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Review

Enzymatic hydrolysis of lignocellulosic biomass from low to high solids loading

Solid state enzymatic hydrolysis (SSEH) has many advantages, such as higher sugar concentration, lower operating costs, and less energy input. It should be a potential approach for the industrial application of lignocellulosic ethanol. The purpose of this work is to review the enzymatic hydrolysis of lignocellulosic biomass from low to high solids loading and introduce its both challenges and perspectives. The limitations of SSEH, including inhibition effects, water constraint, and rheology characteristic, are summarized firstly. Various strategies for overcoming these limitations are proposed correspondingly. Fed batch process and its feeding strategy to improve the SSEH efficiency are then discussed. Finally, several intensification methods, hydrolysis reactor, and pilot- and demonstration-scale operations of SSEH are described. In-depth analysis of main limitations and development of novel intensification methods and reactors should provide an effective way to achieve large-scale implementation of SSEH.

Keywords: Biorefinery / Process intensification / Rheology / Solid state enzymatic hydrolysis / Water constraint

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1 Introduction

In the past decades, rapid economy development in many countries has raised the need for renewable energy resources due to the finite supply of fossil energy and an increasing concern of global warming [1–3]. Biomass refinery for renewable fuels has been since attracted much attention worldwide in response to energy security, environmental concerns, and society development [2,4,5]. Lignocellulosic biomass (LCB), such as agricultural residues, forestry residues, and energy crops, should be primary raw material for renewable fuels production in view of its sustainable source and economical efficiency [6–8]. Lignocellulosic ethanol has been considered as one of most promising renewable fuels due to its high octane number, heat of vaporization, and compatibility with motor vehicles [2, 9, 10].

The procedures of lignocellulosic ethanol production are mainly divided into three steps, pretreatment, hydrolysis, and fermentation [11–13]. Enzymatic hydrolysis is one of the most important unit operations, in which polysaccharides were hydrolyzed into monosaccharides that can be subsequently converted to ethanol by microorganisms. Generally, the solids

content in the enzymatic hydrolysis stream is an important factor affecting the economic feasibility of lignocellulosic ethanol production [14–16]. In conventional conversion process solids loading is low, which results in low products concentration, low utilization efficiency of equipment, massive wastewater discharge, and high capital cost. In the distillation process, > 4% w/w ethanol concentration is needed to bring an efficient economic benefit, and thus the corresponding sugar levels of > 8% w/w are required. For most LCB types, the initial solids loading should be above 20% w/w [15, 16]. The enzymatic hydrolysis operated beyond 15% solids loading (w/w) is generally called high solids enzymatic hydrolysis [16]. Because the high solids enzymatic hydrolysis process has few or no free water and presents a solid state, it is also named solid state enzymatic hydrolysis (SSEH) in this study. SSEH can produce high sugar and ethanol concentrations, and thus it could reduce energy input and costs associated with the distillation operation. In addition, SSEH offers many other advantages, including improved overall productivity, size reduction of equipment, reduced water consumption, and decreased operating costs [15–17]. SSEH should be a potential approach for the industrial application of lignocellulosic ethanol. Some SSEH results of LCB with various pretreatments are given in Table 1 [18–29]. However, there are also several technical challenges hindering the large-scale implementation of SSEH [15–17]. For example, the increased degradation products (DPs) from pretreatment and the increased sugars concentration in enzymatic hydrolysis should inhibit the enzymes activity; the increased solids loading may lead

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Abbreviations: DP, degradation product; LCB, lignocellulosic biomass; SSCF, simultaneous saccharification and cofermentation; SSEH, solid state enzymatic hydrolysis

Table 1. SSEH results of LCB after various pretreatments

Feedstock	Solid loading	Pretreatment	Enzyme loading	Hydrolysis conditions	Conversion (%)	Ref.
Cane bagasse	20% w/w	Steam explosion (with acid impregnation)	0.1 g/g cellulose	50°C, 200 rpm, 96 h	69.2	[18]
Corn stover	28% w/w	Dilute acid	22 FPU/g cellulose	45°C, 130 rpm, 168 h	~73	[19]
Corn stover	27.9% w/w	Steam explosion (without washing)	20 FPU/g dry stover	50°C, 220 rpm, 96 h	70.9	[20]
	30% w/w	Steam explosion (with washing)		50°C, 220 rpm, 96 h	72.5	[20]
Corn stover	20% w/w	Steam explosion	30 FPU/g glucan	pH 4.8, 50°C, 200 rpm, 168 h	~70	[21]
Olive tree pruning	30% w/w	Liquid hot water	15 FPU/g solids	50°C, 150 rpm, 72 h	~50	[22]
Corn stover	20% w/w	Aqueous ammonia	15 FPU cellulase/g glucan, 30 CBU beta-glucosidase/g glucan, 7.5 mg xylanase/g glucan	pH 4.8, 50°C, 200 rpm, 168 h	~63	[23]
Corn stover	18% w/w	Ammonia fiber expansion	20 mg enzyme/g glucan	pH 5.0, 50°C, 250 rpm for 24 h, 150 rpm for 48 h	~61	[24]
Poplar	20% w/w	Organosolv	20 FPU/g cellulose, 80 CBU/g cellulose	-	83	[25]
Corn stover	9 + 3 + 3% w/w	Dilute acid	5 FPU/g solids	50°C, 12 rpm, 96 h	77	[26]
Sweet sorghum bagasse	10 + 5 + 5% w/w	Liquid hot water	30 FPU/g glucan	50°C, 100 rpm, 120 h	60	[27]
	15 + 7.5 + 7.5% w/w	Liquid hot water	30 FPU/g glucan	50°C, 100 rpm, 120 h	54	[27]
Corn stover	12 + 6 + 6% w/w	Steam explosion	10 FPU/g glucan	50°C, 200 rpm, 120 h	62	Our study
Wheat straw	9 + 8 + 7% + 6% w/v	NaOH	9.6 FPU/g solids	50°C, 120 rpm, 144 h	~39	[28]
Corn stover	10 + 10% w/w	Ammonia fiber expansion	15 FPU/g cellulose	50°C, 200 rpm, 72 h	~92	[29]

