. (2020)[56]
Cashew apple bagasse	10% solid loading with HOAc (0–60 v/v) or H_2SO_4 (0–0.8 w/v) at 121 °C for 30–60 min	The pretreated biomass is washed eight times using 200 mL of tap water with the wastewater is discarded	Araujo Padilha et al. (2020)[57]
Wheat straw	10% solid loading with 1–10% NaOH solutions at 190 °C for 240 min	Deionized water is used to wash the pretreated biomass and then discarded	Tsegaye et al. (2019)[58]
Prosopis juliflora biomass	Microwave irradiation power (270–450 W) for 3–5 min with 0.75–1.25% (w/v) NaOH solutions at the liquid-to-solid ratio of 10–20 mL/g	The filtrate is washed with distilled water until the pH becomes neutral and then discarded	Alexander et al. (2020)[59]
Softwood pine	10% solid loading with 0–2% w/v NaOH at 100–180 °C for 1–5 h	The pretreated biomass is washed with distilled water until reaching pH 7 with the wastewater is discarded	Safari et al. (2017)[60]
Poplar biomass	13% solid loading with ethanol (60%, v/v) solution and 1.25% (w/w) of H_2SO_4 at 180 °C for 60 min	The pretreated biomass is washed with 60 °C aqueous ethanol (60%), as the washings are combined with the filtrate and poured into ∼500 mL of deionized water.	Meng et al. (2020)[61]
Hybrid Pennisetum	Four organosolv (γ-valerolactone, tetrahydrofurfuryl alcohol, ethanol, and acetone) assisted	The pretreated biomass is first washed with an equal volume of the organic solvent at least three times to avoid lignin	Tan et al. (2020)[62]

	by 0.05 mol/L H_2SO_4 with a liquid/solid ratio of 12:1 at 100 °C for 2 h	deposition and then washed to neutral with water as the wastewater is discarded	
Spruce and oak sawdust	2% solid loading with 1-ethyl-3-methylimidazolium acetate at 45 °C for 40 min	The pretreated biomass is precipitated by adding two times of ultrapure water and then centrifuged; it is thoroughly washed with ultrapure water as the wastewater is discarded	Alayoubi et al. (2020)[63]
Hornbeam and spruce wood	4% solid loading with biomass to 1-butyl-3-methylimidazolium chloride ratio of 1:4 at 50–150 °C for 0.5–2 h	The pretreated biomass is washed with distilled water to obtain 95–99% removal of the ionic liquids as the wastewater is discarded	Dotsenko et al. (2018)[64]
Wheat straw	10% solid loading with ChCl, guaiacol, and $AlCl_3$ (molar ratio of 25:50:1) at 80–130 °C for 1 h	The pretreated biomass is washed with 200 mL of hot ethanol and excessive water stepwise as the wastewater is discarded	Huang et al. (2021)[65]
Hybrid Pennisetum	10% solid loading with $FeCl_3$ in ChCl/glycerol at 60–140 °C for 1–9 h	The pretreated slurry is washed with 50% acetone/water (100 mL) and then vacuum-filtered and re-washed with 50% acetone/water until the filtrate is colorless as the acetone is distilled from the filtrate	Wang et al. (2020)[66]
Eucalyptus biomass	Pilot-scale pretreatment at 180 °C for 15 min with 2.4 wt.% H_2SO_4 followed by steam explosion	After pretreatment, residual biomass solids were pressed to remove the hydrolyzate, and the steam-explored sample was washed with distilled water until the pH of the filtrate was 6.0.	Mclntosh et al. (2016)[67]

[1]The studies were collected from recent publications with a pretreatment that included liquid hot water, acid, alkaline, organosolv, ionic liquid, deep eutectic solvent, and combined methods.

2.1 Sugar and lignin derivatives

Furans (HMF and furfural) are generally found in the acid-pretreated slurry and derived from cellulose and hemicellulose degradation, respectively.[68–70] Based on the literature review, these molecules are not found to significantly inhibit enzymatic hydrolysis, apart from the work reported that the addition of 2 or 5 mg/mL of furfural to the substrate of cellulose and enzyme decreased glucose recovery by 5% and 9%, respectively.[20] However, it has been widely recognized that they can negatively influence the microbial ethanol fermentation of the pretreated materials.[71–79] In this regard, Roberto et al. demonstrated that furfural at 0.5 g/L had no significant effect on the cell growth of Scheffersomyces stipitis, while furfural at 2 g/L was detrimental to cell growth.[80] Similarly, Nigam found that the presence of furfural at 0.25 g/L was unable to limit the cell growth of Pichia stipitis and ethanol production from wheat straw hydrolysates, while furfural at 1.5 g/L notably reduced ethanol yield and productivity by 90.4% and 85.1%, respectively.[77] Concerning their inhibitory mechanisms, Allen et al. illuminated that furfural triggered the accumulation of reactive oxygen species in Saccharomyces cerevisiae inducing cellular damage via the destruction of mitochondria and vacuole membranes as well as the actin cytoskeleton and nuclear chromatin.[81] During fermentation, HMF and furfural can be metabolized by Saccharomyces cerevisiae into 5-hydroxymethyl furfuryl alcohol and furfuryl alcohol, respectively, indicating their similar inhibitory mechanisms.[75,76] Compared to furfural, HMF was found to have less inhibitory effects on microbial activity due to its lower membrane permeability.[76,82] In addition, weak organic acids such as acetic, formic, and levulinic acids can typically be produced from the dissociation of acetyl groups and furans during pretreatment.[46,82,83]

