Full Length Article

Rheology evolution of high solids content and highly viscous lignocellulose system in biorefinery fermentations for production of biofuels and biochemicals

Weiliang Hou[1], Jialun Kan[1], Jie Bao[*]

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

GRAPHICAL ABSTRACT
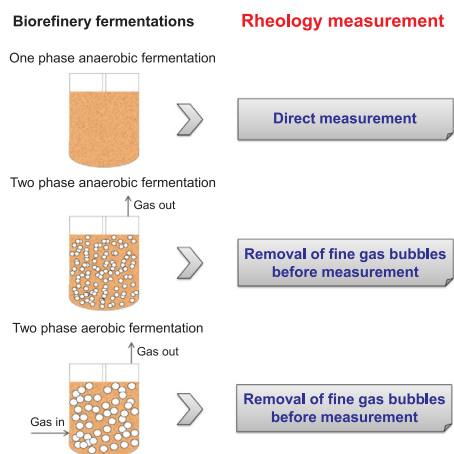
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ABSTRACT

Rheological property of high solids content and highly viscous slurries of lignocellulose biomass in biorefinery fermentations is crucially important for designing large scale reactors. Rheology studies on pre-processing, pretreatment and enzymatic hydrolysis of high solids content lignocellulose biomass have been experimentally investigated and applied to reactor designs of the corresponding steps. Fermentation is the final step to complete the gap of the overall chart of rheology models of lignocellulose biorefinery process. This study experimentally measured rheology behaviors of biorefinery fermentations in three typical forms for biofuel and biochemical production under high solids content and highly viscous slurries. The advanced dry biorefining technology was adopted and excellent fermentation behaviors were achieved using corn stover as material, in which the yield of cellulosic ethanol, L-lactic acid, gluconic and xylonic acids reached up to 81.6, 96.0, 101.5 g/L and 34.4 g/L, respectively. Rheology results found that product generation and simultaneous hydrolysis conversion both played a key role in rheology evolution of fermentation processes. This rheology study provided a crucial basis for large scale bioreactor design in industrial lignocellulose biorefinery operations under high solids loading.

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[*] Corresponding author.
  E-mail address: jbao@ecust.edu.cn (J. Bao).
[1] These authors are equally contributed to this work.

1. Introduction

Biorefining of lignocellulose biomass includes multiple steps of pre-processing, pretreatment, detoxification (conditioning), hydrolysis, fermentation and product recovery [1]. High lignocellulose feedstock loading in each step of biorefining process is important to obtain high product titer and reduce recovery cost in industrial operations [2–4]. Simultaneous saccharification and fermentations (SSF) is the most efficient and practical fermentation form for biorefinery process, because the glucose generated from hydrolysis is simultaneously converted to products and the strong inhibition of glucose on cellulase is relieved. For operating fermentations close to industrial case, high lignocellulose feedstock solids loading is the precondition for achieving high product titer in SSF for biofuels or biochemical production.

To design large scale reactors with high lignocellulose feedstock loading, accurate rheology measurement is required in each biorefining steps. In our previous studies, rheological properties of raw corn stover feedstock (40–100% of solids, w/w) [5], pretreated corn stover feedstock (50–70% of solids, w/w) [6], and corn stover hydrolysate slurry (30% of solids, w/w) had been characterized [7]. Till now, rheology characterization of biorefinery fermentation systems under high solids loading is the last gap to fill the picture of complete biorefinery processing. Biorefinery fermentation evolves in many ways and could be simply classified as three types including anaerobic fermentation without gas generation (lactic acid fermentation), anaerobic fermentation with gas generation (ethanol fermentation) and aerobic fermentation (gluconic acid fermentation). For SSF operation, rheology is more versatile and elusive because of the simultaneous involvement of enzymatic hydrolysis and fermentation. Also, the existence of gas bubbles in fermentation slurry (anaerobic fermentation with gas generation or aerobic fermentation) could disturb rheology measurement by giving a false signal on apparent viscosity value [8,9], hence intensifying difficulty on accurate rheology measurement.