Solid loading is based on the dry matter of pretreated biomass solid.

to water constraint and high viscosity, resulting in the poor efficiency of mass transfer concomitantly. Thus, new strategy should be exploited to overcome these limitations and improve the efficiency of SSEH. The advantages and limitation factors of SSEH are given in Fig. 1.

The purpose of this work is to review the challenges and introduce the perspectives of SSEH. The limitations of SSEH, including inhibition effects, water constraint, and rheology characteristic, are summarized. Various strategies for overcoming these limitations are proposed correspondingly. Fed batch mode and its feeding strategies are then discussed to improve the efficiency of SSEH. Finally, several intensification methods, hydrolysis reactor, and pilot- and demonstration-scale operations of SSEH are described.

2 Limitations of SSEH

2.1 Inhibition effects by DPs

LCB contains cellulose and hemicellulose that can be released by pretreatment and hydrolysis and subsequently fermented to bioproducts such as ethanol by microorganisms. However, DPs are simultaneously generated from cellulose, hemicellulose, and

other polymer compositions such as lignin during pretreatment and hydrolysis according to the LCB types and the pretreatment conditions. DPs mainly include weak acids, furan derivatives, and phenolic compounds [4, 30]. Some DPs and soluble solids inhibit the enzymes activity and hence reduce the enzymatic hydrolysis efficiency. Notably, DP inhibition effects should become serious due to their increasing concentration in SSEH [14, 17, 19]. Zhao and Chen reported the effect of small phenolic compounds in pretreated stover on cellulase activity and found phenolic aldehyde significantly inhibits cellulase activity at 0.05–8 g/L [31]. Phenolic acids show significant inhibition effect at 0.05 g/L and slight stimulation effect at 2–4 g/L on cellulase activity, which indicates a concentration-dependent effect [31]. Most small phenolic compounds in pretreatment are less than 1.0 g/L and should significantly affect the cellulase activity in enzymatic hydrolysis. Tejirian and Xu also reported that oligomeric phenolics present more inhibition on enzymatic hydrolysis compared with simple phenolics due to the inactivate effects on cellulases [32]. Simple and oligomeric phenolics may also inhibit enzymatic hydrolysis by adsorbing onto cellulose [32]. They also found that polyethylene glycol and tannase could bind and degrade the oligomeric phenolics, respectively, and thus mitigated the oligomeric phenolic's inhibition [32]. Hodge et al. found that when insoluble solids

Figure 1. The advantages and limitation factors of SSEH [15, 16].

concentration is below 20%, soluble components (acetic acid, phenolics, furans, sugars) are primary factors contributing to the inhibition of enzymatic hydrolysis [19]. Rajana and Carrier studied the effects of dilute acid pretreatment on DP production and its inhibition on enzymatic hydrolysis [33]. Results showed that the increase of pretreatment severity lead to the increase of DP generation, as well as a 27% reduction in monosaccharide yield at 15% solids loading [33]. In addition, nonproductive adsorption of the enzymes on lignin or molten lignin fragments is an important cause for decreased enzyme activity in enzymatic hydrolysis [8, 14, 34, 35]. Ko et al. confirmed that the adsorption of enzymes on lignin is nonproductive and thus reduce the enzymatic hydrolysis efficiency of liquid hot water pretreated solids [34]. They also found that because the enzyme components have different profiles, such as various molecular weights and hydrophobicity, and their different adsorption behaviors with lignin, the change of enzyme activities ratio is needed in cellulose hydrolysis [34]. Xu et al. also reported that upon mixing with the insoluble substrate, the enzymes are adsorbed and no significant desorption is detected due to the specific binding of cellulose and the nonspecific binding of lignin [35]. The addition of several representative cellulolysis enhancers could enhance the enzymes action on substrates, leading to a significant desorption of the adsorbed enzymes [35].