The individual addition of organic acids to the hydrolysis or fermentation system was noticed to be detrimental,[20,77] which could be attributed to the change in slurry pH inhibiting cell growth. Therefore, their negative effects may be mitigated via alkaline neutralization, which can be seen in the report that H_2SO_4 pretreated slurry after ammonia conditioning could be used for enzymatic hydrolysis and fermentation directly.[84]

In the case of lignin, it has been assessed that non-productive adsorption and steric hindrance are broadly known as the primary mechanism controlling lignin-enzyme interactions,[85] depending on the molecular weight and structural characteristic of lignin.[86] Pseudo-lignin, formed from dilute acid pretreatment,[69,70,87] can also significantly retard cellulose hydrolysis.[88] Besides, the phenolic compounds are reported to be more poisonous than the previously-mentioned derivatives even at lower concentrations, due to their low molecular weight allowing them easily penetrate cell membranes and alter cell morphology.[75,76,82] Even with solid-liquid separation after pretreatment, these degraded products are still partially adsorbed to the surface of the pretreated biomass, thus excessive water is usually used to remove them or reduce their recondensation.[89]

2.2 Residual chemical reagents

Another reason for post-washing the pretreated biomass is to remove the residual chemical reagents used for biomass pretreatment. For dilute acid and alkaline pretreatments, the pH of the pretreated slurry is commonly too severer to be used as a buffer solution for enzymatic hydrolysis and fermentation.[69] The work by Frederick et al. highlighted that rinsing the 0.98% (v/v) H_2SO_4 pretreated biomass with 1.5 or 3 volumes of water reached glucose yields that were seven folds higher than the unwashed treatment.[90] They also mentioned that the H_2SO_4 pretreated biomass washed with 3 volumes of water created the highest ethanol yields (up to 0.43 g/g-glucose) that were significantly higher than those from the unwashed sample (≤ 0.28 g/g-glucose).[90] Based on the same enzymatic hydrolysis and fermentation conditions, it could be considered that the higher glucose and ethanol yields are highly associated with the removal of the residual H_2SO_4. In addition, Karuna et al. pretreated rice straw with NaOH and subsequently conditioned the pretreated slurry to a pH of 5-6 via extensive water washing or acidification with HCl plus water washing.[91] In this case, excessive post-washing removed the disrupted lignin and residual NaOH, while acidification (neutralization between HCl and NaOH) with post-washing precipitated the modified lignin on the surfaces of rice straw, therefore, the former showed higher enzymatic digestibility of rice straw than the latter.[91] In terms of ionic liquid pretreatment, the researchers performed choline acetate (ChOAc) and 1-ethyl-3-methylimidazolium acetate (EmimOAc) pretreatments (0.5 g biomass/5.0 g ionic liquid) of bagasse powder with different water post-washing times (45 mL per time).[92,93] It was found that cellulase and yeast were more sensitive to the residual EmimOAc concentrations in the pretreated biomass than ChOAc, based on their median effective concentrations.[93] Besides, based on the original bagasse the overall ethanol yield after saccharification and co-fermentation of the pretreated bagasse with post-washing 5 times was only 54% for ChOAc and 22% for EmimOAc.[92] This indicates that the residual ionic liquid in the pretreated biomass is dramatically detrimental to enzymatic and microbial activities, therefore, adequate water is crucial to remove the residual ionic liquid. Moreover, to remove the imidazole from the pretreated biomass, 2 volumes of distilled water and 3 volumes of ethanol

(96%) were employed. However, the HCl used for lignin precipitation was found to deprotonate imidazole.[94] Therefore, even if the chemicals are recovered and recycled for biomass pretreatment, their functional integrity is unknown. Additionally, an anti-solvent applied for chemical reagent removal from the pretreated biomass has a significant influence on glucose conversion efficiency.[95]

