CELF Pretreatment Improves Ethanol Titers from High Solids SSF of Hardwood Poplar

Priya Sengupta
University of California Riverside (UCR)
Charles E. Wyman
University of California Riverside (UCR)
Charles M. Cai (ccai@engr.ucr.edu)
University of California Riverside (UCR)

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Abstract

Woody biomass is not only abundant and sustainably available but also contains more glucan than most agricultural feedstocks. However, its structural strength and density make it more recalcitrant to enzymatic breakdown and limit SSF performance, especially at higher solids loadings needed to achieve desired ethanol titers. Because Co-Solvent Enhanced Lignocellulosic Fractionation (CELF) highly enriches glucan contents in pretreated solids by removing and recovering most of the hemicellulose and lignin, we hypothesized that the greater glucan in SSF at solids loadings that inhibit mixing than possible for other pretreatments would make it possible to achieve higher ethanol titers and yields. Application of a fractal kinetic model to data from enzymatic hydrolysis of CELF pretreated poplar indicated that CELF solids could sustain high cellulase enzyme activity over the entire course of hydrolysis with minimal enzyme deactivation. Model parameters further suggested that increasing enzyme loadings beyond 15 mg protein/g glucan in raw poplar would not increase saccharification performance. Based on these predictions, SSF was applied to solids produced by CELF pretreatment of poplar using Cellic® CTec2 cellulase at a loading of 15 mg-protein/ g-glucan in combination with *S. cerevisiae* D5A. At CELF solids loadings of 13, 17, and 20 wt%, SSF realized ethanol titers of 60, 78, and 87 g/L and yields of 87, 84, and 79% of theoretical in respective orders of increasing solids loadings. Furthermore, sugar release was sustained throughout SSF, irrespective of solids loading, suggested that yeast ethanol tolerance and metabolic capability and not glucan digestibility limited SSF performance.

Introduction

The transportation sector is among the greatest contributors to greenhouse gas emissions by the United States.[1] Lignocellulosic biomass is structural/non-food plant material that can provide an abundant, low cost resource for producing clean, sustainable transportation fuels at a large enough scale to impact energy demands and reduce greenhouse gas emissions.[2,3] Poplar and other fast growing trees can be grown as sustainable energy crops from which cellulose and hemicellulose sugars can be released for fermentation into biofuels, chemicals, adhesives, and other biobased products.[4] Biological conversion of such plant-based sugars into fuel typically involves three primary steps 1) disruption of the plant cell wall via pretreatment to increase the availability of polysaccharides for downstream deconstruction, 2) enzymatic hydrolysis of the polysaccharides into fermentable sugars, and 3) fermentation of these sugars into desired products such as ethanol.[5–7] Three primary biological conversion options that have been employed for steps 2 and 3 are i) hydrolysis of polysaccharides to monomers in one step followed by fermentation of the sugars released into ethanol in a separate step (Separate Hydrolysis and Fermentation or SHF), ii) fermentation of sugars to ethanol in the same vessel as used to enzymatically release these sugars (Simultaneous Saccharification and Fermentation or SSF), or iii) consolidation of enzyme production, breakdown of polysaccharides into sugars, and fermentation of these sugars to ethanol all in a single vessel (Consolidated Bioprocessing or CBP).[8–10]

SSF yeast consuming sugars as they are released by enzymatic hydrolysis reduces end-product inhibition of enzymes, resulting in enhanced rates and yields of ethanol production. In addition, ethanol buildup as fermentation proceeds impedes contamination of SSF by bacteria or other unwanted organisms with low ethanol tolerance. However, because most fermentative organisms including those that are thermotolerant for pure sugar fermentations have limited tolerance to the combined stresses of high temperatures (< 35 ºC), high ethanol and low sugar concentrations, SSF must generally be conducted at lower temperatures than optimal for cellulase enzymes, thereby hurting ethanol rates, yields, and titers.[11,12].

Another important consideration is the high ethanol titers realize significant operating and capital cost savings through reducing energy demand and equipment size for fermentation and downstream ethanol recovery.[13,14] Unfortunately, the difficulty of effectively mixing cellulosic biomass at solids loadings above about 20 wt% limits maximum possible ethanol titers to less than 60 g/L for solids produced by many pretreatments.[15,16] However, a novel Co-solvent Enhanced Lignocellulosic Fractionation (CELF) pretreatment employs tetrahydrofuran in the miscible range with water containing dilute sulfuric acid to significantly increase the glucan fraction in pretreated solids through removal of most of the hemicellulose and lignin.[17] The resulting greater glucan content provides the opportunity to reach higher ethanol titers before reaching the mixing limit for a given solids loading.