In this study, we measured rheology evolution of three representative biorefinery fermentation processes under high lignocellulose feedstock loading including anaerobic L-lactic acid fermentation without gas generation, anaerobic ethanol fermentation with gas generation, and aerobic gluconic acid fermentation. Accurate rheology measurement of fermentation slurries was attempted by removing the impact of gas phase. The power law model was used to character rheology property of lignocellulose fermentation slurry. The evolution of rheological properties in fermentation processes was analyzed and associated with product generation and simultaneous hydrolysis.

2. Materials and methods

2.1. Raw materials and enzyme

Corn stover was harvested from Bayan Nur League, Inner Mongolia Autonomous Region, China in fall 2015. The raw corn stover was milled, water-washed then dried until constant weight. The pre-handled corn stover contained 35.4% cellulose, 24.6% hemicellulose, 16.1% lignin, and 3.5% ash on dry weight base (w/w) measured according to NREL protocols [10,11].

Cellulase enzyme Cellic CTec 2.0 was purchased from Novozymes (China), Beijing, China. The filter paper activity was determined as 203.2 FPU/mL according to the NREL protocols LAP-006 [12], the cellobiase activity was 4,900 CBU/mL according to Ghose [13], and the protein content was 87.3 mg/mL according to Bradford [14].

2.2. Strains and cultures

Pediococcus acidilactici TY112 was obtained from China General Microorganism Collection Center (CGMCC, Beijing, China) with the registration number 8664 and used as L-lactic acid fermentation strain

was firstly inoculated into a simplified MRS medium containing 20 g/L of glucose, 10 g/L of peptone, 10 g/L of yeast extract, 2 g/L of diammonium hydrogen citrate, 5 g/L of sodium acetate anhydrous, 0.6 g/L of $MgSO_4 \cdot 7H_2O$, 2 g/L of K_2HPO_4, 0.25 g/L of $MnSO_4 \cdot H_2O$ with 1% of saccharifying enzyme for 12 h, then transferred into two-stage medium for 4 h with 10% inoculation ratio. Culture conditions were 42 °C and 150 rpm.

Saccharomyces cerevisiae XH7 was kindly provided by Prof XM Bao, Shandong University, Jinan, China and used as ethanol fermentation strain with xylose utilization was activated into YPD medium for 12 h. The culture was successively transferred into the medium containing 5% (w/w) of the biodetoxified corn stover, cellulase dosage of 6 mg/g DM, 2 g/L of KH_2PO_4, 2 g/L of $(NH_4)_2SO_4$, 1 g/L of $MgSO_4$, 10 g/L of yeast extract for 12 h, then inoculated into the same medium for 24 h except the detoxified corn stover replaced as 10% (w/w) from 5% (w/w). Culture conditions were 30 °C, 200 rpm and pH 5.5.

Gluconobacter oxydans DSM 2003 was obtained from from German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany and used as gluconic acid fermentation strain was inoculated into seed medium containing 80.0 g/L of sorbitol, 10.0 g/L of yeast extract, 1.5 g/L of KH_2PO_4, 1.5 g/L of $(NH_4)_2SO_4$, 0.5 g/L of $MgSO_4 \cdot 7H_2O$ and cultured at 30 °C, 220 rpm for 15 h.

Amorphotheca resinae ZN1 was isolated in our previous study and stored in CGMCC with the registration number of 7452. *A. resinae* ZN1 was used as biodetoxification strain and maintained on a potato dextrose agar medium (PDA) slant.

2.3. Pretreatment and biodetoxification operations

Corn stover was dry sulfuric acid pretreated according to Zhang et al. [15] and He et al. [16]. Briefly, raw corn stover and sulfuric acid solution at 2.0% (w/w) were pretreated at a solid/liquid ratio of 2:1 (w/w) and 175 °C for 5 min.