Enzymatic hydrolysis is an intermediate step in the LCB conversion process, and the resulting hydrolyzate should be capable of supporting fermentative microorganisms. But, most DPs inhibit the activity of microorganisms [30]. Therefore, some strategies must be employed to overcome DP inhibition effect. The DP inhibition effect can be partly eliminated by the removal or detoxification of inhibitors. There are a number of methods for detoxification. An effective one is to wash the pretreated biomass with water prior to enzymatic hydrolysis. Rajana and Carrier reported that by using six volumes of washing water, 87% of formic acid and 64% of acetic acid are removed in the water

soluble fraction in dilute acid pretreatment of wheat straw [33]. Eighty six percent of furfural and 87% of HMF are removed in the wash water upon washing the solid fraction [33]. Qin et al. found that the glucan conversion of aqueous ammonia pretreated corn stover increases by 15–30% with the increase of solids loading after postwashing [23]. Additionally, they also found that the increasing concentration of phenolics mainly inhibits the enzymatic hydrolysis performance and postwashing can remove this inhibition effect [23]. Lu et al. found that for washed steam exploded corn stover at 30% solids loading, enzymatic hydrolysis can obtain a cellulose conversion of 72.5%, and its hydrolysate shows a good fermentation performance [20]. However, the hydrolysate of unwashed steam exploded corn stover cannot be fermented at all [20]. Postwashing reduces DP inhibition effect and increases the efficiency of SSEH, but it also increases the water input and the treatment cost of wastewater. Lots of fermentable sugars in pretreated liquid fraction are removed, which reduces the utilization efficiency of sugars. Liu et al. found that compared with that at 15 FPU/g glucan, glucan conversion of steam exploded corn stover with increasing solids loading is obviously improved at 60 FPU/g glucan, which implied that the addition of enzymes can reduce DP inhibition effects [21]. It should be noticed that enzymes account for most cost of enzymatic hydrolysis, and thus the economic feasibility of process should be weighed by considering these factors. Other detoxification methods, such as $\text{Ca}(\text{OH})_2$, activated carbon, and fungus treatment etc., have also been reported to remove DP inhibition effects and proved to be effective [28, 30]. Nonetheless, these operations increase the cost of chemicals and materials, waste treatment, and process operations (Table 2). Therefore, various factors should be considered comprehensively in order to reduce DP inhibition effects and increase the efficiency of SSEH, such as the types of LCB, the choice and optimization of pretreatment, the conditions of enzymatic hydrolysis, and the chemicals and materials use.

Table 2. The limitation factors of SSEH and the potential strategies for overcoming these limitations

Limitation factors	Potential strategies	Effect	Disadvantages
Increased inhibitors concentration	Washing pretreated solid	+++	Increase water input and treatment cost of wastewater; reduce the utilization efficiency of sugars
	Detoxification	++	Increase the use of chemicals and the cost of waste treatment and operation
	Choice of suitable pretreatments	+	—
	Optimization of pretreatment conditions	+	—
Sugars feedback inhibition	Increase of enzyme loading	++	Increase the cost of enzyme
	Enzyme synergetic system	++	Increase the cost of enzyme
	Combination with fermentation (e.g. SSF)	+++	Generally, reduce the enzyme activity
	Enzyme feeding with fed batch mode	++	Increase the cost of operation
Water constraint	Solid feeding with fed batch mode	++	Increase the cost of operation
	Optimization of pretreatment conditions	+	—
	Development of novel strengthened method	++	May increase the cost of reactor
	Using the pelleted biomass solids	++	Increase the energy consumption
Rheology characteristics	Solid feeding with fed batch mode	+++	Increase process complexity; increase the cost of operation
	Development of novel strengthened method	++	May increase the cost of reactor
	Development of novel hydrolysis reactor	++	May increase the cost of reactor

major effect = +++; moderate effect = ++; minor effect = +; SSF = simultaneous saccharification and fermentation.

2.2 Sugars feedback inhibition effects

With the increase of solids loading, the sugars concentration increases and the sugars feedback inhibition effects become serious. The enzymatic hydrolysis of LCB is catalyzed by various enzymes, mainly including endo-1,4- β -D-glucanase, exo-1,4- β -D-glucanase, β -glucosidase, and other xylanases. The products of enzymatic hydrolysis include glucose, xylose, cellobiose, and oligosaccharide etc., some of which inhibit the activity of enzymes especially at high concentration [15, 16]. Kristensen et al. found that the decreasing conversion is an intrinsic effect at increased solids loading, describing a linear correlation from 5 to 30% initial total solids loading (w/w) [36]. With the addition of 50 g/L glucose, the initial hydrolysis rate and the final glucan conversion are obviously reduced. Although product inhibition could not take full responsibility for the decreasing conversion, glucose and in particular cellobiose under the increased concentrations plays a role at high solids loading [36]. Qing et al. found the xylose, xylan, and xylooligomers dramatically decrease the conversion rates and yields in enzymatic hydrolysis [37]. They also found that xylooligomers show more inhibition than xylan or xylose. A lower final glucose yield is obtained even at a low xylooligomers concentration of 1.67 mg/mL. At a higher concentration of 12.5 mg/mL, xylooligomers reduce the initial hydrolysis rates of Avicel by 82% and the final hydrolysis yield by 38% [37]. Hodge et al. found that the rates and extents of enzymatic hydrolysis of dilute acid pretreated corn stover decrease with increasing solid loading [19]. The insoluble solids loadings of more than 25% result in lower final yields, mainly due to the combination of sugar feedback inhibition and mass transfer limitations [19]. These studies suggest that the sugars feedback inhibition effect determine the efficiency of SSEH, especially at higher solids loading.

