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Continuous enzymatic saccharification and its rheology profiling under high solids loading of lignocellulose biomass

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ABSTRACT

Continuous saccharification of lignocellulose biomass is an important process option for industrial biorefinery practice. Continuous saccharification in industrial operations needs to be performed under high solids loading to obtain a high titer of fermentable sugars for the consequent fermentation or the simultaneous saccharification and fermentation (SSF), which also produces highly viscous hydrolysates, two orders of magnitude greater than the regular fermentation medium. The rheological properties in the continuous saccharification should be characterized for bioreactor design. This study investigated the process efficiency of continuous enzymatic saccharification of two typical lignocellulose feedstocks (corn stover and wheat straw) under high-solids loading. The rheological change was profiled under varying saccharification parameters such as dilution rate, solids loading, and enzyme dosage. The apparent viscosity of the high solids loading hydrolysates was found in the range of 0.11–0.56 Pa·s, which allowed the efficient transportation by the regular pump in vessels and pipelines. The presence of inhibitors generated from dry acid pretreatment showed a negligible effect on the hydrolysis efficiency of the continuous saccharification and provided an environment away from microbial contaminations in the hydrolysates. The study provided important technical support for the process and reactor designs of continuous saccharification of lignocellulose feedstock under high solids loading.

1. Introduction

Continuous operation is widely applied in industrial biotechnology processes for its fast and stable performance [1–4]. Lignocellulose biorefining is a multi-step and nonregular process composed of pretreatment, hydrolysis, detoxification, fermentation, and purification of solid biomass particles and highly viscous slurries [5–7]. Batch operation in the biorefinery process inevitably causes solids sedimentation and blockage in reactors, vessels, pumps, and pipelines, resulting in a heavy burden of cleaning [1,4,8]. Continuous biorefinery operation releases the burdens of batch procedures by avoiding the frequent, time and labor-consuming steps of emptying, cleaning, and operation switching. The process productivity of the fermentable sugars is also improved by cutting these steps. Correspondingly, the apparent viscosity of the hydrolysates is reduced in a constantly changing process without experiencing the high apparent viscosity in the start-up stage of the enzymatic hydrolysis.

Continuous biorefinery fermentations were tested on a bench-scale using hydrolysate supernatants obtained from batch saccharification of lignocellulose feedstocks. Ahring et al. [9] tested a continuous lactic acid fermentation using corn stover hydrolysate supernatants obtained by batch hydrolysis and solids-liquid centrifugation. Jin et al. [10] used the AFEX pretreated corn stover for batch enzymatic hydrolysis, and then the hydrolysates were applied for continuous ethanol fermentation. Baroi et al. [11] hydrolyzed the acid-pretreated wheat straw in batch operation then the hydrolysate was used for continuous butyric acid fermentation with the reverse electrodialysis for acid recovery.

Continuous enzymatic saccharification of lignocellulose into fermentable sugars had been tested at low solids loading (5–10%). Lonkar et al. [12] tested continuous hydrolysis of alkali-pretreated corn stover at 10% (w/w) solids loading by complicated 16-stage serial reactors and multiple centrifugal operations. Stickel et al. [13] tested continuous enzymatic hydrolysis of corn stover at 5% (w/w) solids loading in an ultrafiltration membrane reactor to obtain clear hydrolysates. Mahboubi et al. [14] used diluted acid-pretreated wheat straw pulp in a submerged membrane bioreactor at ~3% (w/w) solids content