The pretreated corn stover was neutralized to pH value of 5–6 with 20% (w/w) $Ca(OH)_2$ suspension slurry and then biodetoxified in aerobic condition with *A. resinae* ZN1 for 2 days in ethanol fermentation and gluconic acid fermentation or 3 days in L-lactic acid fermentation.

2.4. Fermentation operations

The dry acid pretreated and biodetoxified corn stover was prehydrolyzed at 6 mg cellulase proteins/g dry solids matter (DM) and 30% solids loading, at pH 4.8 and 50 °C for 6 h [17]. Then the SSF for L-lactic acid production was started by inoculating *P. acidilactici* TY112 at pH 5.5, 45 °C for 72 h and the added nutrient contained 10 g/L of peptone, 10 g/L of yeast extract, 2 g/L of diammonium hydrogen citrate, and 0.25 g/L of $MnSO_4 \cdot H_2O$.

Corn stover was prehydrolyzed at 6 mg cellulase proteins/g DM and 30% solids loading, at pH 4.8 and 50 °C for 12 h [4]. Then the SSF for ethanol production was started by inoculating the *S. cerevisiae* XH7 at pH 5.5, 30 °C for 108 h and the added nutrient contained 2 g/L of KH_2PO_4, 2 g/L of $(NH_4)_2SO_4$, 1 g/L of $MgSO_4$, 10 g/L of YE.

Corn stover was prehydrolyzed at 6 mg cellulase proteins/g DM and 30% solids loading, at pH 4.8 and 50 °C for 48 h [18]. Then gluconic acid production was started by inoculating *G. oxydans* DSM 2003 at pH 4.8, 1 vvm, 35 °C for 48 h and no nutrient was added.

2.5. HPLC analysis

Glucose, xylose, L-lactic acid and ethanol in L-lactic acid fermentation and ethanol fermentation, as well as furfural, acetic acid and HMF were measured by HPLC according to Liu et al. [4] and Yi et al. [19]. Glucose and xylose in gluconic acid fermentation were analyzed according to Hou et al. [20].

2.6. Determination of rheological property

Apparent viscosity (η_a) of fermentation slurries was measured at 25 °C on Brookfield DV2T viscometer (Stoughton, Middleboro). Rheological properties were fitted into power law model $\eta_a = K_p \cdot \gamma^{n-1}$, where n was dimensionless power-law index, K_p was consistency coefficient (Pa·s^n), γ was shear rate (s^{-1}) [8]. The K_p and the n values were calculated by measuring the intercept and the slop of the linearized power law model shown in Eq. (1):

$$\log_{10} \eta_a = \log_{10} K_p + (n-1)\log_{10} \gamma \tag{1}$$

L-lactic acid fermentation slurry was directly used for rheological property measurement. In ethanol fermentation and gluconic acid fermentation, the existence of gas phase disrupted accurate rheology measurement. The air or CO_2 bubbles in the fermentation slurry were removed by vacuum evaporation. The living microbial cells (yeast or bacterium) were eliminated by addition of a small dosage of a strong anti-microbial reagent benzoquinone addition (500 mg/L).

3. Results and discussion

3.1. Rheology evolution of cellulosic L-lactic acid fermentation

The anaerobic L-lactic acid fermentation without gas generation was carried out after 6 h prehydrolysis at 30% (w/w) of corn stover by dry acid pretreatment, then *P. acidilactici* seed was inoculated into the hydrolysate to initiate the fermentation along with the simultaneous hydrolysis of corn stover. The rheology property was directly measured on viscometer without special treatment, due to the inexistence of gas in fermentation slurry samples (Fig. 1).

L-lactic acid accumulated gradually in SSF and finally 96.0 g/L of cellulosic L-lactic acid was generated with the yield of 74.0% (Fig. 1a). The maximum apparent viscosity (η_a) of fermentation slurry appeared at the starting point of fermentation (0.94 Pa·s) and rapidly decreased to 0.14 Pa·s with fermentation processes (Fig. 1b). The consistency coefficient (K_p) also quickly decreased, and the dimensionless power-law index (n) gradually increased to 0.22, indicating the cellulosic L-lactic acid fermentation slurry was a typical non-Newtonian shear-thinning fluid.