Various strategies have been developed to reduce or even avoid the sugars feedback inhibition effects of SSEH. The synergistic system of multiple enzymes should be one of the most effective

strategies [38]. Garcia-Aparicio et al. found that endoxylanases improve glucan conversion of steam-exploded barley straw compared with a standard enzymatic mixture [39]. The positive effect of added xylanase is most evident at early stages of enzymatic hydrolysis [39]. Hu et al. investigated the addition of accessory enzymes to enhance the enzymatic hydrolysis performance at high solids loadings (10–20%, w/v) [9]. They found that the pretreatment and the property of feedstock influence the role that accessory enzymes can play in synergistically interacting with cellulases to effectively hydrolyze the substrate. Synergism between cellulases and accessory enzymes improves the SSEH performance and a higher proportion of xylanase is required when the substrate contains a relatively high xylan content at high solids loadings [9]. Di Risio et al. studied the effects of three enzyme cocktail on the efficacy of enzymatic hydrolysis, including cellulase and β -glucosidase with the addition of cellulose + xylanase, cellulose + xylanase + β -glucosidase, and xylanase, respectively [40]. Result showed that the highest glucose yields (44%) are obtained from the enzyme cocktail consisting of cellulase and β -glucosidase with the addition of cellulose + xylanase + β -glucosidase. An interesting result is that the cellulase and β -glucosidase solution supplemented with xylanase actually yield less xylose than the other two enzyme cocktails (39% as compared with 54 and 85%) [40]. Li et al. reported the synergism of cellulase, xylanase, and pectinase on hydrolyzing sugarcane bagasse and found that the replacement of 20% of cellulase by xylanase enhances the glucose yield by 6.6, 8.8, and 9.5% from steam explosion, NaOH-, and H₂O₂-pretreated sugarcane bagasse, respectively. Results indicated that the degree of synergism between cellulase and xylanase has positive relationship with xylan content and is obviously affected by hydrolysis time [41]. Chen et al. found that cellobiose accumulates in the hydrolysate due to low cellobiase activity in *Trichoderma reesei* cellulase and lead to 65.9% yield of enzymatic hydrolysis of sodium hydroxide pretreated maize straw at 48 h [42]. Supplementary cellobiase from *Aspergillus niger* ZU-07 greatly

reduces the inhibitory effect caused by cellobiose, and the enzymatic hydrolysis yield at 48 h is improved to 81.2% [42]. Hsieh et al. studied the cellulase inhibition by high concentrations of monosaccharides and found that exo- and endoglucanases are most inhibited by monosaccharides such as mannose and galactose [43]. However, other factors, such as types of substrate, particle size, enzyme ratios, enzyme loadings, solids loadings, ab-/adsorption, and surfactants, also affect the synergistic system of multiple enzymes [9, 38, 42, 44]. Enzymatic hydrolysis conducted with fermentation together, which is usually called as simultaneous saccharification and fermentation or simultaneous saccharification and cofermentation (SSCF), is also an effective strategy to reduce sugars feedback inhibition effects. Additionally, the fed-batch mode of enzymes should reduce sugars feedback inhibition effects. These strategies have their respective advantages and disadvantages and should be further studied (Table 2).

2.3 Water constraint effects

Water plays a key role in enzymatic hydrolysis because it is the medium through which enzymes diffuse to and products diffuse away from the reaction sites of LCB [15, 16]. Water is also a reactant in the enzymatic hydrolysis reaction of the glycosidic bonds within the carbohydrates [45, 46]. There are mainly five different water pools existing in LCB suspensions due to the different interaction forces of biomass and water, including primary bound water, secondary bound water, capillary bound water, restricted bulk water, and free water (Fig. 2) [45–48]. With the increase of solids loading, water is constrained in solid substrate and free water content decreases or disappears, resulting in the poor efficiency of mass transfer and hence the low performance of SSEH [15, 16, 47].

Selig et al. investigated the effects of polymers (hemicelluloses, pectins, and lignin) on water constraint and its correlations with the cellulose hydrolysis efficiency [45]. They found that time domain NMR relaxometry shows spin-spin, T_2 , relaxation time curves generally get close to zero faster for most inhibitory polymer preparations (10% dry solids, w/w), indicating that all these polymers contribute to water constraint. Results presented that water constraint and cellulase inhibition have a close correlation, which may decrease the enzymatic hydrolysis efficiency [45]. Roberts et al. studied the effects of water pools distributions on the mass transfer and the enzymatic hydrolysis efficiency at high solids loading [46]. Results showed that the increase of solids loading results in the increase of water constraint in the suspensions. The addition of either glucose or mannose further increases water constraint. The presence of soluble sugars can reduce the enzymatic hydrolysis efficiency simply by increasing water constraint in cellulose suspensions before impacting enzyme activity. Water constraint in cellulose suspensions and the diffusivities of enzyme and monosaccharides within the system have strong correlations. With solids loading increasing from 5 to 20%, the effective diffusivity of bull serum albumin decreases by 61.5%, while the calculated intrinsic diffusivity decreases by 51.2% [46]. Felby et al. studied the cellulose–water interactions during enzymatic hydrolysis and found that different mechanisms of