Results And Discussion

CELF increases potential ethanol titers at limiting solids loadings in SSF

The viscosity of suspensions of cellulosic biomass solids in water increases rapidly with increasing loadings and can become too thick to effectively mix in conventional fermenters at solids levels above about 20–25%, depending on solids water absorption. Improper mixing at higher solids loadings results in uneven heat and mass transfer that is a particular problem in commercial sized fermenters as well as limited water contact for enzymes and microbes to interact with the substrate and sugars in the system, respectively.[15,16] For these example solids loading limitations, pretreated solids containing about 60% polysaccharides could not produce sugar concentrations greater than 12 to 15% sugars, respectively, even if the polysaccharides were completely hydrolyzed to sugars. On the other hand, increasing polysaccharide content to 90% in pretreated solids could translate into as much as 18 to 22.5% sugars in solution, respectively. If we assume that the enzymes could release 90% of the potential sugars into solution and 90% of those sugars could be converted into ethanol with a yield of 51.1 grams of ethanol/gram of sugar, the maximum ethanol titer would increase from 4.9 to 6.2% from 60% polysaccharides in the solids to 7.4 to 9.3% for solids containing 90% polysaccharides, respectively. Thus, pretreatments that remove lignin, ash, and other nonfermentable components as well as hemicellulose offer the potential to achieve higher ethanol titers in SSF for a given solids loading limitation. Furthermore, delignification of biomass reduces non-productive binding of enzymes to non-sugar

components and improves enzyme effectiveness through increasing the specific accessibility of fungal enzymes to glucan.[18–20]

CELF pretreatment can realize this advantage through removing most of biomass lignin and hemicellulose to produce pretreated solids with high glucan levels that can enhance ethanol titers at high solids loadings. Figure 1 shows the conditions that achieved the highest sugar yields from CELF pretreatment of poplar. The solids recovered post pretreatment contained 89% glucan, 3% xylan, and 5% lignin, Furthermore, close to 96% of the glucan in raw poplar was recovered in CELF pretreated solids, while the rest of the glucan and 60% of the xylan (C5 sugars) was recovered as soluble sugars in the CELF liquid fraction. In addition, 89% of the lignin extracted from poplar was recovered from the liquid hydrolysate as a highly pure lignin powder amenable to valorization. In prior studies, incorporation of CELF lignin from poplar into polyurethanes resulted in high strength and good mechanical properties.[21] These pretreatment conditions have been reported previously to be optimal for total sugar digestibility when CELF pretreatment of poplar was combined with consolidated bioprocessing (CBP) using *Clostridium thermocellum*.[22]

Celf Pretreated Poplar Solids Are Highly Digestible At A Low Enzyme Dosages

Fungal cellulolytic enzymes have been estimated to cost up to $1.20/gal ethanol produced, too expensive for commercial 2nd generation biorefining. [21] Thus, significantly reducing enzyme loadings while still achieving high yields from SSF is paramount. [23] In addition, enzyme loadings must be increased even more for high solids SSF to overcome end-product inhibition and enzyme deactivation by non-productive binding.[24–26] Therefore, this study focused on determining how increasing solids loadings impacted enzymatic digestion of glucan in CELF pretreated poplar solids. Accordingly, CELF solids prepared at the conditions shown in Fig. 1 with the corresponding compositions was enzymatically hydrolyzed with Cellic® CTec2 enzyme over a range of protein loadings initially at a 1 wt% glucan loading to avoid end-product inhibition of enzymes confounding determination of ultimate glucan digestibility. Glucan digestibility results, presented in Fig. 2, show that the rate of sugar release was highest at 65 mg protein per g glucan in raw biomass followed by 30 mg protein per g glucan. However, 96% of the glucan in the solids was hydrolyzed to glucose in just 24 h at a lower enzyme dose of 15 mg protein per g glucan. Furthermore, almost all the glucan was converted in 4 days by 7.5 mg protein and in 8 days at an even lower enzyme dose of 5 mg protein per g glucan in raw poplar.