The diminution of water insoluble solids content (WIS) in fermentation slurry was a key reason on viscosity decrease [21,22]. WIS reduced 26.8% in L-lactic acid production (Fig. 4), in which 18.4% of reduction came from the heavy dilution of neutralization alkali to maintain pH stabilization with L-lactic acid generation, and the other reduction could come from the outcome of cellulose hydrolysis in SSF. The result indicated that rheology evolution was substantially caused by L-lactic acid generation, and secondarily simultaneous hydrolysis.

3.2. Rheology evolution of cellulosic ethanol fermentation

For anaerobic biorefinery fermentation with gas generation, the existence of gas could impact on rheology measurement. Ethanol fermentation as a typical example of such anaerobic fermentations in which no aeration is needed but CO_2 is spontaneously generated was used for evaluating rheology measurement in the gas containing system.

We conducted ethanol fermentation by SSF operation after 12 h prehydrolysis at 30% (w/w) of corn stover content (Fig. 3). Glucose was rapidly consumed to an extreme low level in a short period (12 h) and 81.6 g/L of ethanol was obtained accompany with efficient utilization of xylose after 108 h (Fig. 3a). CO_2 gas is spontaneously generated in ethanol fermentation and its impact on rheology measurement was evaluated. When CO_2 bubbles were removed from fermentation slurry by vacuum evaporation, the measured apparent viscosity decreased 20.6% compared with that without any treatment. The effect of living yeast cells in the slurry on rheology property was also assayed by
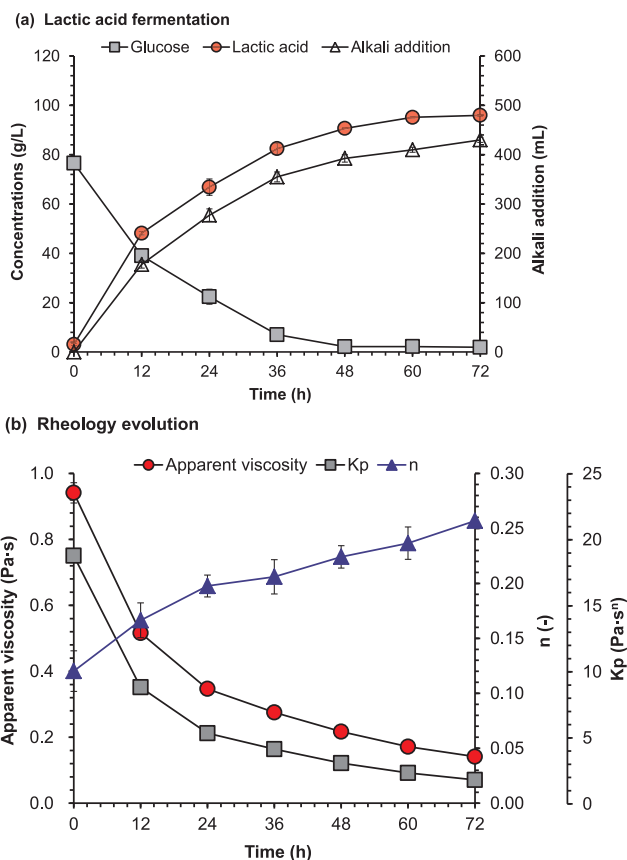

Fig. 1. Rheology evolution of cellulosic L-lactic acid fermentation. (a) Sugars consumption and L-lactic acid generation; (b) Rheology evolution. Prehydrolysis was operated in 5 L bioreactor at 30% solids loading (w/w), 6 mg cellulase protein /g DM, 50 °C, pH 4.8, 150 rpm for 6 h; then SSF was carried out by inoculation of *P. acidilactici* TY112 at inoculum size 10%, 42 °C, pH 5.5, 150 rpm for 72 h. Apparent viscosity was shown at shear rate of 30 s^{-1} measured by a rotational viscometer. The consistency coefficient K_p and dimensionless power-law index n was calculated using the measured apparent viscosity at different shear rates based on power-law model. The error bars representing standard deviation of the repeated experiments.

complete extinction using benzoquinone, but no observable change of rheology property was found between the slurry samples with and without living yeast cells. Therefore, for high solids loading cellulosic ethanol fermentation, as well as the general gas containing fermentation systems, gas bubble removal by vacuum evaporation is necessary and sufficient for rheology property measurement.

