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On-site measurement and modeling of rheological property of corn stover hydrolysate at high solids content

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Hydrolysis of lignocellulose at high solids content is the prerequisite condition to obtain high ethanol titer broth and reduce the distillation cost. Design of large scale bioreactors requires the determination of rheological properties of the hydrolysate, but regular rheometers are no longer applicable to high solids content hydrolysate feedstocks. This study reported an on-site method using the torque meter equipped on hydrolysis reactors for its rheological property measurement. The measured torque data were transformed into the apparent viscosity and then the rheological parameters of the power law model after correlation. The determined parameters were applied to develop the computational fluid dynamics (CFD) model for simulating mixing efficiency and power consumption. This study provided a practical method for measurement of rheological parameters and design of optimal structure of large scale bioreactors of high solids content hydrolysis system.

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1. Introduction

Design and structure analysis of hydrolysis reactors on lignocellulose hydrolysis require characterization of its rheological properties of the hydrolysate system [1–3]. In practical applications, high feedstock solids are loaded into bioreactors for the purpose to obtain high fermentable sugar containing sugars or high ethanol titer fermentation broth [4]. Generally, rheological property of lignocellulose hydrolysate is measured using rheometer. The vane style rheometer were the most commonly used machine for measurement of rheological properties of lignocellulose hydrolysate and these high viscous slurries showed obvious shear thinning properties [5–10]. The plate style rheometer and helical ribbon agitated rheomerter were also used for measurement of rheological property at high lignocellulose solids content hydrolysate and the apparent viscosity and yield stress increased with increasing solids concentration [11–13]. On the other hand, special methods such as magnetic resonance imaging (MRI) method coupled with pressure drop measurement [14] was used to measure the yield stress of pretreated corn stover. These rheometer based measurement of rheological properties are well applied to the low solids content hydrolyssi systems, but not accurate for the hydrolysate containing considerably high solid lignocellulose particles due to the irregular signal response [15] and particle sedimentation, especially in the early stage of hydrolysis at which the maximum power input and most intensive mixing are required.

In this study, an on-site method based on torque measurement using torque meter equipped on hydrolysis reactors was applied to the enzymatic hydrolysis of pretreated corn stover (CS) at the solids loading up to 30% (w/w). The determined torque values were transformed into the rheological parameters and then applied to establish the CFD model of high solids content corn stover hydrolysate for hydrolysis reactor design. This study provides a method for concentrated hydrolysis or fermentation system at high solid loading.

2. Materials and methods

2.1. Raw materials and reagents

Corn stover was harvested in fall 2011, Dancheng, Henan, China. 36.18% of cellulose, 19.83% of hemicellulose, and 4.98% of ash were contained in corn stover measured according to NREL protocols [16,17]. The pre-handling of corn stover included water-washed to remove the field dirt, stones and metals, then dried and milled using a beater pulverizer and screened through a mesh with the

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circle diameter of 10 mm. The pre-handled corn stover was stored in sealed plastic bags until use.

Cellulase enzyme Youtell #6 was provided by Hunan Youtell Biochemical Co., Yueyang, Hunan, China. Its filter paper activity of Youtell #6 was 135 FPU/g according to the NREL protocols LAP-006 [18], and the cellobiase activity was 344CBU/g using the method described by Ghose [19]. All chemicals including H_2SO_4, NaOH, and $CaCO_3$ were purchased from the local supplier Linfeng Chemical Reagent Co., Shanghai, China.

2.2. Pretreatment and Enzymatic Hydrolysis Operations

Corn stover was pretreated using dry dilute sulfuric acid pretreatment (DDAP) according to Zhang et al. [21] and He et al. [20]. Briefly, dry corn stover and 5% (w/w) sulfuric acid solution were co-currently fed into the pretreatment reactor at a solid/liquid ratio of 2:1 (w/w) under helically agitated mixing at 50 rpm, and then pretreated at 175 °C for 5 min. The solids content of the pretreated slurry was around 50% (w/w) and no wastewater was generated. The pretreated materials were cooled down and stored at ambient temperature (18–25 °C) until use.