3. Common strategies for reducing water consumption

3.1 Increasing solid loading

Biomass pretreatment has been often performed at lower solid loadings (≤10%) to efficiently fractionate biomass into cellulose-concentrated solid fraction and hemicellulose- and lignin-derived liquid fraction.[96] In keeping with the idea of reducing water consumption, high solids loading (>10%) for biomass pretreatment was widely promoted. However, undesired side effects such as weak pretreatment effectiveness, accumulation of inhibitory compounds, and high viscosity were often observed.[38,96] In terms of pretreatment effectiveness, Xu et al. conducted the EmimOAc pretreatment of corn stover assisted with/without NaOH and aqueous ammonia (10%, v/v) at 36% (w/w) solid loading but performed the enzymatic hydrolysis of pretreated biomass at 1% (w/v) glucan loading after three times (10 mL/g biomass) of post-washing. In this study, only 60.65–64.82% of total glucose yields were obtained at a higher enzyme loading,[97] indicating that the accessibility of cellulose to enzymes after pretreatment was relatively low. Besides, the increment of solid loading for biomass pretreatment significantly reduced xylan and lignin removal as reported by Chen et al. who carried out the ternary deep eutectic solvent pretreatment of switchgrass under 10-35% of solid loading.[98]

The effects of solid loading used for pretreatment on the accumulation of inhibitory compounds in the pretreated slurry have not been investigated yet, but it can be inferred that an increase in solid loading would increase their concentrations.[38,96] Therefore, the nominal reduction in water consumption may be offset by the heavy water post-washing operation needed for the removal of residual chemical reagents. Additionally, the resultant slurries with high solids tend to be super viscous,[99] due to the water-biomass interaction.[37] Viamajala et al.[100] reported that biomass size reduction can decrease slurry viscosity, but a large amount of energy is required for milling.[101] Moreover, it has been demonstrated that feedstock pump ability could only be achieved at solid loading below 15%.[102] In this regard, Dãrãban et al. found that pumpable wood-based slurry containing 20% solids can be prepared using recycled biocrude as a carrier fluid, given that the particle sizes of biomass were smaller than 0.125 mm.[102] However, this phenomenon has been typically overlooked, instead, excessive water was used to flush the slurry out.[98,103,104] Based on the previous analysis, it might be debatable whether high solid loading for pretreatment can save water because of the subsequent challenges.

3.2 Physicochemical detoxification

To render the dilute acid pretreated biomass and hydrolysate (liquid fraction) more acquiescent for microbial fermentation, many physicochemical [membrane filtration,[105,106] alkaline neutralization,[107] ion exchange resin,[108–110] liquid-liquid extraction,[22,111] and activated charcoal adsorption[21,112,113]] and biological [laccase treatment,[114,115] microbial degradation,[116,117] and engineered strain[118–120]] detoxification methods have been investigated. A summary of these representative studies is presented in terms of strengths and drawbacks (Table 2). The comprehensive collection and comparison among studies in this respect can be found elsewhere.[10,11] but several crucial discoveries can be extracted from them: (1) alkaline addition is unavoidably required to neutralize the residual acid in both pretreated biomass and hydrolysate before enzymatic hydrolysis and fermentation regardless of which methods are applied; (2) the cost of chemicals and enzymes for detoxification should be taken into consideration since the end product-bioethanol is considerably sensitive to materials input;[34] (3) excessive pursuit of HMF and furfural removal efficiency and negligence of their subsequent recovery are undesirable because they are especially high-value platform molecules for biofuels and chemicals conversion; and (4) almost all techniques only focus on the acid pretreated hydrolysate and ignore the residual inhibitors in the pretreated biomass. Concerning the simple operation and low capital investment, alkaline neutralization and liquid-liquid extraction might be relatively preferable. The former can create optimal conditions that result in an analogous fermentability comparing a synthetic sugar solution without inhibitors,[107] whereas the latter can entirely extract HMF and furfural as high-value coproducts with the extraction solvent can be recycled into the system.[111]

Table 2. Different physicochemical and biological detoxification methods at lab and pilot scales with their advantages and disadvantages.

Samples	Methods	Advantages	Disadvantages	Ref.