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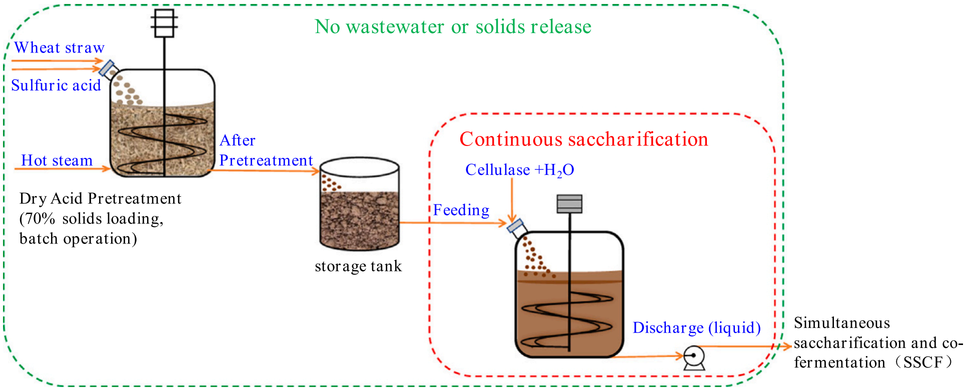

Fig. 1. Schematic illustration of continuous saccharification of dry acid pretreated and non-biodetoxified wheat straw or corn stover.

with multiple filtrations to obtain the low titer of fermentable sugars (~15 g/L). These low-solids content (Less than 15% w/w) continuous saccharification only generated low-titer fermentable sugars and end products in consequent fermentation steps but resulted in considerably high wastewater generation and energy consumption in the consequent purification step of the biorefinery chain.

Industrial biorefinery operations require a high titer of fermentable sugars (glucose, xylose, and cello-oligo sugars) as the carbon source for consequent fermentation or simultaneous saccharification and fermentation (SSF). Hence, to ensure an increased yield of fermentable sugars production, it is necessary to perform the saccharification with a higher lignocellulose content, specifically solid loadings above 15% of the total hydrolysis system [15]. This means that enzymatic hydrolysis needs to be completed under a higher lignocellulose content. With a high solid content in enzymatic saccharification, high viscosity and high concentration of solid particles (lignin, etc.) of the hydrolysate pulp make common filter press, centrifugal, or membrane solid-liquid separation hard to operate. Moreover, the hydrolysate contains a high concentration of cello-oligosaccharides that is not capable of direct fermentable monosaccharides. Therefore, the hydrolysate from high solid-content saccharification can only be used directly for subsequent simultaneous saccharification and fermentation rather than for the clear hydrolysate through solid-liquid separation. On the other hand, the high solids loading of the biomass generates challenging situations for the operation, such as high initial viscosity limiting the mass transfer, difficulties in mixing, high stirring power consumption, and formation of inhibitors in the enzymatic hydrolysates [16,17]. Therefore, the rheological property of high solids containing hydrolysates is critically important for the design of impellers geometry, mixing rate, and power consumption of continuous saccharification reactors [18–20]. Until now no reports on high solids loading continuous saccharification and rheological profiling are available [21].

Conventional dilute acid pretreatment has been used due to its high wastewater generation, high production of inhibitors including furfural, 5-hydroxymethylfurfural (HMF), and acetic acid and lignin, the presence of which limits the action of cellulase. In this study, the dry dilute acid pretreatment method established by the group in previous work was applied, where the lignocellulosic feedstock was added to the pretreatment reactor as dry particles with high solids content (~ 70% w/w), while a small amount of dilute acid catalyst solution was added and left the reactor as solid dry particles (~ 50% w/w), with relatively low process energy consumption and inhibitor content and no wastewater discharge [5–7,22]. The present work aimed to evaluate the feasibility of continuous saccharification of typical lignocellulose feedstocks (wheat straw and corn stover) under high solids content (30%, w/w) required by the practical biorefinery applications. The changes in the apparent viscosity of the hydrolysate during continuous saccharification were investigated under various operation parameters for the purpose of bioreactor designs.

2. Materials and methods

2.1. Raw materials and reagents

Wheat straw and corn stover were harvested from Nanyang City, Henan Province, China in the fall of 2019. The raw materials were coarsely chopped, washed to remove field dirt and air dried, and milled to pass through the mesh of 10 mm in diameter. The wheat straw contained 34.36 ± 1.40% of cellulose, 25.12 ± 0.98% of hemicellulose, 16.69 ± 0.16% of lignin, and 10.63 ± 0.10%, and the corn stover contained 38.77 ± 2.39% of cellulose, 24.56 ± 0.11% of hemicellulose, 15.19 ± 0.64%of lignin, and 7.98% ± 0.18%of ash on dry weight base (w/w) according to NREL protocols [23,24].