The enzymatic hydrolysis was carried out in a 5 L bioreactor with the inner diameter of 166 mm and the height of 240 mm equipped with a helical impeller as described in our previous study [22]. Pretreated corn stover was fed into the bioreactor to reach the solids content of 30% (w/w) by adding distillated water at the cellulase dosage of 15 FPU/g DM, 50 °C for 12 h. The constant pH of 5.0 was maintained by automatic addition of 5 M NaOH solution.

2.3. Apparent viscosity measurement using rheometer

5 mL of hydrolysate sample was withdrawn from the bioreactor periodically and boiled for 5 min to inactive enzyme activity. Then the apparent viscosity of the sample was measured using an Anton Paar MCR501 rheometer (Ashland, VA, USA) with parallel plate geometry. Samples were pre-sheared 1 min at 100 s^{-1} shear rate, and then determined apparent viscosity at different shear rates for fitting rheological parameters.

2.4. Apparent viscosity measurement using on-site method

Mixing torque of the pretreated corn stover and high solids content hydrolysate on the same 5 L bioreactor was measured using a torque meter (HX-901, Huaxin Mechanical and Electrics Co., Beijing, China) installed on the bioreactor. The torque data were recorded every 5 s and lasted for one min at different agitation rates (50–150 rpm) for pretreated corn stover and the specific agitation rate of 150 rpm for hydrolysate slurry during enzymatic hydrolysis. The zero torque value T_{i0} was defined as the torque data in the empty bioreactor without feedstock loading. The torque value with full loading of feedstock was recorded as T_i. The mixing torque value was defined as $M = T_i - T_{i0}$.

The calculation method of apparent viscosity η_a was referred to Zhang et al. [23]. Briefly, the dimensionless power number N_p is correlated to Reynolds number Re_m as $N_p = C \times {Re_m}^x$, where C is a geometry parameter of the reactor used and independent of the fluid properties, x is a dimensionless factor with the values of $C = 147.12$ and $x = -1$ for the reactor used [23]. Re_m is calculated by $Re_m = \frac{\rho N d^2}{\eta_a}$ where ρ is the density of the fluid (kg/m^3) which was measured by a graduated cylinder, N is the impeller rotation rate (rev/s), d is the impeller diameter (m), η_a is the apparent viscosity (Pa s). Then N_p can be represented as $N_p = C \times Re_m^x = \frac{147.12\eta_a}{\rho N d^2}$.

On the other hand, N_p can be calculated in a stirred vessel by $N_p = \frac{P}{\rho N^3 d^5}$ according to Metzner et al. [24], where the power consumption P (W) is represented as $P = 2\pi N M$. Combining the two N_p expressions together to give the working equation for calculating the apparent viscosity η_a using the measurable parameters M, N, and d :

$$\eta_a = \frac{2\pi M}{C N d^3} = \frac{\pi M}{73.56 N d^3} \tag{1}$$

2.5. CFD modeling

A commercial grid-generation tool, ICEM CFD 14.0 (Ansys Inc., PA, USA) was used to generate the 3D grids of the reactor model in Autodesk Inventor 2014 (Autodesk Inc., CA, USA) for running Fluent 14.0 (Ansys Inc., PA, USA). The multiple reference frame approach was used to the helical ribbon agitated reactors. The upper surface of the liquid was defined as symmetry boundary, and a non-slip wall condition was used for both the walls and the surface of the impellers. The model was performed under laminar flow condition and chemical reaction was negligent. The material used was assumed to be incompressible, non-Newtonian fluid and the rheological properties were correlated by power law model. The tracer was same in physical properties as the background fluid and at the moment of injection the mass fraction of tracer in the injected area equals 1 while at all other areas it equals 0. Convergence was achieved when all normalized residuals reached values smaller than 10^{-4}.