cellulase enzymes can be observed through their effects on the cellulose–water interactions, and the synergistic effects between endoenzymes and exoenzymes can be easily detected by time domain NMR. It is possible to link the state and location of water within the cellulose fiber with structural changes upon enzymatic hydrolysis [47]. Selig et al. investigated the effects of solute concentration on high solids enzymatic hydrolysis of LCB [48]. Results indicated that solute concentration and its impact on water availability play a significant role in adverse effects associated with enzymatic hydrolysis at high solids loading. The concentration of soluble compositions, such as sugar alcohols, low molecular weight enzyme preparation components, and monomer hydrolysis products significantly decreases the conversion rates and is shown to correlatively lower water activity in enzymatic hydrolysis. Insoluble complex carbohydrates, including the nonhydrolysable cellulose, constrain water more significantly than high concentrations of soluble compositions [48]. Tsuchida et al. studied water accessibility in cellulose of pretreated sugarcane bagasse and found that the stronger interaction of water with pretreated bagasse is consistent with better enzymes accessibility to cellulose and higher efficiency of the enzymatic hydrolysis [49]. Selig et al. studied the hydration and hydrolysis of cellulose I_β , II, and III_I at increasing solids loadings [50]. They found cellulose III_I is more readily hydrolysable and less susceptible to increased solids levels than cellulose I_β and II through observing the hydrolysis by cellulases at arrayed solids loadings. Under equally hydrated conditions, time domain NMR results showed that cellulose II constrain water most while cellulase III_I have most free water. Cellulose III_I have the most restricted water pool and the changes in water distributions in hydrolysis are most dramatic with respect to cellulose III_I compared with celluloses I_β and II [50].

Although much research has been done to reveal the effect mechanisms of water constraint on the enzymatic hydrolysis efficiency, the results are currently still inconclusive. Many questions should be further solved. For instance, what are the main factors affecting on water constraint and how these factors correlate with the efficiency of SSEH? Do different states and locations of water play their unique role in SSEH? What strategies can be developed to change water constraint and hence increase the efficiency of SSEH? Additionally, should the types of LCB, the choice and conditions of pretreatment, the conditions of enzymatic hydrolysis, and the intensification methods be considered together to reveal the correlations between the water constraint and the efficiency of SSEH?

2.4 Rheology characteristic

Rheology is a branch of mechanics, which mainly focuses on the deformation and flow pattern of material under stress, strain, temperature, humidity, and other conditions. In SSEH, in-depth analysis of the rheological characteristic is crucial for the reactor design and the process optimization [51–53]. Rheological characteristic of LCB suspension depends on the types, particle size, fiber flexibility, and other physicochemical properties of LCB. In addition, it also depends on the pretreatment methods and conditions [15, 16, 51]. When solids loading is beyond 15%, the lubricating phenomenon of enzymatic hydrolysis system disappears

Figure 2. Schematic displaying water pools of LCB in multilength scale of its conversion process at high solids loading.

and the rheological characteristic obviously changes [15, 16]. Enzymatic hydrolysis at high solids loading results in many challenges of process handling. Roche et al. studied the yield stress of dilute acid pretreated corn stover in SSEH and found that the saccharified dilute acid pretreated corn stover liquefy to the point of being pourable ($\tau_y < 10$ Pa) at a total biomass conversion of about 40% after around 2 days [51]. They developed mass balance and semiempirical relationships to connect the enzymatic hydrolysis progress with particle concentration and yield stress. Wildemuth–Williams model is used to express yield stress (τ_y) as a function of volume fraction (ϕ) [51]:

$$\tau_y(\phi) = [A(\phi/\phi_{m0}) / (1/\phi/\phi_{m\infty})]^{1/m} \quad (1)$$

where ϕ_{m0} is the maximum volume fraction without the presence of shear; $\phi_{m\infty}$ is maximum packing fraction in the limit of infinite shear; A and m relate to the mechanics of the shear-induced breakdown of the microstructure of the suspension. The results of experimental data show good agreement with the proposed relationships. Using the method of combining mass balance relationships and theoretical models, a variety of material and rheological characteristic can be correlated to sugar concentrations for any arbitrary composition of LCB [51]. Dasari et al. investigated the effect of varying initial particle sizes, including $33 \mu\text{m} < x \leq 75 \mu\text{m}$, $150 \mu\text{m} < x \leq 180 \mu\text{m}$, $295 \mu\text{m} < x \leq 425 \mu\text{m}$, and $590 \mu\text{m} < x \leq 850 \mu\text{m}$, on enzymatic hydrolysis rates and rheological characteristic of sawdust slurries [54]. Higher enzymatic reaction rates and conversions of cellulose to glucose are observed at lower initial particle sizes. For the equivalent initial solids loading, smaller particle sizes led to lower viscosity. For instance, at a solids loading of 10% w/w, the viscosity decreases from 3000 cP for $150 \mu\text{m} < x \leq 180 \mu\text{m}$ particle size slurries to 61.4 cP for $33 \mu\text{m} < x \leq 75 \mu\text{m}$ particle size slurries. As a result, smaller particle size may reduce the time required for enzymatic hydrolysis, and the corresponding reduction in viscosity may enable higher solids loading and reduce reactor sizes during large-scale processing [54]. Viamajala et al. studied the rheological characteristics of untreated and dilute acid pretreated corn stover at high solids loading and found that corn stover slurries