Cellulase enzyme Cellic CTec 2.0 was purchased from Novozymes China, Beijing, China. The filter paper activity was determined as 256 FPU/mL according to the NREL protocols LAP-006 [25], the cellobiase activity was 4653.3 CBU/mL according to Ghose et al. [26], and the protein content was 86.3 mg/mL according to Bradford et al. [27].

2.2. Pretreatment operation

Dry acid pretreatment was performed by feeding a 3.8% (w/w) sulfuric acid solution co-currently with the wheat straw or corn stover into a 20 L helical ribbon impeller-driven reactor at the ratio of dry feedstock to the sulfuric acid solution was 2:1 [5–7,28]. The pretreatment was conducted under the (dry) solids loading of 67% by weight percentage. Briefly, 1200 g of feedstock (dry base) and approximately 500 ~ 600 g of 3.8% (w/w) dilute sulfuric acid solution (depending on the moisture content of the feedstock) were concurrently fed into the pretreatment reactor at the solids/ liquid ratio of 2:1 (w/w). After stirring for 3 min at mild mixing, the hot steam was jetted into the reactor, took 6 min to the required temperature (175 ℃) and maintained for 5 min. Then the pretreated solid feedstocks were discharged gravitationally from the bottom outlet port (the process takes 2–3 min). All the dilute acid solution and the condensed water were completely adsorbed into the solids to form approximately 50% (w/w) of the dry pretreated feedstock solids with the pH around 2.0. The sulfuric acid in the pretreated biomass solids was neutralized to a pH value of 4.8–5 using $CaCO3$ powder. No wastewater was generated during the pretreatment process. The water content of the pretreated wheat straw and corn stover was 50.23% and 48.36%, respectively.

2.3. Continuous enzymatic hydrolysis operations

The continuous enzymatic saccharification was operated in a 5 L bioreactor equipped with helical ribbon impeller agitation as described in Zhang et al. [5] and Liu et al. [7]. Pretreated wheat straw or corn stover solids were fed into the bioreactor with the proper cellulase enzyme solution and deionized water to maintain the total solids content

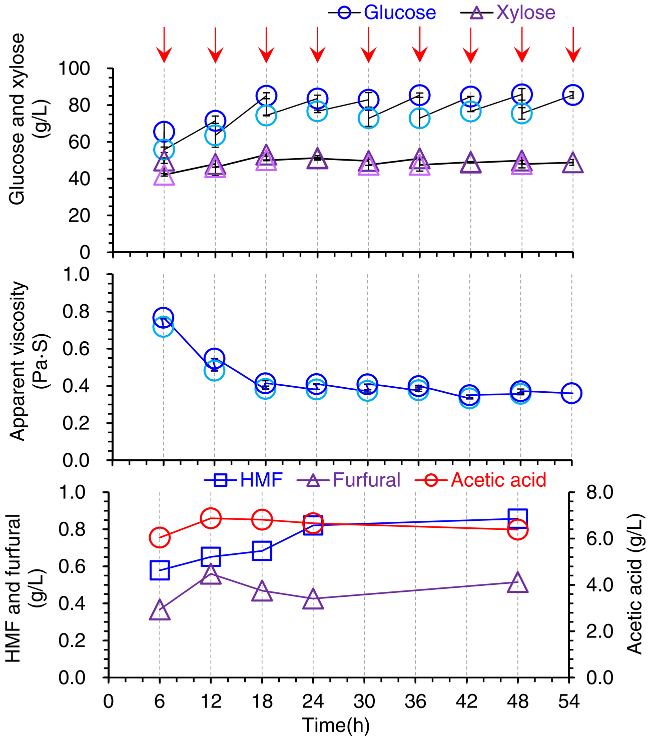

Fig. 2. Continuous saccharification of dry acid pretreated wheat straw without biodetoxification at a dilution rate of 0.02 h^{-1}. 5 L bioreactor at 30% solids (w/w), 4 mg cellulase proteins per gram of dry matter, feeding, and discharging (indicated by the arrow↓) were performed every 6 h.