H_2SO_4 pretreated corn stover hydrolysate[1]	Trialkylamine extraction	73.3% of HOAc, 45.7% of HMF, and 100% of furfural are removed	The concentration process of hydrolysate and post-washing of pretreated biomass are needed	Zhu et al. (2011)[22]
Stepwisely liquid hot water and H_2SO_4 pretreated sugarcane bagasse hydrolysate	A sequence of treatments including $Ca(OH)_2$ neutralization, IR-120 resin, activated charcoal, and IRA-67 resin	Inhibitors such as HMF, furfural, HOAc, and formic acid are removed	Various chemicals [$Ca(OH)_2$, resin, and activated charcoal] input with tedious operation procedures increases production baseline cost	Vallejos et al. (2016)[109]
H_2SO_4 pretreated seaweed hydrolysate	Activated carbon, the over-liming method with $Ca(OH)_2$, and the ion exchange method with polyethyleneimine	Activated carbon shows the best performance for HMF removal with simple operation	Ion exchange leads to a significant loss of fermentable sugars; higher energy is demanded to produce activated carbon	Nguyen et al. (2019)[112]
H_2SO_4 pretreated spruce hydrolysate	NH_4OH, NaOH, and $Ca(OH)_2$ neutralization	It is practical to operate with the mild optimal conditions (pH 9.0/60 °C for NH4OH; from pH 9.0/80 °C to pH 12.0/30 °C for NaOH treatment)	Sugars are partially lost; removal efficiency of phenols is relatively low; HMF and furfural removal highly depends on the alkaline concentration	Alriksson et al. (2006)[107]
H_2SO_4 pretreated sugarcane bagasse	Vacuum evaporation followed by liquid-liquid extraction using 1-butanol, isobutyl acetate, or methyl isobutyl ketone	Methyl isobutyl ketone leads to 69.0% of phenolics, 85.4% of HOAc, and 100.0% of HMF and furfural removal	Vacuum evaporation increases energy input; organic solvents are costly and can be partially dissolved in the hydrolysate	Roque et al. (2020)[111]

Modeled hydrolysate	Ten nanofiltration and reverse osmosis membranes with low molecular weight cut-off	High rejection performances (97% for sugars and 80% for HMF and vanillin)	The operation process is costly; inhibitors can only be selectively removed; sugars are partially lost	Nguyen et al. (2015)[106]
Simulated hydrolysate	Activated charcoal in a fixed-bed column adsorption system	HMF, furfural, and phenolic compounds can be efficiently removed	The affinity of activated charcoal with H_2SO_4 and HOAc is weak; activated charcoal is costly	Lee et al. (2020)[21]
HNO_3 pretreated corncob hydrolysate	Ion exchange resin	70% of the nitrate salt, phenolic content, and HMF are removed	Pore diffusion is slow and required a high processing time	Kumar et al. (2018)[108]
H_2SO_4 pretreated corn stover hydrolysate	Laccase treatment	84% of the phenolic compounds are removed	Laccase is costly and cannot be recycled; the treatment is time-consuming	Fang et al. (2015)[114]
H_2SO_4 pretreated sugarcane bagasse hydrolysate	The isolated bacterium (Bordetella sp. BTIITR) treatment	100% of furfural, 94% of HMF, and 82% of HOAc are removed	The treatment is time-consuming, as incubation took 16 h	Singh et al. (2017)[116]
Dry H_2SO_4 pretreated corn stover	Co-culture of xylose-utilizing and inhibitor-tolerant Saccharomyces cerevisiae	Ethanol yield and concentration are enhanced	Large amounts of sugar are left in the final slurry; complex operation procedures are needed	Zhu et al. (2016)[120]
Pilot-scale supercritical water with H_2SO_4 catalyst	The hydrolysate was centrifuged to remove the sediments and then treated with 4% (w/v) of activated carbon. The pH of the filtered	The hydrolysate can be fermented to ethanol with a yield of 14.1% based on biomass	Solid/liquid loading (1:50 w/v) for pretreatment is too low; detoxification (activated	Jeong et al. (2017)[121]

pretreated hydrolysate	hydrolysate was adjusted to 5.5 by 5 N NaOH		charcoal) and concentration (evaporation) of hydrolysates are costly	

[1]Hydrolysate was denoted as a liquid fraction after pretreatment and solid-liquid separation.

3.3 Black liquor recycling

In the case of alkaline pretreatment, the black liquor from the pretreated hydrolysate has typically been recycled for biomass pretreatment (Table 3). Seemingly, it is a promising strategy to reduce water and chemical consumption during biomass valorization. Several drawbacks make this method controversial: (1) the extra water and NaOH usually need to be replenished into the black liquor;[122–126] (2) the pretreated biomass is commonly subjected to excessive water post-washing and then combined with the fresh buffer before enzymatic hydrolysis and fermentation;[124,125,127,128] and (3) the pretreatment effectiveness (lignin removal, sugar conversion, ethanol yield, etc.) often decreases as recycling time increases.[122–124,127–129] Based on the previous analysis, it is difficult for a single acid and alkaline pretreatment to make up for their shortcomings in a comprehensive way.

Table 3. Recycling of black liquor from alkaline pretreated biomass hydrolysate for biomass pretreatment.

Biomass	Initial pretreatment conditions	Post-treatment process	Pretreatment effectiveness and findings	Ref.