3. Result and discussion

3.1. Determination of rheological properties of pretreated corn stover hydrolysate

The enzymatic hydrolysis of the dilute sulfuric acid pretreated corn stover at the solids loading of 30% (w/w) was carried out for 12 h (Fig. 1). Glucose and xylose concentrations (Fig. 1a) rapidly increased, while insoluble solids content (Fig. 1b) and particle size (Fig. 1c) obviously decreased with hydrolysis processes. For rheological property measurement, two methods described in the Methods section were conducted. The regular method was conducted by taking hydrolysate samples from the hydrolysis reactor and measuring on rheometer. The samples of the pretreated corn stover materials for rheometer measurement were taken only after 30 min of hydrolysis because the initial hydrolysate samples contained too many solids and were not able to use rheometer. Instead, an on-site method using torque meter equipped on the reactor was tested by measuring mixing torque value of hydrolysis reactor and then transformed into the values of apparent viscosity according to the working equation Eq. (1) (Fig. 2). No sampling was required thus the measurement started from the very beginning to the end of the hydrolysis. The apparent viscosity values determined by the on-site method well agreed to the data measured using the rheometer in the late stage of hydrolysis when larger fiber particles hydrolyzed (Fig. 2). At the early stage of hydrolysis (0–2 h), the apparent viscosity values between the two methods showed difference due to the inaccuracy of rheometer on the early stage samples with high solids content. The results suggest that the on-site method for apparent viscosity measurement was applied to the high solids content hydrolysis system and could be used as general method in the similar system.

The apparent viscosity results determined by the two methods (Fig. 2) show that the apparent viscosity of pretreated corn stover decreased significantly at the early stage of hydrolysis (first one hour in the present hydrolysis condition), then changed slowly with the hydrolysis processes. The tendency of apparent viscosity did not exactly correspond to the changes in sugar generation (glucose and xylose) and water insoluble solids (WIS) (Figs. 1a and b), but did correspond to the particle size distribution in which the size

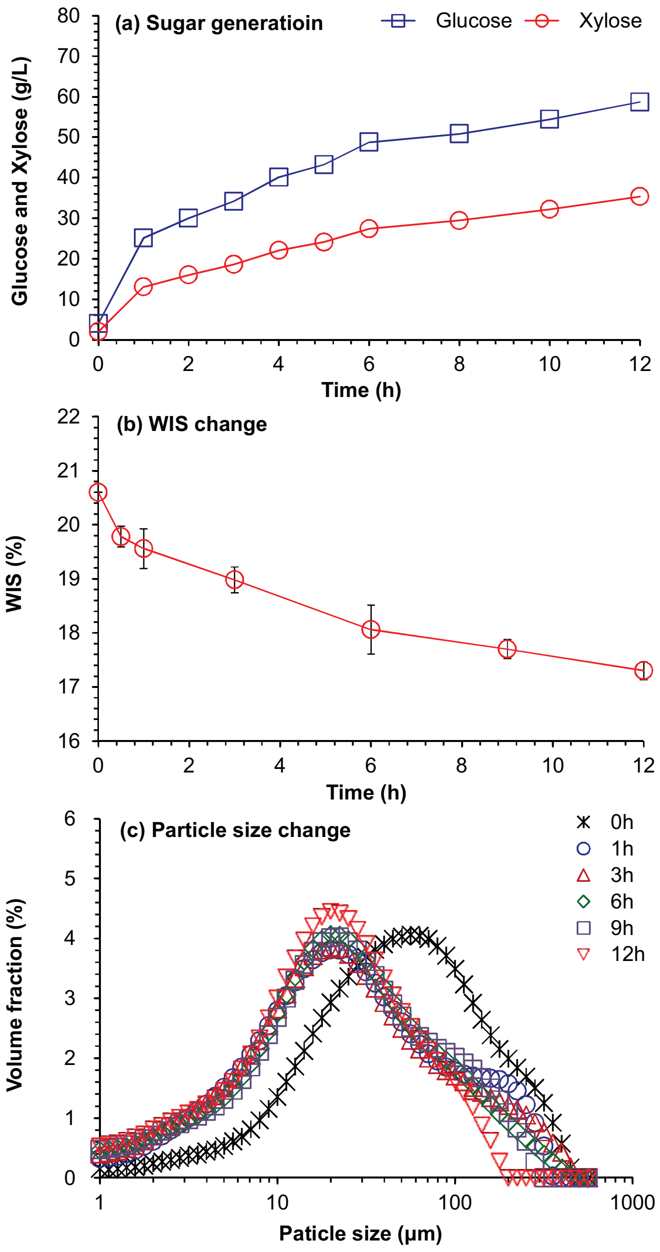

Fig. 1. Enzymatic hydrolysis of dry dilute acid pretreated corn stover. (a) Glucose and xylose; (b) insoluble solids; (c) particle size distribution. Conditions: Solids content at 30% (w/w), 15 FPU/g DM cellulase (10 mg cellulase protein/g DM), pH 5.0, 50 °C for 12 h.

distribution changed significantly in the first one hour then the changes maintained at relatively constant size distribution (Fig. 1c).