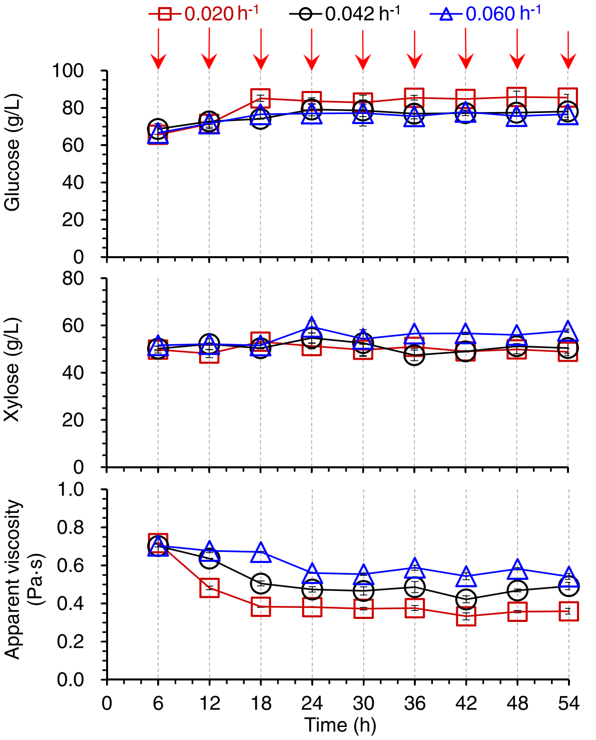

Fig. 3. Continuous saccharification process under varying dilution rates. Conditions: feeding and discharge (indicated by the arrow↓) were performed every 6 h in a 5 L bioreactor at 30% solids (w/w), 4 mg cellulase protein/ g dry matter.

of 20~30% (w/w). The wheat straw or corn stover was fed into the saccharification reactor directly using a conical funnel feeder without autoclave. The pH was maintained at 4.8 by automatically addition of 5 M $Ca(OH)_2$ slurry. The temperature was set to 50 °C and the stirring at 150 rpm. According to the different dilution rates (0.020 h^{-1},0.042 h^{-1} and 0.060 h^{-1}), the pretreated lignocellulosic solids, water and cellulase were fed into the reactor at the equal mass of the discharged hydrolysates. The operation of the hydrolysate discharge and the feedstock feeding was repeated every 6 h (four times a day). When continuous feeding and discharging make the fermentable sugar concentration and apparent viscosity of the saccharification solution do not change significantly, it is considered to have reached a stable operating state, and its operation can be regarded as a continuous saccharification process (Fig. 1). The samples were taken periodically for measurement of soluble monosaccharide and inhibitor concentrations, as well as rheological properties.

2.4. Analysis

5 mL hydrolysate samples were removed from the bioreactor and the apparent viscosity of the hydrolysate was determined using a Brookfield DV2T viscometer (Stoughton, Middleboro) at 50 °C and 43.5 s^{-1} shear rate.

Glucose, xylose, furfural, 5-hydroxymethylfurfural (HMF), and acetic acid were analyzed by the Shimadzu HPLC system equipped with a Bio-rad Aminex HPX-87 H column and RID-10A detector. Twenty microliters of the sample were subjected and analyzed at 60 ℃ using 5 mM H_2SO_4 as eluent with a flow rate of 0.6 mL/min [28].