Sugarcane bagasse	A steam explosion under 190 °C for 15 min, followed by NaOH (1%, w/v) delignification at 98–100 °C for 1 h with 5% solid loading	The black liquor is recycled as a delignification solution with the pH adjusted to 13; the pretreated biomass is washed with water until pH 6	NaOH can be recycled as black liquor but it is vital to keep the pH in the 12.6–13.3 range before each new delignification cycle	Rocha et al. (2014)[122]
Rice straw	10% solid loading with NaOH (0.5 M, pH=13.9) at 121 °C for 2 h	The black liquor is ultrafiltered by a ceramic membrane; the permeate is recycled as a delignification solution with its pH readjusted to 13.9; the membrane is cleaned at 50 °C with an aqueous NaOH solution of 1% (w/v) for 1 h and then washed with deionized water	Glucose yields from enzymatic hydrolysis at 10% solid loading with citrate buffer range from 40–50% as the black liquor is recycled four times	Li et al. (2015)[123]
Sugarcane bagasse	10% solid loading with NaOH (2%, W/V) at 80 °C for 2 h	The pretreated solid is washed with 600 mL water three times; the black liquor is added 0–1.5% (w/v) of NaOH and recycled for pretreatment	Enzymatic hydrolysis efficiency decreases as the recycling times of black liquor increase	Wang et al. (2016)[127]
Miscanthus sacchariflorus	Twin-screw extrusion NaOH (0.6 M) pretreatment at 100 °C	The black liquor is recycled for pretreatment; the pretreated biomass is used for enzymatic hydrolysis directly without details	Lignin removal and sugar yields decrease as recycling times increase	Cha et al. (2016)[129]

Corn stover	10% solid loading with H_2O_2 (7.5%, v/v) solution with pH of 11.58 at 25 °C for 1 h	The pretreated biomass is washed with distilled water until neutral pH; the black liquor is recycled for pretreatment	Lignin and hemicellulose removal and sugar yields decrease as recycling times increase	Alencar et al. (2017)[124]
Corn stover	3 kg solids with 0.165 kg sodium hydroxide pellets and 29.6 kg of tap water at 80 °C for 2 h	The black liquor is combined with water and 0.165 kg of NaOH; the pretreated biomass is washed with 30 kg of fresh water in the paddle reactor for 30min	The accumulation does not lower acetyl and lignin removal during pretreatment, resulting in comparable sugar yields in enzymatic hydrolysis	Chen et al. (2018)[125]
Cogongrass	10% solid loading with NaOH (2%, W/V) at 85 °C for 90 min	The pretreated biomass is washed with 300 mL of water in three stages; the black liquor is diluted by wasted water and replenished with 1% (W/V) NaOH	Ethanol yield decreases from 90.8% (zero recycle) to 66.4% (tenth recycle) at 3% solid loading of enzymatic hydrolysis	Goshadrou (2019)[128]
Sugarcane bagasse	10% solid loading with vacuum-assisted NaOH 2% (w/v) pretreatment at 121 °C for 1 h	The pretreated biomass is washed to neutral pH in hot deionized water; the black liquor is supplied by fresh deionized water and adjusted to a pH of 13.70	Glucose yields are not significantly different between pretreatment with fresh NaOH and recovered black liquor; ethanol yields obtained from the unwashed biomass are significantly higher than those from the washed biomass	Fan et al. (2020)[130]

4. Novel processes for reducing water consumption

4.1 Omitting water-washing after solid-liquid separation

Eliminating the washing step after biomass pretreatment is required to reduce water use, therefore, it is vital to distinguish alternative pretreatment strategies to minimize the generation of inhibitory compounds.[40] Previous studies have reported the potential of unwashed pretreated biomass from solid-liquid separation for enzymatic hydrolysis and fermentation. For example, Lu et al.[131] achieved an ethanol concentration of 56.28 g/L via Tween 40 pretreatment of unwashed pretreated reed straw and further fed-batch fermentation (Fig. 1). Wang et al. used the unwashed NaOH pretreated sugarcane bagasse for fed-batch enzymatic hydrolysis and fermentation after pH adjusting with glacial HOAc (Fig. 2) and reached an ethanol production of 44.53 g/L and 87.35% of theoretical ethanol yield.[126] Furthermore, a pretreatment method of densifying biomass with acid or alkali chemicals followed by an autoclave has been proposed to achieve high ethanol concentration (> 70g/L) through fed-batch hydrolysis and fermentation.[132–136] If water washing is omitted, the acid or alkali used for densifying biomass remains in the pretreated biomass. Therefore, the residual chemicals might destroy the structure and activity of the enzymes by changing the pH of the slurry during fed-batch fermentation. Unfortunately, it was only mentioned that no washing and detoxification were needed after pretreatment, whether the activity of enzymes changed and how to adjust the pH of slurry again when loading the pretreated biomass were not mentioned in detail. Additionally, the operation of liquid discarding and solid drying after solid-liquid separation may be a challenge for industrial applications.