Fractal Kinetic Modeling Provided Insights Into The Effect Of Substrate-cellulase Interactions On Enzyme Performance

Cellulases must first attach to insoluble crystalline cellulose chains that are then hydrolyzed by processive action of the enzyme along the cellulose. The following fractal kinetics model was applied to the glucan hydrolysis results to provide insights into substrate-enzyme interactions at different enzyme loadings:

$\frac{dC}{dt} = -k_t C$, where $k_t = kt^{-h}$

In which k is the instantaneous rate constant, k_t is a time independent rate coefficient, h reflects the rate decay over time, and correlation of k_t/k with glucan conversion shows the effect of a particular enzyme dose on enzymatic hydrolysis rates. A high "k_t/k" and low "h" indicate more effective enzyme-substrate interaction and faster hydrolysis rates.[27] Fig. 3 shows how k_t/k values change over the course of hydrolysis for different enzyme doses. For the highest enzyme loading of 30 mg protein per g glucan in raw Poplar, the correlation value of 1 indicates that the initial rate of reaction is not limited by diffusion. The drop in initial values as enzyme loadings drop in order of 15, 7.5, and 5 mg per g glucan show a proportional relationship between enzyme dose and initial reaction rate. However, the steep drop in k_t/k values with increasing glucan conversion at the highest enzyme dose compared to that for lower enzyme loadings could result from a greater ratio of enzyme to remaining free active sites on the cellulose crowding the active sites and interfering with hydrolysis. The correlation values at the lower enzyme loadings drop more slowly, apparently because hydrolysis is consistently more limited by the slow processive action of the cellulase along the active sites of the cellulose chain through the course of saccharification.

The h values have been attributed to structural characteristics and cellulose accessibility to the enzyme. Furthermore, lower h values have been interpreted to indicate greater substrate-enzyme interaction and a higher rate of reaction.[27] An interesting observation from Fig. 4 is that although the rate constant increased linearly with enzyme loading, the fractal exponent h could be fit by a parabolic polynomial that first dropped with increasing enzyme dose and then drastically increased. The high h value at the lowest enzyme loading suggests low substrate-enzyme interaction, possibly due to cellulase inhibition by sugars released or slow enzyme movement from one active site to another. The dropping trend of h values with increasing enzyme loading up to 15 mg protein per g glucan indicated improved glucan hydrolysis and better substrate-enzyme interactions in the presence of more cellulase. The dramatic increase in h at 30 mg protein per g glucan result from a "jamming effect" of the cellulose binding domain due to the high amount of active enzyme competing for the few remaining cellulose active sites, especially towards the end of the hydrolysis. Combining the information from Figs. 2, 3, and 4 shows that higher enzyme loadings improved hydrolysis rates but did not necessarily improve hydrolysis effectiveness or efficiency. Moreover, a possible jamming effect near hydrolysis completion indicates enzyme activity was sustained throughout hydrolysis, possibly as a result of the low lignin content in the solids reducing unproductive cellulase binding.

Prior fractal kinetics models fit to data from enzymatic hydrolysis of the glucan in solids produced by CELF and Dilute Acid (DA) pretreatments of corn stover led Nguyen et.al. to hypothesize that extensive delignification by CELF pretreatment reduced unproductive cellulase-lignin binding and thereby kept hydrolysis rates nearly constant throughout saccharification.[28] Similarly, the fractal kinetic results reported here show that solids produced by CELF pretreatment of Poplar exhibit such good substrate-enzyme reactivity that glucan can be hydrolyzed completely without loading more than 15 mg enzyme protein per g glucan. Based on the enzymatic saccharification data in Fig. 2 and fractal kinetic model parameters shown in Figs. 3 and 4, an enzyme loading of 15 mg protein per g glucan in raw Poplar was selected for SSF at high solids loadings.

Ethanol tolerance of Saccharomyces cerevisiae D5A constrains SSF ethanol yields at higher solids loadings

Because high ethanol concentrations in the fermentation broth negatively impacts cell viability, growth, and metabolism[29], ethanol tolerance of fermentative microorganisms limit the titers and therefore yields as SSF solids loading are increased. In order to estimate the ethanol tolerance of the D5A *Saccharomyces cerevisiae* variant used in this study, control fermentations were conducted at glucose concentrations of 180 and 200 g/L at 37°C. As shown in Fig. 5, D5A reached an ethanol titer close to 87 g/L from 180 g/L of glucose, a yield of 90% of theoretical, in about 48 hours. However, for 200 g/L of glucose, the titer and theoretical ethanol yield dropped to 81 g/L and 78%, respectively, with unfermented glucose left in solution. This result indicated that the maximum ethanol titer using D5A was close to 87 g/L.