The accurately measured rheological properties show that the η_a value of ethanol fermentation slurry quickly decreased from 0.55 Pa·s to 0.08 Pa·s with fermentation processes (Fig. 3b). The corresponding power law model parameters K_p and n values were correlated by power law model.

The WIS of fermentation slurry decreased 15.5% in the cellulosic ethanol fermentation (Fig. 2). Because of ethanol as solvent being completely dissolved into water, the generated ethanol (81.6 g/L, also 103.4 mL/L) achieved 10.3% dilution of fermentation slurry and decrease on WIS, while the half glucose simultaneously hydrolyzed in SSF could be another reason for the reduction of WIS. Therefore, the dilution of ethanol produced and simultaneous hydrolysis mainly effected on rheology evolution in cellulosic ethanol fermentation.

3.3. Rheology evolution of cellulosic gluconic acid fermentation

Aerobic gluconic acid production was conducted after 48 h prehydrolysis at high corn stover solids content (30%, w/w) (Fig. 4).
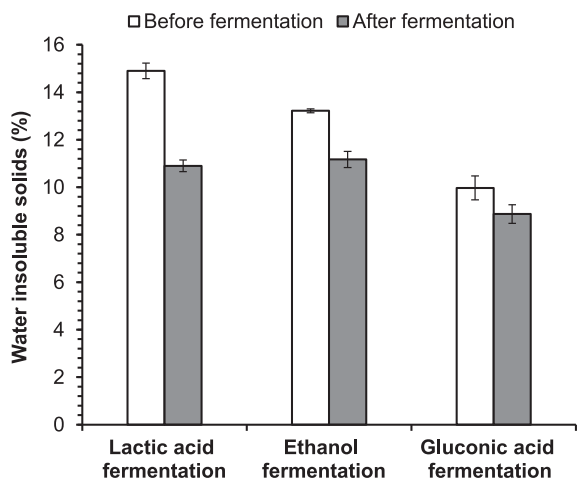
Fig. 2. Water insoluble solids content of biorefinery fermentations under high solids loading. L-lactic acid fermentation: prehydrolyzed at 50 °C, pH 4.8 for 6 h, 6 mg cellulase protein /g DM under 30% solids loading (w/w), then SSF by inoculation of *P. acidilactici*, 42 °C, pH 5.5 for 72 h. Ethanol fermentation: prehydrolyzed at the same conditions for 12 h, then SSF by inoculation of *S. cerevisiae*, 30 °C, pH 5.5 for 108 h. Gluconic acid fermentation: prehydrolyzed at the same conditions for 48 h, then fermentation was carried out by inoculation of *G. oxydans* and 1 vvm, at pH 4.8 and 35 °C for 72 h. The error bars representing standard deviation of the repeated experiments.