3. Results and discussion

3.1. Evaluation of hydrolysis efficiency and rheological change of high solids continuous saccharification

Fig. 2 shows that the dry acid pretreated wheat straw (without detoxification) was enzymatically hydrolyzed at 30% (w/w) solid content by the periodical feedstock feeding and hydrolysate discharging at 12% of the total hydrolysates every 6 h, corresponding to 0.020 h^{-1} of dilution rate (equivalent to the average hydrolysis time of 50 h). After three feeding and discharging operations within 18 h, the glucose concentration increased from 65.5 g/L to 85.1 g/L, and the apparent viscosity of the hydrolysate decreased significantly from 0.718 Pa·s to 0.383 Pa·s and remained relatively stable. The apparent viscosity of the hydrolysate immediately increased after each feeding but quickly decreased and remained relatively stable afterward. The power consumption of the enzymatic hydrolysis process was closely related to the rheological properties of the hydrolysate. The apparent viscosity of the continuous saccharification process can be greatly reduced in a short time, thus contributing to the reduction of process energy consumption.

The dry acid pretreatment generated a high concentration of inhibitors in the pretreated wheat straw (5.21 mg furfural, 3.19 mg 5-hydroxymethylfurfural (HMF), 17.32 mg acetic acid per gram of dry wheat straw, and considerable phenolic aldehydes). The continuous enzymatic saccharification was conducted without biodetoxification, and the inhibitors were released into the hydrolysate as the solid cellulose was hydrolyzed (~6 g/L of acetic acid, ~0.9 g/L of furfural, and ~0.5 g/L of HMF). No negative effect on cellulase activity and enzymatic saccharification efficiency were observed for the high solids content continuous saccharification process comparing to total fermentable sugars (glucose + xylose, ~135 g/L) obtained by batch saccharification after removal of inhibitors by biodetoxification [29–31].

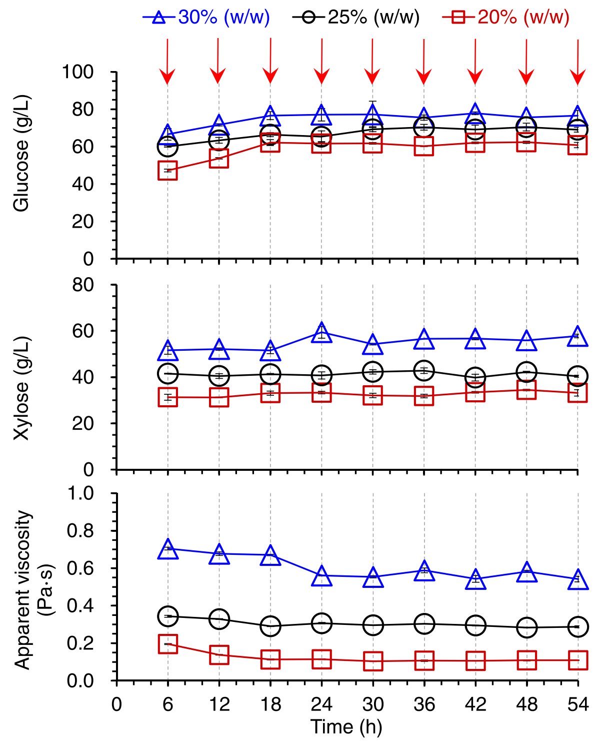

Fig. 4. Continuous saccharification process under varying solids content. Conditions: feeding and discharge (indicated by the arrow↓) were performed every 6 h, with dilution rates of 0.06 h^{-1} in a 5 L bioreactor, 4 mg cellulase protein/g dry matter.

3.2. *Rheological profiles of continuous saccharification under varying parameters*

Dilution rate determines the productivity of continuous operation and is regulated by varying the feeding or discharging rate. The effect of dilution rate on the hydrolysis yield and rheology property of the continuous saccharification of pretreated wheat straw were investigated (Fig. 3). When the dilution rate increased from 0.020 h^{-1} to 0.042 h^{-1} (the average saccharification time decreased from 50 h to 23.8 h), the glucose generation decreased from 84.7 g/L to 77.9 g/L; the further dilution rate increase to 0.06 h^{-1} (the saccharification time 16.7 h) only led to the slight decrease of glucose to 76.6 g/L. Xylose recovery maintained constant because nearly 90% of xylan was hydrolyzed into xylose during the pretreatment operation [5]. On the other hand, the increase in dilution rate led to a sharp increase in the apparent viscosity (0.36, 0.47, and 0.56 Pa·s at the dilution rate of 0.020, 0.042, and 0.060 h^{-1}, respectively), although the glucose and xylose concentrations maintained relatively stable. This phenomenon may come from the generation of relatively low-solubility oligo-saccharides under high solids content, instead of easily soluble monosaccharides (glucose and xylose) generation [32]. The high dilution rate in continuous saccharification resulted in the increased volumetric productivity of fermentable sugars with relatively low sugars concentration (the volumetric productivity of fermentable sugars was 1.9, 5.4, and 8.0 $g\cdot L^{-1}\cdot h^{-1}$ at the dilution rate of 0.020, 0.042, and 0.060 h^{-1}, respectively), while the lower sugars concentration lessened product (glucose) inhibition on the cellulase enzyme activity and further led to the higher hydrolysis yield.