3.2. Rheological parameters calculation and CFD modeling

The apparent viscosity of the pretreated corn stover material at different hydrolysis times was measured using the on-site torque method and the rheometer method under different shear rates (Fig. 3). The obvious shear shinning performance was observed at each stage of hydrolysis and confirmed the non-Newtonian fluid property of the pretreated corn stover and the hydrolysate. Then the apparent viscosity η_a of the pretreated corn stover and hydrolysate was correlated using power law model $\eta_a = K_p \times \gamma^{(n-1)}$, similar to that of virgin corn stover suspensions [23]. The model parameters, the consistency coefficient K_p and the dimensionless power-law index n, were obtained as follow: Apparent shear rate γ (s^{-1}) in laminar flow can be represented by impeller speed N (rev/s) as $\gamma = K_s N$ according to Metzner and Otto [25] and combining with the two equations $\eta_a = K_p \times \gamma^{(n-1)}$ gives the working equation for determination of K_p and n :

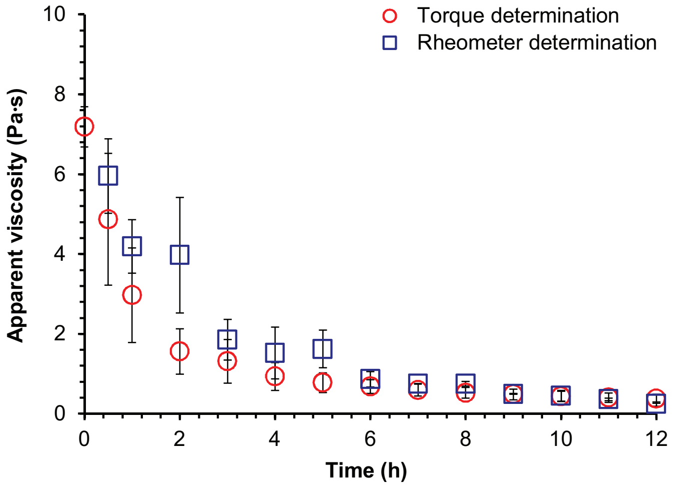

Fig. 2. Time course of apparent viscosity of pretreated corn stover during hydrolysis. Measured by both the on-site torque method and on rheometer at the agitation rate of 150 rpm. Conditions: Solids content at 30% (w/w), 15 FPU/g DM cellulase (10 mg cellulase protein/g DM), pH 5.0, 50 °C for 12 h.