Ethanol titers from high solids SSF of CELF pretreated Poplar solids were similar to those from pure glucose fermentations

To mix up to 20 wt% solids in shake flasks, a press driven by a hydraulic jack was used to reduce the moisture content in CELF pretreated solids from 72 to 61% (see Materials and Methods). SSF at a Cellic® Ctec2 cellulase loading of 15 mg protein per g glucan in raw Poplar was then applied to 13, 17, and 20 wt% loadings of these CELF solids to give glucan loadings of 11, 15, and 18 wt%, respectively. As shown in Fig. 6, the result was ethanol titers of 60 g/L, 78 g/L, and 87 g/L in just 7 days with no to little residual sugars left in the broth. These yields corresponded to theoretical ethanol yields of 87%, 84%, and 79%, respectively, from the increasing progression in glucan loadings. Figure 6 (a) shows that at the lowest solid loading of 13 wt%, glucan was completely hydrolyzed and all the sugars were totally consumed in 7 days. Almost complete hydrolysis of glucan was also observed for the next higher solid loading of 17 wt% in 10 days, but now glucose began to accumulate on day 8, at which point ethanol production slowed down substantially, as shown in Fig. 6 (b). At the highest solid loading of 20 wt%, Fig. 6 (c) shows that close to 92% of the glucan was completely hydrolyzed in 10 days. However, the ethanol concentration that could have reached up to 94 g/L (at 90% conversion of all the available sugars to ethanol) plateaued at 87 g/L at 6 days despite continued glucose release, consistent with pure glucose fermentations also stopping at this titer.

Achieving greater than 50 g/L ethanol titers while sustaining high yields at low enzyme loadings reduces the cost of biological processing of cellulosic biomass to ethanol.[24] The results reported here show that high saccharification extents can be achieved for enzymatic hydrolysis of poplar at 13, 17, and 20 wt% loadings of CELF pretreated solids. This outcome is distinctive from results for solids produced by other pretreatments of cellulosic biomass.[30] However, even though high conversion of glucan in CELF solids was achieved for all solids levels, ethanol concentrations were limited to about 87 g/L, beyond which glucose accumulated. The latter result points towards ethanol toxicity being the roadblock to attaining higher ethanol yields with the D5A yeast employed. Hence, ethanol yields from SSF of CELF solids were not limited by substrate digestibility but by yeast strain ethanol tolerance. Thus, a higher yield is likely if future research could improve the ethanol tolerance of the fermentative organism employed in an SSF environment.

Conclusions

Application of a fractal kinetic model to saccharification data revealed that although increasing enzyme loadings for SSF of CELF pretreated Poplar might enhance saccharification rates, it would not improve enzyme effectiveness or substrate-enzyme interactions. Based on fractal model insights, a Cellic® CTec2 cellulase loading of 15 mg protein per g glucan in raw poplar was applied in combination with a D5A yeast strain for SSF of 20 wt% CELF pretreated poplar solids to achieve an ethanol titer of 87 g/L in 6 days, This ethanol concentration is nearly the same as the 7 day 86 g/L ethanol titer reached from a 23 wt% solids loading of CELF pretreated corn stover, a far less recalcitrant material, using Accellerase® 1500 cellulase at a dose of 10 mg protein per g glucan in raw corn-stover in combination with the same D5A strain.[24] However, although CELF pretreated poplar solids were almost completely hydrolyzed at this high solids loading, the leveling off of the ethanol concentration at 87 g/L while glucose continued to accumulate showed that D5A's ethanol tolerance and not the enzymatic digestibility of CELF solids limited ethanol concentrations and yields. Overall, this study shows that in addition to CELF removal of most of the hemicellulose and lignin from poplar achieving highly digestible glucan, the resulting enhanced glucan content at limiting solids loadings results in higher titers than realized for solids with lower glucan content from other pretreatments. However, it also points out the need for fermentative organisms with greater tolerance to ethanol and other SSF stress factors if higher ethanol concentrations are to be reached by SSF.