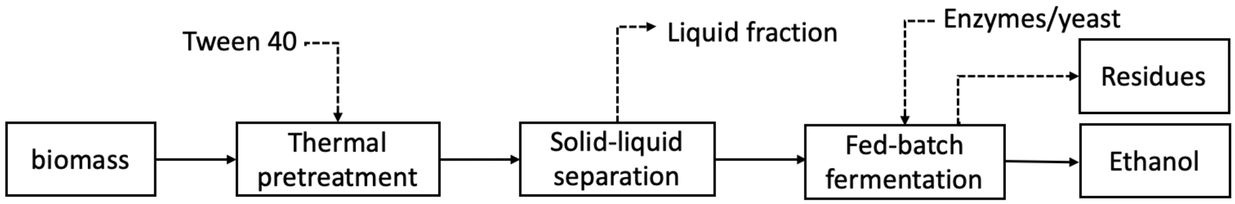

Fig. 1. The schematic flowchart of Tween 40 pretreatment followed by solid-liquid separation and fed-batch fermentation for ethanol production.

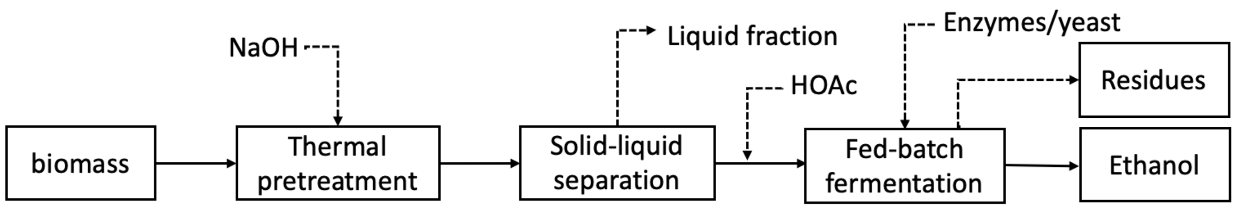

Fig. 2. The schematic flowchart of NaOH pretreatment followed by solid-liquid separation, pH adjusting with HOAc, and fed-batch fermentation for ethanol production.

4.2 Omitting water-washing without solid-liquid separation

The direct hydrolysis and fermentation of the pretreated slurry without solid-liquid separation may be the ideal pathway to reduce water consumption. Zheng et al.[137] reported that the slurry of pretreated sugarcane bagasse at 5% (w/v) solid loading can be hydrolyzed and fermented to ethanol after pH adjusting with 4 mol/L NaOH solution (Fig. 3). Whether it can be applied to the pretreatment scenarios with high solid loadings remains unknown. Romaní et al.[138] conducted non-isothermal autohydrolysis pretreatment of Eucalyptus globulus wood and performed simultaneous saccharification and fermentation of the pretreated slurry at a liquid/solid (g/g) of 6.4 to achieve an ethanol concentration of 50.2 g/L. However, in this study, the method of pH adjustment was not introduced. Rana et al.[139] conducted the wet explosion pretreatment of loblolly pine with a solid loading of 25% at a pilot plant and then performed the enzymatic hydrolysis of the pretreated slurry after the pH was adjusted to 5.0 using 4 M KOH. Remarkably, a 96% glucose and nearly 100% hemicellulose yield was reached, even though HMF, furfural, and HOAc were produced.[140] Note that just because a pretreated slurry can be hydrolyzed by enzymes after pH adjustment does not mean that it can be fermented by strains into ethanol as discussed in Section 2.1. This can be also verified by our previous study where H_2SO_4 (pH = 1.12) or NaOH (pH = 13.53) pretreated slurry at initial solid loadings of 10 and 20% was subjected to enzymatic hydrolysis and fermentation after pH adjusting with 10 M NaOH and 10% H_2SO_4, respectively.[141] Results showed that both scenarios can generate high sugar concentration and yield, but only the hydrolysate from NaOH pretreatment with 10% initial solid loading can be efficiently fermented to bioethanol.[141]

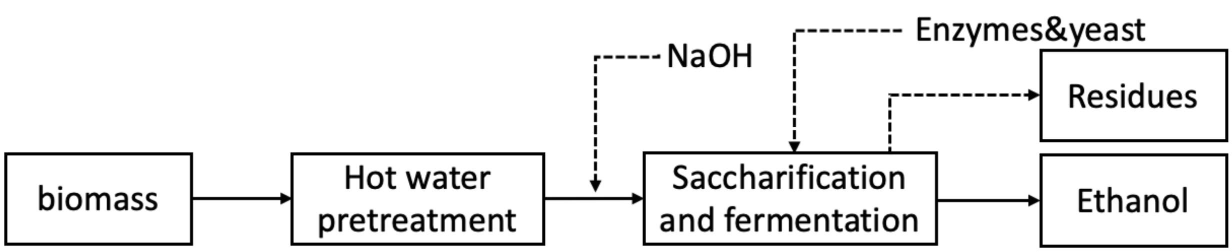

Fig. 3. The schematic flowchart of liquid hot water pretreatment followed by pH adjusting with NaOH and fermentation for ethanol production.