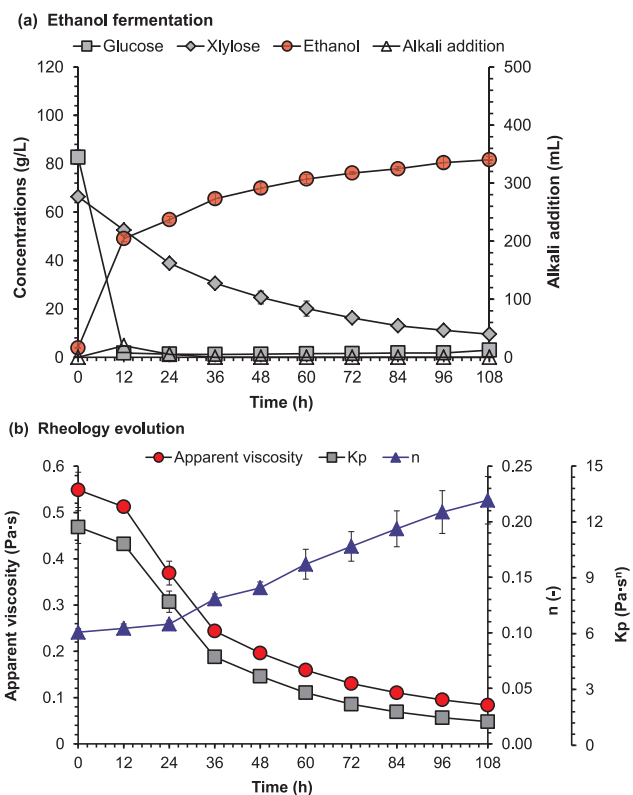
Fig. 3. Rheology evolution of cellulosic ethanol fermentation. (a) Sugars consumption and ethanol generation; (b) Rheology evolution. Prehydrolysis was operated at 30% solids loading (w/w), 6 mg cellulase protein /g DM, 50 °C, pH 4.8, 150 rpm for 12 h; then SSF was carried out by inoculation of *S. cerevisiae* XH7 at inoculum size 10%, 30 °C, pH 5.5, 150 rpm for 108 h. Apparent viscosity was shown at shear rate of $30\,\mathrm{s}^{-1}$ measured by a rotational viscometer. The consistency coefficient K_p and dimensionless power-law index n was calculated using the measured apparent viscosity at different shear rates based on power-law model. The error bars representing standard deviation of the repeated experiments.
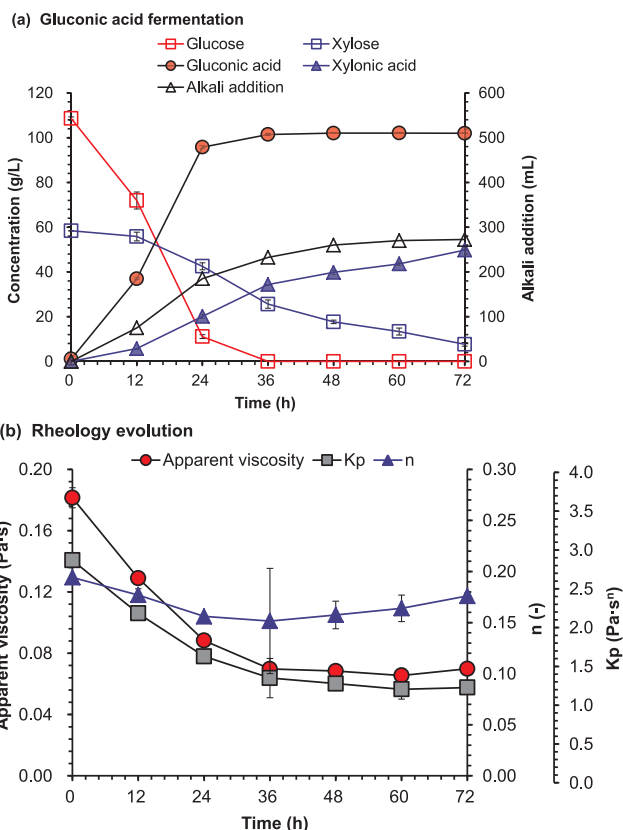
Fig. 4. Rheology evolution of cellulosic gluconic acid fermentation. (a) Sugars consumption and acids generation; (b) Rheology evolution. Prehydrolysis was operated at 30% solids loading (w/w), 6 mg cellulase protein/g DM, 50 °C, pH 4.8, 150 rpm for 48 h; then gluconic acid fermentation was carried out by inoculation of *G. oxydans* at inoculum size 5%, 500 rpm and 1 vvm, at pH 4.8 and 35 °C for 72 h. Apparent viscosity was shown at shear rate of $30\,\mathrm{s}^{-1}$ measured by a rotational viscometer. The consistency coefficient K_p and dimensionless power-law index n was calculated using the measured apparent viscosity at different shear rates based on power-law model. The error bars representing standard deviation of the repeated experiments.