The solids content of the pretreated wheat straw significantly affected the hydrolysis efficiency and rheological property of the continuous saccharification (Fig. 4). When the solids content increased from 20% to 30%, the glucose concentration (at the steady-state) increased by 24.7% (from 61.47 g/L to 76.63 g/L), and the apparent viscosity increased by almost five folds (from 0.11 Pa·s to 0.56 Pa·s, but still in the range of regular pump transportation capacity), and the

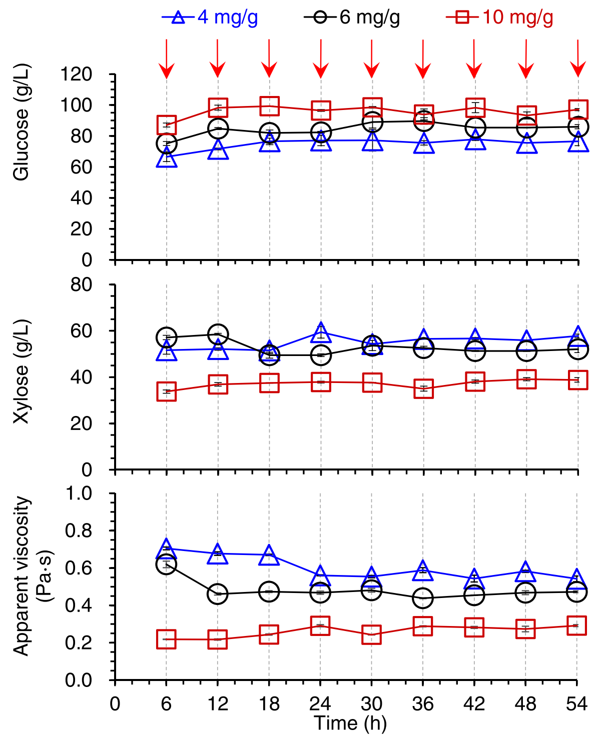

Fig. 5. Continuous saccharification process under varying cellulase enzyme dosage. Conditions: 5 L bioreactor at 30% solids (w/w), dilution rate of 0.06 h^{-1}, feeding, and discharge (indicated by the arrow↓) were performed every 6 h.