4.3 Perspective on CaO pretreatment with an acid neutralization

Based on the finding in our previous study,[141] could the toxicity of NaOH pretreated slurry with 20% initial solid loading after pH adjusting with H_2SO_4 to yeast cells be caused by the high concentration of Na_2SO_4? If so, the $CaSO_4$ produced by substituting NaOH with CaO turns into a precipitate that does not affect the growth of yeast cells, and the tiny amount of dissolved calcium ions may also be used by yeast (Fig. 4). In this regard, CaO [or $Ca(OH)_2$] pretreatment of lignocellulosic biomass has been well established.[142–146] Surprisingly, it was reported that $Ca(OH)_2$ (0.15 g/g biomass) pretreated corn stover slurry after pH adjusting with H_2SO_4 can be efficiently converted to ethanol.[147] There might be some concern about how to achieve the practical handling of $CaSO_4$ and whether it affects downstream ethanol distillation. Replacing the H_2SO_4 with HOAc in the pH-adjusting pretreated slurry will lead to the formation of calcium acetate which can be separated from the fermented slurry before ethanol distillation. Compared to less-value $CaSO_4$, calcium acetate is indeed a functional calcium salt. In addition, CO_2 could be an alternative for the neutralization of $Ca(OH)_2$ to form $CaCO_3$. Experiments and techno-economic analyses are needed to confirm whether the idea is feasible. In addition, in the process of thermochemical pretreatment with high solid loading, more or less inhibitory compounds will be produced in the pretreated slurry, so it is essential to screen and select robust enzymes and strains with high activity and tolerance.