exhibited shear-thinning behavior, which can be described using a Casson model as follows [55]:

$$\tau^{0.5} = \tau_{Cy}^{0.5} + K_C \gamma^{0.5} \quad (2)$$

where τ is the shear stress, Pa; τ_{Cy} is the apparent Casson yield stress, Pa; γ is the shear rate, s^{-1} ; K_C is a Casson constant, $\text{Pa}^2 \text{s}^2$. With the increase of solids loading, the greater interaction among particles likely increased the apparent viscosity and yield stress properties of pretreated solid slurry. These results suggest that rheological characteristic depended on the composition of corn stover as well as its physical properties such as particle size [55]. Um et al. performed the experimental determination of the viscosity, shear stress, and shear rate relationships at 10–20% solids loading with a variable speed rotational viscometer (2.0–200 rpm) [56]. The viscosities of enzymatic hydrolysis liquid are in ranges of 0.0418–0.0144, 0.233–0.0348, and 0.292–0.0447 Pa·s for shear rates up to 100 reciprocal seconds at 10, 15, and 20% initial solids (w/v), respectively. Four rheological models, including Herschele–Buckley model, Bingham model, Casson model, and Power Law, were used to present the experimental data and to determine the yield stress of the slurries. The results indicated that the Herschel–Bulkley model fits the data satisfactorily over the whole experimental range at 10–20% solid loading [56]. These researches indicate that the rheological characteristic of enzymatic hydrolysis system significantly changes with the increase of solids loading and the progression of enzymatic hydrolysis. They are closely related with the enzymatic hydrolysis performance of LCB. The rheological analysis of SSEH in the bioreactor should be helpful for the optimization of operational parameters and the design of reactors (Table 2). Therefore, further understanding of the rheological characteristic of LCB should facilitate the efficiency of SSEH.

3 Fed-batch strategy overcoming the limitations of SSEH

Fed-batch process has been investigated as an effective strategy to improve the efficiency of SSEH [15, 57, 58]. Fed-batch enzymatic

hydrolysis offers several advantages. For example, fed-batch process reduces the initial viscosity of enzymatic hydrolysis system and provides time for the solids to liquefy. Fed-batch process maintains a lot of free water in the initial stage of enzymatic hydrolysis, which minimizes or altogether avoids the diffusion and mixing limitations. Additionally, fed-batch process should be helpful for the recycle of enzymes and the reduction of inhibition effect [15, 57, 58].

Wang et al. investigated the SSEH of liquid hot water pretreated sweet sorghum bagasse and found that fed-batch enzymatic hydrolysis method produces total sugar of 17.06 g/L more than batch enzymatic hydrolysis and raises the solids loading to 30% [27]. Yang et al. obtained the high concentration sugars from pretreated corn stover by fed-batch enzymatic hydrolysis [59]. In their experiment, fed-batch enzymatic hydrolysis was initiated at 12% w/v solids loading with an enzyme loading of 20 FPU/g solid. Six percent solids were then fed consecutively at 12, 36, and 60 h. After 144 h, the final concentrations of reducing sugar, glucose, cellobiose, and xylose reached 220, 175, 22, and 20 g/L, respectively. The final total biomass conversion is 60% in fed-batch enzymatic hydrolysis [59]. Chen et al. reported that fed-batch enzymatic hydrolysis of sodium hydroxide pretreated maize straw was initiated with a solids loading of 80 g/L, with solids added at 6 and 12 h to get a final solids loading of 110 g/L [42]. After 72 h of enzymatic hydrolysis, the reducing sugars concentration is 89.5 g/L with a hydrolysis yield of 83.3%. The hydrolysate contains 56.7 g/L glucose, 23.6 g/L xylose, and 5.7 g/L arabinose, which is suitable for subsequent fermentation process [42]. Hodge et al. utilized both the insights obtained from experimental work and kinetic modeling to develop an optimization strategy for fed-batch enzymatic hydrolysis at insoluble solids beyond 15% w/w (equivalent to a 25% initial solids loading) that was the upper limit solids loading for enzymatic hydrolysis of pretreated corn stover, otherwise the mixing in stirred tank reactors would become problematic [57]. The final cellulose conversion is approximately 80% of theoretical for fed-batch stirred tank reactors at a total solids loading of 25% w/w initial insoluble solids. However, one thing to note is that the reaction time for fed-batch stirred tank reactors is longer than that for general hydrolysis. Results suggested that faster rates for fed-batch stirred tank reactors can be obtained either by using higher enzyme loadings or by improving enzyme preparations with increased tolerance to sugar inhibition [57]. Tai et al. developed a mathematical optimal control strategy of fed-batch enzymatic hydrolysis of dilute acid pretreated LCB based on a modified epidemic model [60]. Using the optimal control feeding strategy, glucose concentration and accumulated cellulose conversion is 77.31 g/L and 72.08% within 100 h, respectively, which is 108.76 and 37.50% higher than in batch enzymatic hydrolysis process [60]. Gao et al. examined the optimal initial solids loading, feeding time, and quantities in the fed-batch enzymatic hydrolysis of alkali-pretreated sugarcane bagasse at increased solids loading [61]. The enzymatic hydrolysis system was initiated with 12% w/v solids loading in flasks, where 7% fresh solids were then fed consecutively at 6, 12, and 24 h to get a final solids loading of 33% with the addition of all the requested cellulase loading (10 FPU/g substrate) at 0 h. At 120 h, the maximal concentrations of cellobiose, glucose, and xylose are 9.376, 129.50, 56.03 g/L, respectively. The final total glucan conversion rate attains to 60% [61].