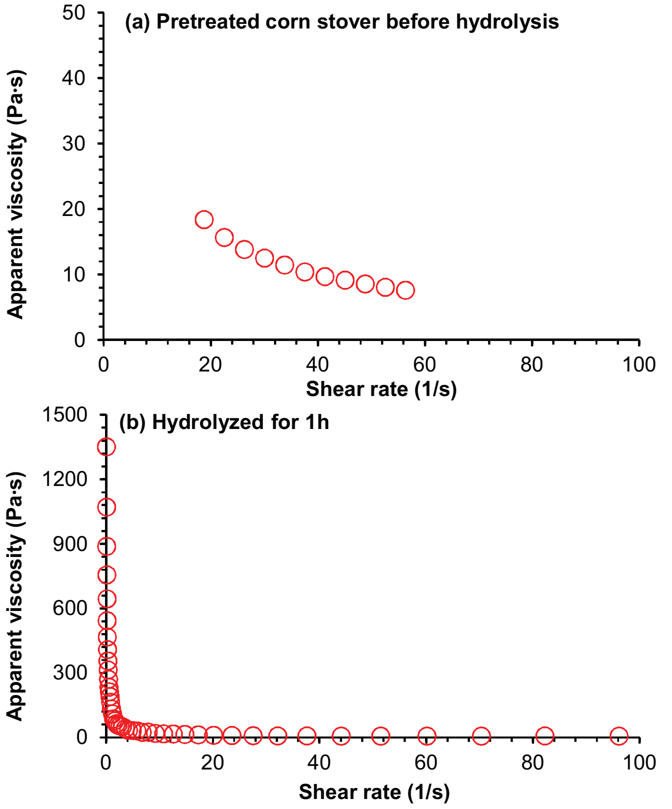

Fig. 3. Apparent viscosity under changing shear rate measured by the on-site method (a) and on rheometer (b). Conditions: Solids content at 30% (w/w), 15 FPU/g DM cellulase (10 mg cellulase protein/g DM), pH 5.0, 50 °C for 12 h.

$$\begin{aligned}\log_{10}\eta_a &= \log_{10}K_P + (n-1)\times\log_{10}(K_S\times N)\\ &= [\log_{10}K_P + (n-1)\times\log_{10}K_S] + (n-1)\times\log_{10}N \qquad (2)\end{aligned}$$

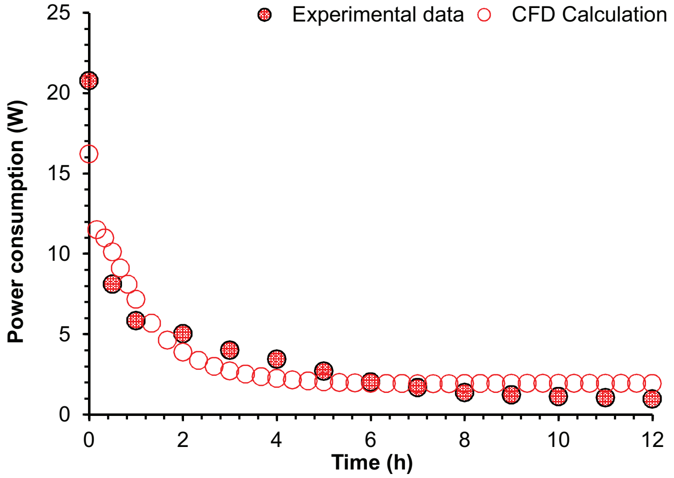

Fig. 4. Power consumption of the hydrolysis of pretreated corn stover calculated by torque measurement and CFD simulation. Conditions: Solids content at 30% (w/w), 15 FPU/g DM cellulase (10 mg cellulase protein/g DM), pH 5.0, 50 °C for 12 h.

where the apparent viscosity η_a was calculated by Eq. (1) $\eta_a = \frac{\pi M}{73{,}56 N d^3}$ from the reactor diameter d, the torque data M and the mixing agitation rate N. The Metzner constant K_s was calculated by for the helical impeller in the range of $0 < n < 0.45$ [26].

$$k_s = \frac{2}{N_r}\frac{S^{2/n}}{S^2}\frac{S_e^2 - 1}{S_e^{2/n} - 1\pi^2}\frac{C}{(l/d)}\left[\frac{n}{2-n}\frac{S^{(2/n)-1}}{S-1}\right]^{1/(n-1)} \quad (3)$$

in which

$$S_e = \frac{D}{d_e} = \frac{S}{S - \frac{2w/d}{\log_{10}\left[\frac{S-(1-2w/d)}{S-1}\right]}} \quad (4)$$

where S is the diameter ratio of D/d of the helical ribbon impeller (HRI), w is ribbon width (m), d is the impeller diameter (m), l is the immersed height of helical ribbon (m), D is the reactor diameter (m), N_r is the number of helical ribbon.

Table 1
Rheological property change of pretreated corn stover at high solids content during enzymatic hydrolysis.