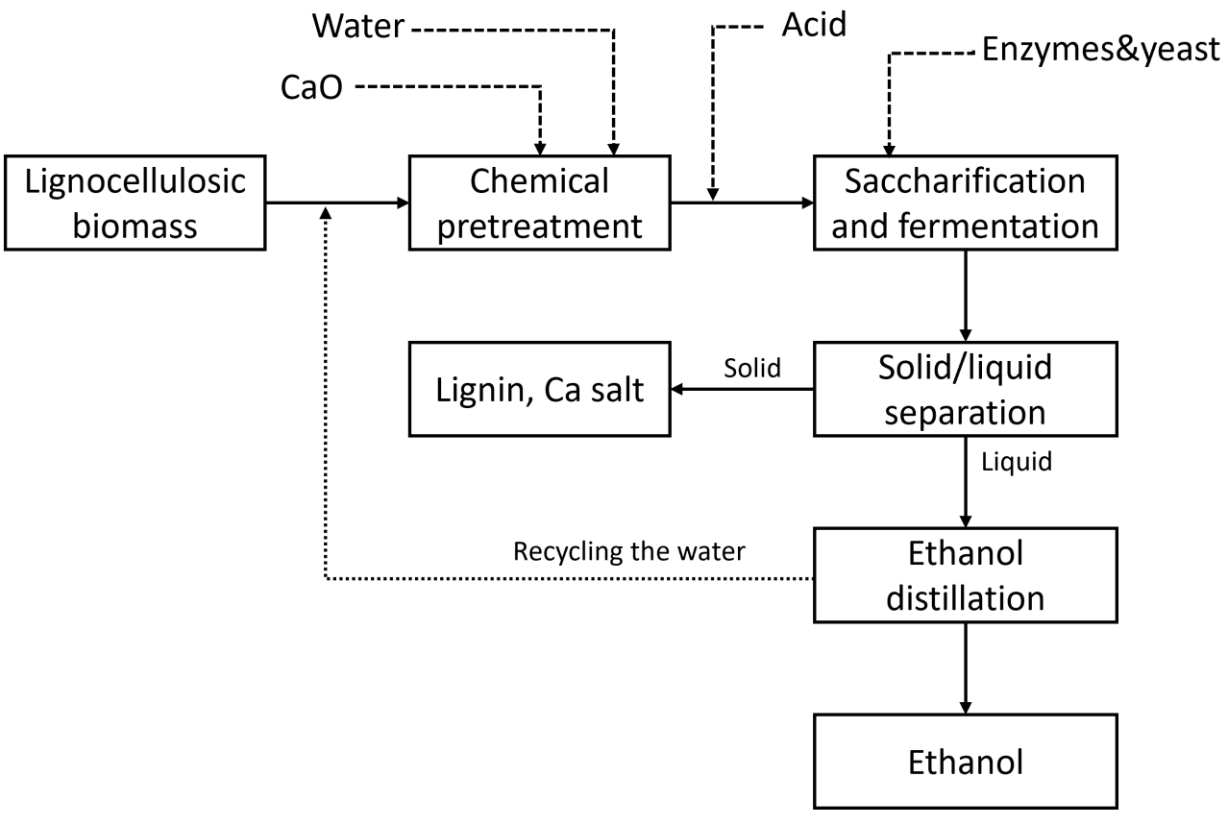

Fig. 4. The schematic flowchart of CaO pretreatment followed by pH adjusting with acid for ethanol production without water washing and solid-liquid separation.

5. Challenges and prospects

The production of biofuels from lignocellulosic biomass has great potential to reduce the dependence on fossil fuels. However, no robust pretreatment technologies are available yet for commercialization even though H_2SO_4 pretreatment has been highly promoted due to its low production costs. One of the economic challenges could be associated with water and chemicals overconsumption. For example, Ovejero-Pérez et al.[148] demonstrated that increasing water washing volumes of the pretreated biomass increased production costs but washing with 5.5 g water/g ionic liquid was approved to be the most economic option as it achieved a minimal total ionic liquid recovery cost of \$16/kg of biomass. Increasing solid loading for biomass pretreatment might be able to reduce water use to some extent, but the resulting side effects including ineffective pretreatment performance, more byproduct generation, and high viscosity cannot be ignored. For acid pretreatment, physicochemical and biological detoxification technologies have been applied to render the liquid fraction amenable to enzymes and microbes. Alkaline neutralization and liquid-liquid extraction could be doable based on their simple operation and low cost. For alkali pretreatment, recycling black liquor for biomass pretreatment provides a pathway to minimize water and chemical consumption, but several disadvantages such as declining pretreatment effectiveness should be considered. Up to now, several studies have removed the water washing step after pretreatment and achieved high ethanol concentration with/without solid-liquid separation, which offers a new pathway to reduce water use. Among them, CaO pretreatment followed by acid neutralization enables enzymatic hydrolysis and fermentation directly without solid-liquid separation and detoxification. To demonstrate the effect of water use during pretreatment on the economic and environmental aspects of the refinery, it is highly needed in future studies to obtain quantitative evaluation at a pilot scale. In addition, the development of robust strains that can keep effective digestion ability under severe conditions could also solve this challenge to a certain extent.

6. Conclusions

Physicochemical pretreatment strategies have been considerably developed to render lignocellulosic biomass amenable to enzymes and strains for ethanol production. However, a large amount of wastewater generation and discarding after biomass pretreatment might stall commercial exploration. The inhibitory effects of derivatives and residual chemicals on enzymes and microbes necessitate the excessive water washing of the pretreated slurry. The undesired side effects such as weak pretreatment effectiveness, high concentration of inhibitory compounds, and high viscosity of slurry might offset the advantages of operating at high solid loading. Physicochemical detoxification of the acid-pretreated liquid fraction faces technoeconomic challenges due to the additional investment of chemicals and materials. Seemingly, black liquor recycling has great potential to reduce water and chemical consumption, however, the alkali-pretreated biomass is substantially washed with water and then mixed with the fresh buffer for enzymatic hydrolysis and fermentation. Nevertheless, the additional water and alkali generally are replenished to the black liquor, because the pretreatment effectiveness inevitably decreases as black liquor recycling time increases. Recent studies on excluding water washing with/without solid-liquid separation provide new perspectives for water conservation. In particular, lime pretreatment followed by pH adjusting with acid may offer great promise for high-loading pretreatment and fermentation without water washing and solid-liquid separation to minimize water consumption.

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Notes

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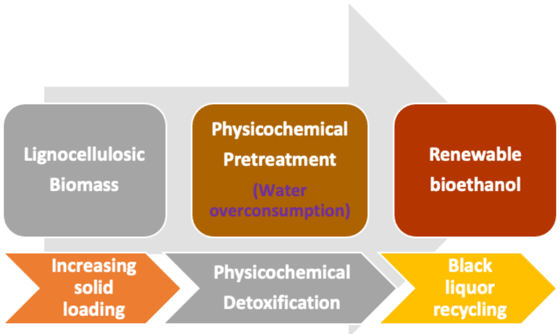

Synopsis

This review reveals wastewater generation during lignocellulosic bioethanol production and presents facile processes to reduce water consumption.