These results indicate that fed-batch process has many obvious advantages and should improve the efficiency of SSEH to some extent. However, previous studies did not draw a unified conclusion. Some problems should be solved before using fed-batch enzymatic hydrolysis for lignocellulosic ethanol industrialization. Fed-batch mode increases process complexity and leads to the increase of process cost. The increases in enzymatic conversion and process cost should be balanced. The feeding of enzymes should be evaluated together with the feeding of solids. In addition, fed-batch process factors including the types of LCB, the methods of pretreatment, and the conditions of enzymatic hydrolysis should be fully considered (Table 2).

4 Stirring method of SSEH

In SSEH, the limitation factors generated from high solids loading are interacted with each other. With the increase of solids loading, the concentration of inhibitors and produced sugars increase, resulting in more serious inhibition effects. The increases in solids loading reduce free water content and lead to water constraint and poor efficiency of mass transfer. The viscosity of SSEH increases concomitantly, which further reduces the mass transfer efficiency and increases the energy consumption of mixing. These limitation factors hinder the large-scale implementation of SSEH. Therefore, new strategy such as novel intensification method and hydrolysis reactor should be exploited to overcome these limitation factors and improve the efficiency of SSEH.

Zhang et al. developed a novel helical impeller to enhance the SSEH efficiency of steam explosion pretreated corn stover compared with a rushton impeller [62]. They found that the rheological viscosity of enzymatic hydrolysis system increases with the increase of solids loading, which is similar to the stirring power consumption. Additionally, the mixing energy consumption by the helical impeller obviously reduces compared with that by the rushton impeller. The results indicated that the mixing of the helical impeller is more effective than that of the rushton impeller [62]. Wang et al. investigated the effects of two impellers on SSEH of liquid hot water pretreated sweet sorghum bagasse [27]. Compared with the double-curved blade impeller, the plate-and-frame impeller improves glucose production by 10%. The total sugar concentration produced by plate-and-frame impeller is approximately 17.9% higher than that by double-curved blade impeller at 20% solids loading. The results showed that the axial flow and radial flow of solution have the same stirring intensity for plate-and-frame impeller, which is better for mixing solution and lead to higher total sugar concentration than that of double-curved-blade impeller [27]. Zhang et al. investigated the feasibility of using a laboratory peg mixer to enhance the SSEH performance of LCB [25]. The enzymatic hydrolysis of unbleached hardwood pulp and organosolv-pretreated poplar at 20% solids loading leads to a high glucose concentration. The peg mixer was found to be capable of providing effective mixing of unbleached hardwood pulp at high solids loading. It only takes 1.0 h to liquefy unbleached hardwood pulp at 20% solids loading in the peg mixer compared with 40 h in a shake flask [25]. Sun et al. studied the effects of ball oscillation, shakers, and static state on the enzymatic hydrolysis of steam exploded corn stover, finding that

ball oscillation increases the adsorption–desorption efficiency of cellulase, and hence increases the cellulose conversion and the efficiency of enzymatic hydrolysis at 10% solids loading [63]. Liu and Chen reported a novel intensification method, periodic peristalsis, to enhance the efficiency of SSEH compared with water baths shaker [64, 65]. The results showed that glucan and xylan conversion at 18% solid loading using periodic peristalsis is increased by 3.4–5.8 and 4.5–6.2%, respectively, compared with that using water baths shaker [64, 65]. These studies suggested that the development of intensification method should be effective strategies to improve the efficiency of SSEH. In addition, it should be noticed that the stirring speed, the paddle size, and the proportion of paddle size to reactor internal diameter determine the efficiency of mass transfer and hence the performance of SSEH [15, 16, 62, 63].

5 Novel enzymatic hydrolysis reactors and pilot-scale platforms

Several reactors have been reported to increase the efficiency of SSEH. Jørgensen et al. developed a reactor system for enzymatic liquefaction at high solids loading [14]. The reactor system was based on free fall mixing using a horizontally placed drum with a horizontal rotating shaft mounted with paddlers for mixing. The results showed that before 10 h, the enzymatic hydrolysis system at 40% solids loading changes from intact particles (length 1–5 cm) into paste/liquid that could be pumped. Liquefaction and hydrolysis for 96 h with an enzyme loading of 7 FPU/g dry matter and 40% dry matter result in a glucose concentration of 86 g/kg [14]. Roche et al. investigated the laboratory-scale reaction vessels, roller bottle reactors, for SSEH of LCB [66]. The results of enzymatic hydrolysis of five biomass pretreatments with different severities and two enzyme preparations suggested that this system works well. The mass transfer limitations of SSEH are significant but can be mitigated with a relatively low amount of mixing input. The reactor system is shown to be scalable between bench (125 mL) and floor (2 L) scales at 20% initial insoluble solids loadings. The results suggested that the reactor system and its ability to accommodate numerous reaction vessels will be useful in screening new biomass pretreatments and advanced enzyme systems at high solids loading [66]. Dasari et al. employed an 8 L scraped surface bioreactor for enzymatic hydrolysis to handle high solids loading and as a means for scale-up from laboratory-scale shake flasks [67]. In this system, the scraping action of the blades keeps the reactor surface clear and thus improves the heat transfer characteristics. The horizontal rotation of the shaft and blades provides mixing and prevents particle settling much more effectively compared with conventional stirred tanks. Enzymatic hydrolysis tests are performed for initial solids loadings between 10 and 25%, and the efficiency factor is the highest for 20% initial solids loading. As a result, the scraped surface bioreactor proves to be more efficient than shake flasks [67].