Determination method	Hydrolysis time (h)	n (−)	K_p (Pa s^n)
On-site torque method	0	0.214	179
Rheometer method	0.5	0.128	151
	1	0.183	83.7
	2	0.249	42.6
	3	0.293	23.7
	4	0.344	12.6
	5	0.340	13.8
	6	0.421	7.45
	7	0.467	7.10
	8	0.460	7.20
	9	0.516	3.90
	10	0.538	3.00
	11	0.548	2.55
	12	0.563	1.35

Note: K_p is the consistency coefficient, n is the dimensionless power-law index. Enzymatic hydrolysis processes were carried at the solids loading of 30%, 15 FPU/g DM cellulase dosage, pH 5.0, 50 °C and 150 rpm agitation rate for 12 h.

The n value was calculated by the slope value of Eq. (2) using the logarithm of agitation rates (50–150 rpm) as independent variable and the logarithm of apparent viscosity as dependent variable. The K_s value was calculated by Eqs. (3) and (4) using the n value and the consistency coefficient K_p was calculated by the intercept of Eq. (2). The calculated K_p and n values at varying time point of the hydrolysis are shown in Table 1, in which the values at 0 h was determined by the on-site method and the rest were by rheometer method. The CFD model of hydrolysis bioreactors in both lab bench scale of 5 l and large scale of 2.5 m^3 was developed as described in Methods section using the rheological parameters determined. The power consumption calculated by CFD simulation approximately fitted with the experimentally determined value by $P = 2\pi NM$ (Fig. 4), indicating that the determined rheological parameters met the requirement of power consumption calcula-

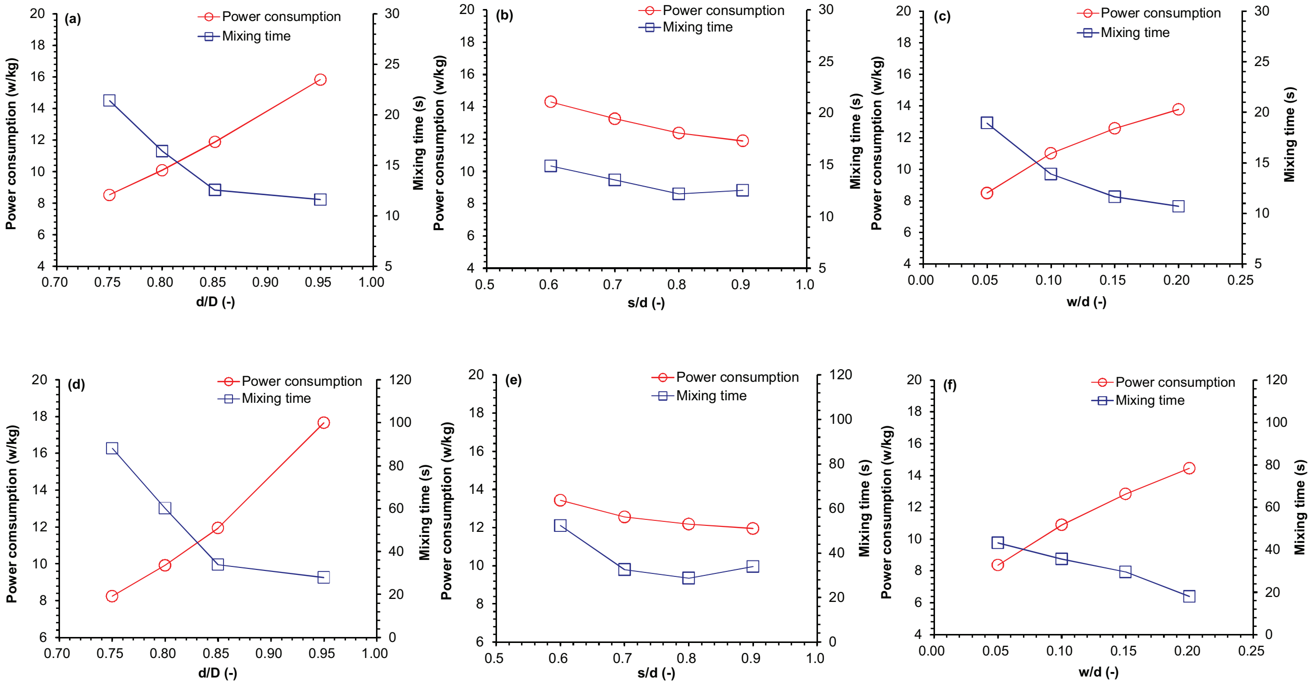

Fig. 5. Geometry design of hydrolysis bioreactors based minimum power consumption and mixing time in the scale of 5 L (a–c) and 2.5 m^3 (d–f). Parameters: d is the diameter of impeller; D is the reactor diameter; s is the pitch size of the helical ribbon; w is the ribbon width.