Materials And Methods

Populus trichocarpa woody biomass used from this study was generously provided through the BioEnergy Science Center (BESC) from Pacific Northwest National Laboratory. The composition of the raw Poplar as determined by following NREL LAP (version 08-03-2012) was 47.0% glucan, 16.9% xylan, and 21.2% acid-insoluble lignin.[31] The biomass was air-dried and knife milled using a laboratory mill (Model 4, Arthur H. Thomas Company, Philadelphia, PA) to pass through a 1 mm internal sieve size. Novozymes® generously provided Cellic® Ctec 2 cellulase with a protein content estimated using a

Pierce BCA analysis kit of 250 mg/ml. The National Renewable Energy Laboratory (NREL) generously provided the D5A yeast strain, a *Saccharomyces cerevisiae* variant.

CELF Pretreatment: Prior to CELF pretreatment, milled Poplar wood chips were soaked overnight at 4°C in a 1:1 ratio (weight basis) of THF to water containing 0.5 wt% H_2SO_4. A dry biomass loading of 7.5 wt% of the total working mass was CELF pretreated in a 1 L Hastelloy Parr autoclave reactor (236HC Series, Parr Instruments Co., Moline, IL) equipped with a double stacked pitch blade impeller rotating at 200 rpm. A series of pretreatments were carried out at 160°C for 15 minutes, i.e., conditions that resulted in maximum sugar recovery for CELF followed by enzymatic hydrolysis (not published). Temperature inside the reactor was measured by an in-line thermocouple (Omega, K-type), and all reactions were maintained within ± 2°C using a 4 kW fluidized sand bath (Model SBL-2D, Techne, Princeton, NJ). At the end of each pretreatment, the reactor was cooled by submerging quickly in a large room temperature water bath. The solids were then separated from the liquor by vacuum filtration at room temperature through glass fiber filter paper (Fisher Scientific, Pittsburgh, PA). The mass and density of the liquid fractions were measured to calculate yields and close mass balances. The solids collected were washed with water until clear water ran through the solids which were then hydraulically pressed to reduce the moisture content to 61%.

Enzymatic hydrolysis of CELF pretreated Poplar: Batch enzymatic hydrolysis was performed by following the standard NREL protocol[32] using 125 mL flasks with a total working volume of 50 mL. For each, CELF pretreated biomass was loaded to a 1 wt%, glucan loading. In addition, 50 mM of citrate buffer (pH 4.5) along with millipore water and 0.02% sodium azide as an antimicrobial agent were added to 50 mL to reach a final pH of 4.8. Triplicates were run with the appropriate amount of substrate. The flasks were placed in a 50°C incubator shaker (Infors HT Multitron, MD) and allowed to equilibrate for 1 h at 150 rpm. Appropriate amounts of Cellic® Ctec2 enzyme were added to the flasks at target protein loadings, and the flasks were then returned to the incubator shaker. 1 mL samples were taken at the times reported, centrifuged (Centrifuge 5424, Eppendorf, Germany) at 15000 rpm for 10 min, diluted, and analyzed for sugar concentration in the broth.

Fractal modeling of enzymatic hydrolysis kinetics: The following fractal model based on first order breakdown of glucan to form glucose with a rate coefficient k_t that varied with time raised to the -h power was used to describe cellulose hydrolysis over hydrolysis time:

$\frac{dC}{dt} = -k_t C$, where $k_t = kt^{-h}$ 1

The k, k_t, and h parameters in Eq. 1 were fit to enzymatic hydrolysis data by MATLAB.

Seed inoculum preparation: *Saccharomyces cerevisiae* (D5A) yeast were grown in 10 mg/mL of yeast extract (Becton, Dickinson and Company, Redlands, CA), 20 mg/mL of peptone (Becton, Dickinson and Company, Redlands CA), and 50 mg/mL of glucose to reach exponential growth and then stored in glycerol (~ 14 wt%). When needed, the frozen stock was thawed and grown overnight in 10 mg/mL yeast extract, 20 mg/mL peptone, and 50 mg/mL glucose in a 250 mL baffled flask in a 37°C, 130 rpm shaking

incubator (Infors HT Multitron, MD). The inoculum was then centrifuged and resuspended in sterile deionized (DI) water to reach an optical density (O.D.) of 0.5 as determined by (SpectraMax ABS Plus, Molecular Devices, CA) at 600 nm.