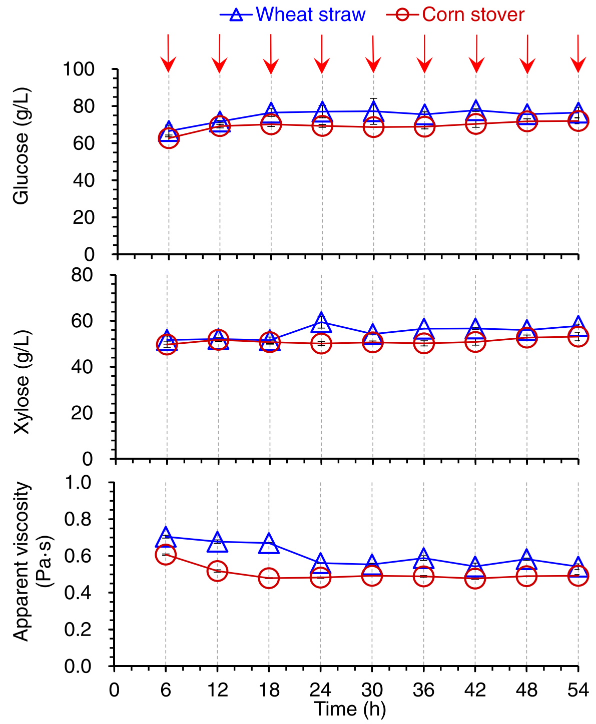

Fig. 6. Continuous saccharification process under different feedstocks. Conditions: 5 L bioreactor at 30% solids (w/w), 4 mg cellulase protein/g dry matter, feeding, and discharge (indicated by the arrow↓) were performed every 6 h, dilution rates 0.06 h^{-1}.

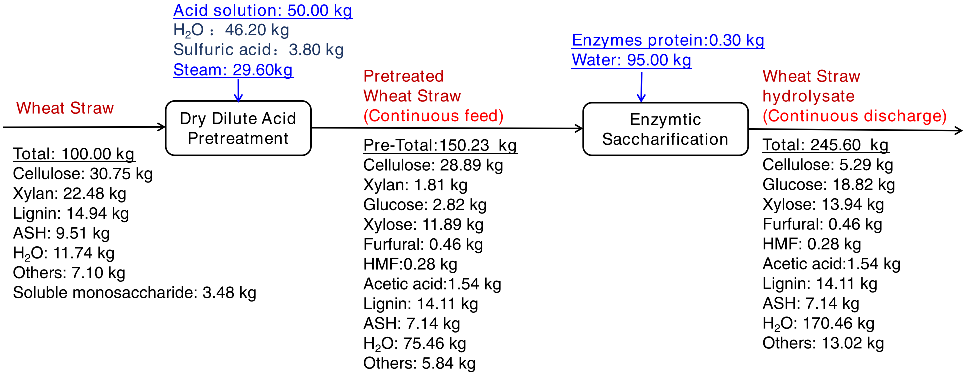

Fig. 7. Mass balance of continuous saccharification process at steady state. The dilution rate of 0.06 h-1as shown in Fig. 3.

overall volumetric productivity of the fermentable sugars by more four folds (from 1.9 $g·L^{-1}·h^{-1}$ to 8 $g·L^{-1}·h^{-1}$). As the concentration of solids increases, the apparent viscosity increases as the free water decreases, and it does not affect the hydrolysate transport in the continuous process. The high viscosity indicates the presence of partially unhydrolyzed cellulose oligosaccharides in the saccharification solution, which can continuously provide a carbon source for the subsequent simultaneous saccharification and fermentation processes, increasing the product flow while reducing the use of water [33,34].

In the continuous enzymatic saccharification process, a high level of enzyme activity must be maintained over time by adding enzymes in order to maintain a constant sugar concentration[35]. High cellulase dosage enhances the saccharification efficiency but also increases the enzyme cost (Fig. 5). Glucose concentration increased by 26% (from 76.6 g/L to 96.3 g/L) but the apparent viscosity sharply decreased by 50% (from 0.56 Pa·s to 0.28 Pa·s) when the enzyme dosage increased from 4 mg to 10 mg cellulase protein per gram of dry wheat straw. The viscosity reduction was found to be more sensitive to the cellulase enzyme dosage, but the modest cellulase dosage (4–6 mg cellulase protein/g dry matter) was still preferred because of the acceptable hydrolysis yield and pumpable transportation viscosity. Since the cellulase cost accounts for nearly half of the final cellulosic ethanol product [36], the modest cellulase dosage was the proper option for continuous saccharification at the high solid content for industrial biorefineries.

Two major lignocellulose feedstocks, wheat straw, and corn stover were tested for continuous saccharification under high solids content (Fig. 6). The glucose yields and apparent viscosities of the two feedstocks were essentially the same with a slightly smaller apparent viscosity for corn stover (0.49 Pa·s) than that of wheat straw (0.54 Pa·s).