tion of high solids content hydrolysis by CFD modeling. The overall energy cost for mixing the high solids content hydrolysis of pretreated corn stover was integrated using Origin software (OriginLab Co., Northampton, Massachusetts, USA) based on the power consumption curve in Fig. 4. The calculated energy cost per kilogram of dry material was approximately 0.075 kWh and this data could be applied for estimation of mixing energy cost in large scale reactors.

The CFD model was applied to design different hydrolysis bioreactors by considering the minimum power consumption and mixing time (Fig. 5). In general, the power consumption per kg of dry pretreated corn stover feedstock is similar in the two reactors with volume of 5 L and 2.5 m^3 in the range of geometrical size tested, but the mixing time in 2.5 m^3 reactor is several folds greater than that in 5 L reactor at varying geometrical size of the hydrolysis reactors. This is in agreement of virgin corn stover mixing performance [23]. The mixing time of hydrolysate slurry decreased with reducing impeller diameter (d) until the ratio of d to the reactor diameter (D) reaches 0.85, while the power consumption constantly increased with increasing d/D value (Fig. 5a and d). On the other hand, the screw pitch height (s) of helical ribbon impeller has the least effect on both power consumption and mixing time, in which the ratio of s to impeller diameter (d) at 0.8 leads to the minimum mixing time (Fig. 5b and e). The increasing ribbon width (w) leads to the increasing power consumption and decreasing mixing time (Fig. 5c and f). Balancing the energy cost and mixing performance, the proper range of reactor structure parameters for the 2.5 m^3 hydrolysate reactor were around 0.8–0.9 in d/D, 0.7–0.9 in s/d and 0.1–0.2 in w/d. The performance of reactor designs will be tested in the scale-up of lignocellulose hydrolysis in the future commercial plant.

4. Conclusion

The on-site method for measurement of apparent viscosity of the hydrolysate at 30% (w/w) of pretreated corn stover solids was established and applied to obtain the rheological parameters for CFD modeling. The power consumption and mixing efficiency of the high solids content corn stover during enzymatic hydrolysis were calculated and analyzed using the CFD model. The geometrical structure parameters of the 2.5 m^3 hydrolysis reactor were optimized based on the minimum power consumption and mixing time on hydrolysis of pretreated lignocellulose feedstock.

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References

[1] D. Carvajal, D.L. Marchisio, S. Bensaid, D. Fino, Enzymatic hydrolysis of lignocellulosic biomasses via CFD and experiments, Ind. Eng. Chem. Res. 51 (2011) 7518–7525.

[2] B.X. Wu, Computational fluid dynamics investigation of turbulence models for non-newtonian fluid flow in anaerobic digesters, Environ. Sci. Technol. 44 (2010) 8989–8995.

[3] B.X. Wu, CFD simulation of mixing for high-solids anaerobic digestion, Biotechnol. Bioeng. 109 (2012) 2116–2126.

[4] M. Galbe, P. Sassner, A. Wingren, G. Zacchi, Process engineering economics of bioethanol production, in: L. Olsson (Ed.), Biofuels (2015) 303–327;
J.S. Knutsen, M.W. Liberatore, Rheology of high-solids biomass slurries for biorefinery applications, J. Rheol. 53 (4) (2007) 877–892.

[5] J.J. Stickel, J.S. Knutsen, M.W. Liberatore, W. Luu, D.W. Bousfield, D.J. Klingenberg, C.T. Scott, T.W. Root, M.R. Ehrhardt, T.O. Monz, Rheology measurements of a biomass slurry: an inter-laboratory study, Rheol. Acta 48 (2009) 1005–1015.

[6] C.M. Roche, C.J. Dibble, J.S. Knutsen, J.J. Stickel, M.W. Liberatore, Particle concentration and yield stress of biomass slurries during enzymatic hydrolysis at high-solids loadings, Biotechnol. Bioeng. 104 (2) (2009) 290–300.