Pure sugar fermentations and growth: Pure sugar fermentations by D5A were carried out in 125 mL flasks at glucose concentrations specified in the Results and Discussion section. The appropriate glucose amount was dissolved in Millipore water (Milli-Q, EMD Millipore, Darmstadt, Germany) and added to a flask and bubble trap assembly. Duplicates and a substrate blank were sterilized at 121°C for 35 min in an autoclave (HA-300MII, Hirayama Manufacturing Corporation, Japan) and cooled in a laminar flow hood (Baker and Baker Ruskinn, Sanford, ME) to prevent contamination followed by adding water to adjust for evaporative losses. 50 mM citrate buffer (pH 4.5) was added to reach a final pH of 4.8 in 50 mL, and 40 mg/L of tetracycline along with the seed inoculum were used in 48 h fermentations shaking at 130 rpm and 37°C. 0.75 mL samples were taken every 2 h until the stationary phase was reached. These samples were centrifuged at 15000 rpm for 10 min, diluted, and analyzed for ethanol and sugar concentrations.

Simultaneous Saccharification and Fermentation (SSF): Batch SSF experiments were performed in 125 mL flasks with a total working volume of 25 mL containing CELF pretreated biomass corresponding to desired glucan loadings to which were added 50 mM citrate buffer (pH 4.5) to reach a final pH of 4.8 in 25 mL followed by 40 mg/L tetracycline (Sigma Aldrich, St. Louis, MO) as an antimicrobial agent. Cellic® Ctec2 cocktail was loaded at 15 mg-protein per g-glucan-in-raw poplar, and the yeast inoculum was added next. Flasks with attached bubble traps were loaded with Millipore water and the appropriate amount of substrate. Duplicates with substrate as well as substrate blanks were sterilized at 121°C for 35 min in an autoclave (HA-300MII, Hirayama Manufacturing Corporation, Japan). Next, the flasks were cooled in a laminar flow hood (Baker and Baker Ruskinn, Sanford, ME) to prevent contamination and reweighed to determine how much water to add to restore the intended solids loading. After adding buffer, antimicrobial agent, enzyme cocktail, and yeast inoculum, SSF was carried out for 10 days at 37°C at 130 rpm in an incubator shaker (Infors HT Multitron, MD). 1 mL samples were taken periodically, centrifuged at 15,000 rpm for 10 min, diluted, and analyzed for sugar and metabolite concentrations in the liquid.

Measuring sugar and ethanol concentrations: Liquid samples along with appropriate calibration standards were analyzed by HPLC (Waters Alliance 2695 system, Waters, Milford MA) equipped with a Bio-Rad Aminex® HPX-87H column (BIO-RAD, Hercules CA) and Waters 2414 RI detector with a 5 mM sulfuric acid eluent flow rate of 0.6 ml min^{-1}. Chromatograms were integrated by the Empower® 2 software package (Waters Co., Milford MA).

Model equations: Percent glucan conversion to glucose via enzymatic hydrolysis of a 1 wt% glucan loading was calculated as follows:

$$Percentglucanconversiontoglucose = \frac{C_{Glucose} \times 0.9 \times WV}{M_G} \times 100 \quad 2$$

In which,

$C_{Glucose}$ is the concentration of glucose in the enzymatic hydrolysis liquid at a given time, mg/ml,

WV is the working volume in the flask, ml (50 ml),

M_G is the mass of glucan initially added, g.

At lower solid loadings, i.e., < 5 wt%, the density of the solvent phase was assumed to be the same as the density of water. As the insoluble solid fraction increased, the density of the liquid fraction first increased due to increasing sugar concentrations and then dropped slightly due to increasing ethanol concentration. The fluid density was measured directly.

The ethanol yield as a percent of the theoretical maximum was calculated as follows:

$$Percent\ Theoretical\ Ethanol\ Yield = {(C_{Eth} \times V_L \times 0.9 \times 100)}/{(0.511 \times M_G)} \quad 3$$

with

$$V_L = (M_W + M_{DS})/\rho \quad 4$$

$$V_L = (M_W + V_W \times (C_{Eth} + C_{Glucose} + C_{Glycerol} + etc.))/\rho \quad 5$$

In which C_{Eth} is the ethanol concentration in the fermentation broth, mg/ml,

$C_{Glucose}$ is the ethanol concentration in the fermentation broth, mg/ml,

$C_{Glycerol}$ is the ethanol concentration in the fermentation broth, mg/ml,

V_L is the volume of liquid fraction in the fermentation medium, ml,

M_G is the mass of glucan initially added, g,

M_W is the mass of water initially added, g,

M_{DS} is the mass of dissolved solids in the fermentation medium at any given time point, g, and

ρ is the density of the medium, g/ml.