3.3. Mass balance of continuous saccharification and process evaluation

The mass balance of wheat straw in high-solids loading continuous saccharification was calculated based on the experimental results (Fig. 7). The calculation basis was 100 kg of dry wheat straw starting from dry acid pretreatment (batch operation) to the continuous saccharification at a dilution rate of 0.06 h^{-1}. Continuous hydrolysis of wheat straw yielded 76.63 g/L glucose and 56.77 g/L xylose, which were similar to the sugar yields from batch enzymatic hydrolysis [30,31]. The pretreatment hydrolyzed ~6% cellulose and ~92% hemicellulose to glucose and xylose. The continuous saccharification led to ~83% of cellulose conversion, but the glucose yield was only ~67%, indicating approximately 16% of the sugars were still in the form of oligosaccharides and not completely converted into monosaccharides. While the major purpose of the saccharification step in the biorefinery process chain is to liquefy lignocellulosic into liquid form, the saccharification at this step is to obtain the pumpable viscosity for biorefinery processing transportation. When this purpose is achieved, the partial saccharification of the lignocellulosic substrates to soluble oligosaccharides is sufficient and the complete saccharification is to be conducted in the consequent simultaneous saccharification and fermentation (SSF) in a lessened sugar inhibition on cellulase activity. The total fermentable sugar productivity during continuous saccharification (0.023 kg sugars per kg feedstock per hour) was similar to that of the batch enzymatic hydrolysis (0.025 kg sugars per kg feedstock per hour) [31]. Conclusively, the present continuous saccharification significantly achieved the feasibility of pumpable viscosity of high-solids loading hydrolysates (0.56 Pa·s) in a short period for complete utilization of glucose or xylose to end-products in the consequent SSF step.

The inhibitors generated from pretreatment harshly curb microbial growth and metabolism but showed a limited effect on cellulase activity [37]. The continuous saccharification of dry acid pretreated wheat straw or corn stover in this study showed a similar saccharification efficiency to that in the batch saccharification. An additional advantage was the protection of the pretreated wheat straw or corn stover from microbial contaminations for open operations of saccharification and transportation without considering contamination troubles. In the consequent process steps, the inhibitors are degraded by biodetoxification without loss of fermentable sugars before simultaneous saccharification and fermentation for production of ethanol and other products [28,38,39].

The transportation of highly viscous liquids in the biorefinery process is a critical technical difficulty. The viscosity of the hydrolysate slurry in the saccharification section under high solids content is approximately 2–3 orders of magnitude higher than that of conventional industrial fermentation broth (water, 0.55×10^{-3} Pa·s at 50 ℃; the hydrolysates, 0.56 Pa·s). This study used a bioreactor equipped with helical ribbon impeller agitation for solid-liquid mixing. By adjusting process parameters such as dilution rates, solids content, and enzyme dosage, the apparent viscosity of the hydrolysate slurry was controlled in the range of 0.11–0.56 Pa·s, which is allowed the routinely pumping transportation for the continuous saccharification process.

4. Conclusion

The continuous enzymatic saccharification process with high solids content was operated by periodically feeding feedstock and discharging hydrolysate slurry at an equal quantity from non-detoxified wheat straw and corn straw as raw materials. The process performance of continuous saccharification at high solids content (30%, w/w) and their rheological characteristics of continuous hydrolysis were evaluated. The viscosity of the hydrolysate slurry was controlled in the range of 0.11–0.56 Pa·s by adjusting the process parameters dilution rate, solid content, and enzyme dosage for continuous saccharification to achieve an open pumping transport and does not require consideration of microbial contamination. The efficiency of continuous saccharification was similar to batch saccharification of the feedstocks after biodetoxification. This study provides a basis for continuous saccharification reactor design for lignocellulosic feedstocks at high solids loadings.

CRediT authorship contribution statement

Ya Wang: Conceptualization, Data curation, Investigation, Writing – original draft. **Hucheng Yang:** Investigation, Methodology. **Bin Zhang:** Investigation, Methodology. **Xiucai Liu:** Conceptualization, Supervision, Funding acquisition. **Jie Bao:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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