[7] J. Du, F.Z. Zhang, Y.Y. Li, H.M. Zhang, J.R. Liang, H.B. Zheng, H. Huang, Enzymatic liquefaction and saccharification of pretreated corn stover at high-solids concentrations in a horizontal rotating bioreactor, Bioprocess. Biosyst. Eng. 37 (2014) 173–181.

[8] R.K. Dasari, K. Dunaway, R.E. Berson, A scraped surface bioreactor for enzymatic saccharification of pretreated corn stover slurries, Energy Fuels 23 (2009) 492–497.

[9] K.W. Dunaway, R.K. Dasari, N.G. Bennett, R.E. Berson, Characterization of changes in viscosity and insoluble solids content during enzymatic saccharification of pretreated corn stover slurries, Bioresour. Technol. 101 (10) (2010) 3575–3582.

[10] S. Viamajala, J.D. McMillan, D.J. Schell, R.T. Elander, Rheology of corn stover slurries at high solids concentrations-effects of saccharification and particle size, Bioresour. Technol. 100 (2009) 925–934.

[11] N.V. Pimenova, T.R. Hanley, Measurement of rheological properties of corn stover suspensions, Appl. Biochem. Biotechnol. 105 (2003) 383–392.

[12] N.V. Pimenova, T.R. Hanley, Effect of corn stover concentration on rheological characteristics, Appl. Biochem. Biotechnol. 114 (2004) 347–360.

[13] D.M. Lavenson, E.J. Tozzi, M.J. McCarthy, R.L. Powell, Yield stress of pretreated corn stover suspensions using magnetic resonance imaging, Biochem. Biotechnol. 108 (2011) 2312–2319.

[14] M.R. Ehrhardt, T.O. Monz, T.W. Root, R.K. Connelly, C.T. Scott, D.J. Klingenberg, Rheology of dilute acid hydrolyzed corn stover at high solids concentration, Appl. Biochem. Biotechnol. 160 (2010) 1102–1115.

[15] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D. Crocker, Determination of structural carbohydrates and lignin in biomass. Golden, CO., National Renewable Energy Laboratory, (2008).

[16] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, Determination of structural carbohydrates and lignin in biomass. Golden, CO., National Renewable Energy Laboratory, (2008).

[17] B. Adney, J. Baker, Measurement of cellulase activities. Laboratory analytical procedure (LAP-006). National Renewable Energy Laboratory, Golden, CO, (1996).

[18] T.K. Ghose, Measurement of cellulase activities, Pure Appl. Chem. 59 (1987) 257–268.

[19] J. Zhang, X. Wang, D. Chu, Y. He, J. Bao, Dry pretreatment of lignocellulose with extremely low steam and water usage for bioethanol production, Bioresour. Technol. 102 (6) (2011) 4480–4488.

[20] Y.Q. He, L.P. Zhang, J. Zhang, J. Bao, Helically agitated mixing in dry dilute acid pretreatment enhances the bioconversion of corn stover into ethanol, Biotechnol. Biofuels 7 (2014) 1.

[21] J. Zhang, D. Chu, J. Huang, Z. Yu, G. Dai, J. Bao, Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor, Biotechnol. Bioeng. 105 (4) (2010) 718–728.

[22] L.P. Zhang, J. Zhang, C.H. Li, J. Bao, Rheological characterization and CFD modeling of corn stover–Water mixing system at high solids loading for dilute acid pretreatment, Biochem. Eng. J. 90 (2014) 324–332.

[23] E.L. Paul, V.A. Atiemo-Obeng, S.M. Kresta, Handbook of industrial mixing, John Wiley & Sons, Inc, New Jersey, 2004.

[24] A.B. Metzner, R.E. Otto, Agitation of non-Newtonian fluids, AIChE J. 3 (1) (1957) 3–10.

[25] G. Delaplace, R. Guerin, J.C. Leuliet, R.P. Chhabra, An analytical model for the prediction of power consumption for shear-thinning fluids with helical ribbon and helical screw ribbon impellers, Chemical. Eng. Sci. 61 (10) (2006) 3250–3259.