Declarations

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Figures

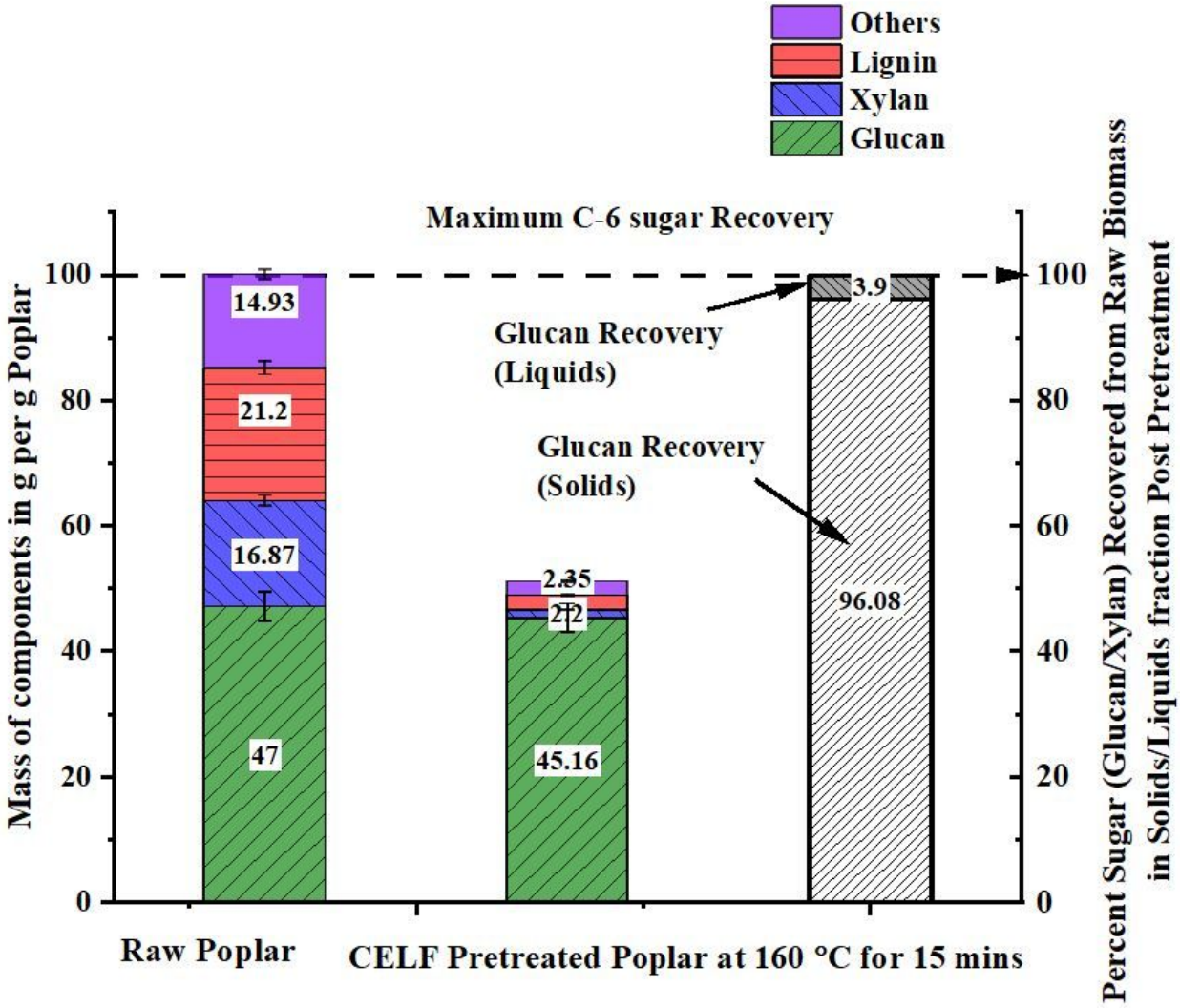

Figure 1

Compositional analysis of raw and CELF pretreated Poplar solids and glucan recovered in solid and liquid fractions post pretreatment at 160 °C and 15 min.