Chen et al. developed an industrial-level system of non-isothermal simultaneous solid state hydrolysis, fermentation, and separation to alleviate the problems of low solids, non-isothermal, and sugars feedback inhibition effects (Fig. 3) [68]. In this integrated process, the enzymatic hydrolysis and fermentation that are connected together by the hydrolyzate loop

Figure 3. Nonisothermal simultaneous solid state hydrolysis, fermentation, and separation (NSSSFS) system. (A) Condensing water circulation device; (B) filter plate; (C) outer cylinder with heating jacket; (D) thermal insulation inner column; (E) ethanol fermentation zone; (F) enzymatic saccharification zone; (G) float valve; (H) CO₂ circulation pump; (I) hydrolyzate circulation pump; (J) valve; (K) adsorption column of activated carbon. The arrows mean the direction of gas and liquid flow [2, 67]

can be conducted at around 50 and 37°C, respectively, at 25% solids loading. Glucose produced from enzymatic hydrolysis is timely consumed by yeast, and the ethanol is then separated online through gas stripping followed by activated carbon adsorption. Based on the pilot level of 300 L enzymatic hydrolysis and fermentation reactor, a novel industrial level of 110 m³ SSEH, fermentation, and ethanol separation plant has been successfully established [2, 13, 68]. A semicontinuous conversion process of LCB is designed to achieve a capacity of 4000 L ethanol and operate at solids loading of > 20% w/w by National Renewable Energy Laboratory (Golden, CO, USA) [15]. In this system, after about 24–30 h liquefaction, the slurry is pumped into vertical and stirred tank reactors to complete the enzymatic hydrolysis of the pretreated solids. This operation is capable of processing about 0.5–1.0 ton dry LCB into ethanol each day [15]. A demonstration-scale operation for ethanol production from LCB has been established by integrating feedstock handling, steam explosion, and SSCF at high solids loading in Jilin province, China [13]. The major equipment of this industrial system includes six 50 m³ SE reactors, sixteen 400 m³ simultaneous saccharification and fermentation reactors, and one ethanol distillation tower. The SSCF is carried out at above 20% solids loading, while the enzyme loading is reduced by 25.0% using the synergistic enzyme systems compared with only cellulose addition. More than 4% w/w ethanol concentration is obtained, corresponding to 72.3% of the ethanol's theoretical yield. Additionally, lignin plastic composite material and compress natural gas are coproduced. As a result,

this operation system obviously reduces the cost of ethanol production and facilitates the industrial application of LCB refinery [13].

6 Direction of future work

Although adequate research on SSEH has been carried out, several challenges should be overcome to achieve large-scale implementation of SSEH. In-depth analysis of main limitations should be conducted to reveal the key factors affecting the efficiency of SSEH. In order to increase the efficiency of SSEH, various factors should be considered comprehensively such as the types of LCB, the choice and optimization of pretreatment, the conditions of enzymatic hydrolysis, and the intensification methods. The synergistic system of multiple enzymes should be further constructed to reduce the sugars feedback inhibition effects. The main factors affecting water constraint and their correlations with the efficiency of SSEH should be further investigated. The advantages of fed-batch process and effective feeding strategies should be fully exploited. Most importantly, new intensification methods and enzymatic hydrolysis reactors should be developed to change the characteristics of SSEH system, increase the mass transfer efficiency, and reduce the energy consumption of mixing. The technological and economic analysis should be conducted to ensure the economic feasibility of the industrialized application of SSEH process.

7 Concluding remarks

SSEH offers several advantages including high sugars concentrations, low capital and operating costs, and less energy input, and thus it should be a potential approach for the industrialization of lignocellulosic ethanol. However, various strategies should be combined together to overcome the limitation factors and improve the SSEH efficiency. The synergistic system of multiple enzymes and the fed-batch mode should be further exploited. Novel intensification methods and hydrolysis reactors should be exploited to increase the mass transfer efficiency, reduce the energy consumption of mixing, and improve the SSEH performance.

Practical application

Solid state enzymatic hydrolysis (SSEH) has many advantages, such as higher sugar concentration, lower operating costs, and less energy input. It should be a potential approach for the industrial application of lignocellulosic ethanol. However, there are several technical challenges of SSEH hindering its large-scale implementation, including water constraint, rheology change, and inhibition effect, etc. Various strategies for overcoming these limitations are proposed to improve the SSEH performance. In-depth analysis of main limitations and development of novel intensification methods and reactors should provide an effective way to achieve large-scale implementation of SSEH.

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