Before measuring rheology evolution of gluconic acid fermentation slurry, air bubbles in the slurry were removed by vacuum evaporation following the same procedure to ethanol fermentation slurry.

Glucose and most of xylose were converted into gluconic acid (101.5 g/L) and xylonic acid (34.4 g/L) within 36 h (Fig. 4a) and the η_a value of fermentation slurry reduced quickly with the formation of gluconic acid and xylonic acid (Fig. 4b). The corresponding K_p value also decreased and the n value maintained relatively constant during the fermentation.

The addition of neutralization alkali with gluconic acid generation diluted 12.0% of fermentation slurry (Fig. 4a), which was consistent with the reduction of WIS in fermentation process (Fig. 2). The result indicated that the change of WIS, as well as rheology could be mainly led by gluconic and xylonic acids generation, and the impact of simultaneous hydrolysis could be slight.

3.4. General rule of rheology evolution in biorefinery fermentations

This study systematically investigated rheology evolution of three representative fermentation processes in biorefinery fermentation step under high solids loading condition for the first time. For common anaerobic fermentation slurry without gas generation, rheological properties could be directly measured by rheometers or viscometers. For anaerobic fermentation with gas generation and aerobic fermentation such gas containing fermentation system, gas bubbles in
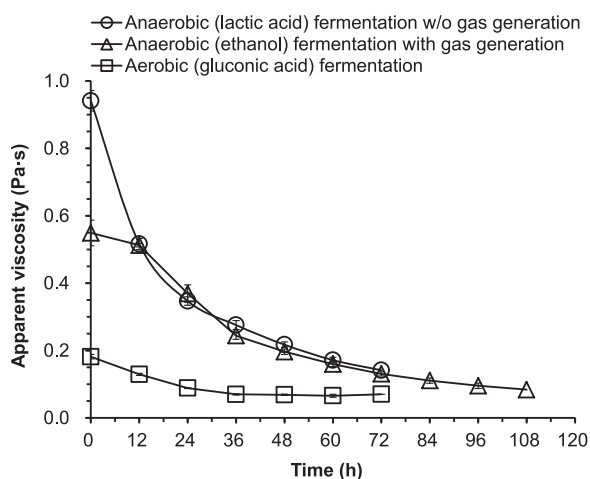
Fig. 5. Rheology evolution of biorefinery fermentations under high solids loading. Apparent viscosity was determined at shear rate of $30 \, s^{-1}$ using rheological properties. The error bars representing standard deviation of the repeated experiments.

fermentation samples should be removed by vacuum evaporation for obtaining accurate rheological properties.

The accurately measured results indicated that rheology evolution was mainly caused by product generation and then simultaneous hydrolysis, and showed the decreasing tendency with the progress of fermentation in three representative fermentation processes, but varied at different ranges (Fig. 5). The difference in apparent viscosity values came from the variation of prehydrolysis times, products, strains and so on. Therefore, experimental measurement of rheology evolution is required in each biorefinery fermentation process to obtain accurate rheological parameters.

4. Conclusion

We experimentally investigated rheology behaviors of three representative biorefinery fermentations in high solids content and highly viscous slurries of lignocellulose feedstock and removed the impact of gas on rheology measurement in gas containing fermentations (such as ethanol fermentation and gluconic acid fermentation). The accurate rheology measurement showed that product generation and simultaneous hydrolysis conversion played a key role in the rheology evolution of biorefinery fermentations. The rheology parameters measured in this study could be used in the CFD modeling for designing the large scale bioreactors in industrial application of lignocellulose biorefinery.

Acknowledgement

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