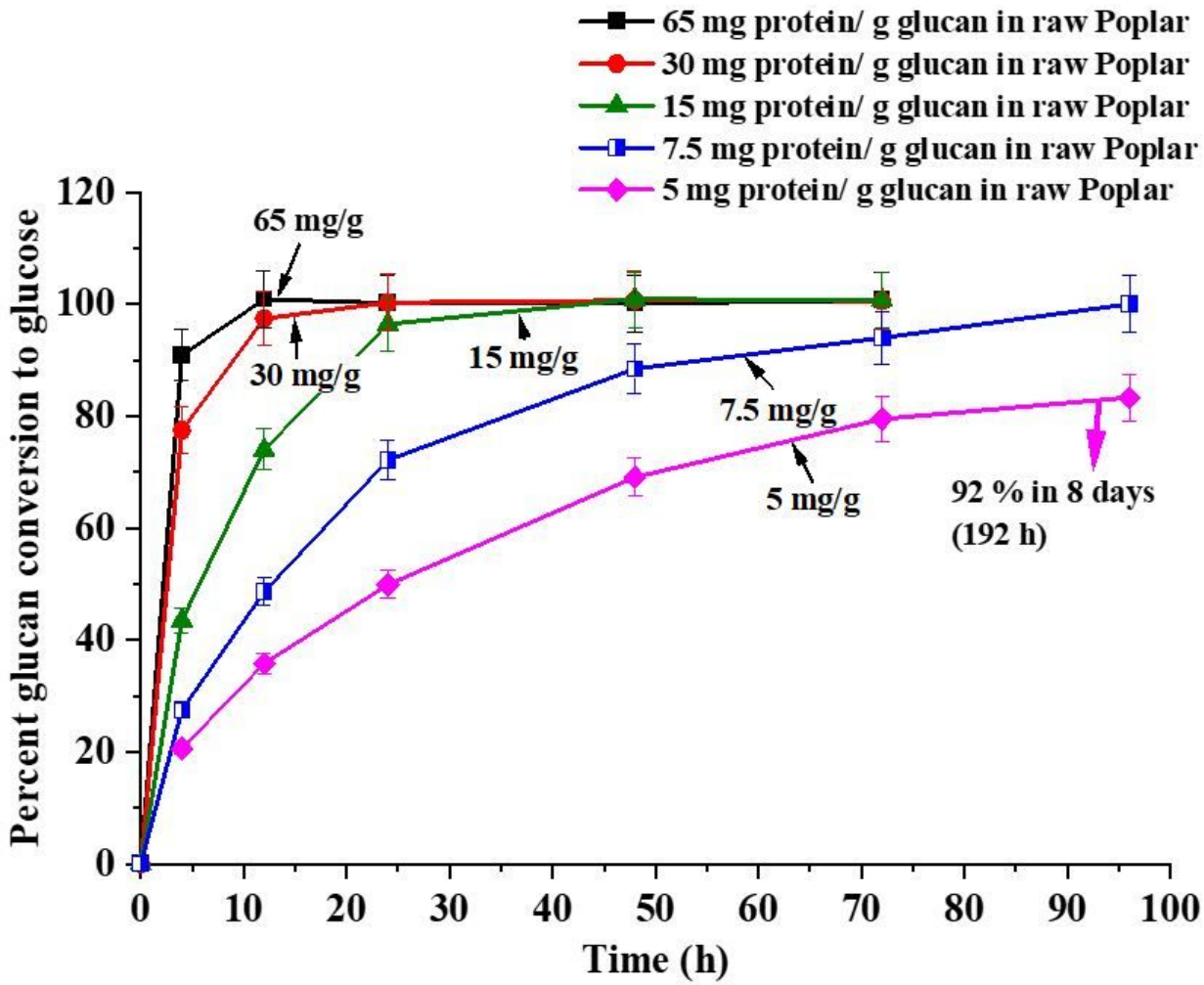

Figure 2

Glucan hydrolysis by Cellic® Ctec2 cellulase over a range of enzyme loadings (mg protein per g glucan in raw Poplar) of a 1 wt% glucan loading of Poplar solids resulting from CELF pretreatment at 160 °C for 15 min.

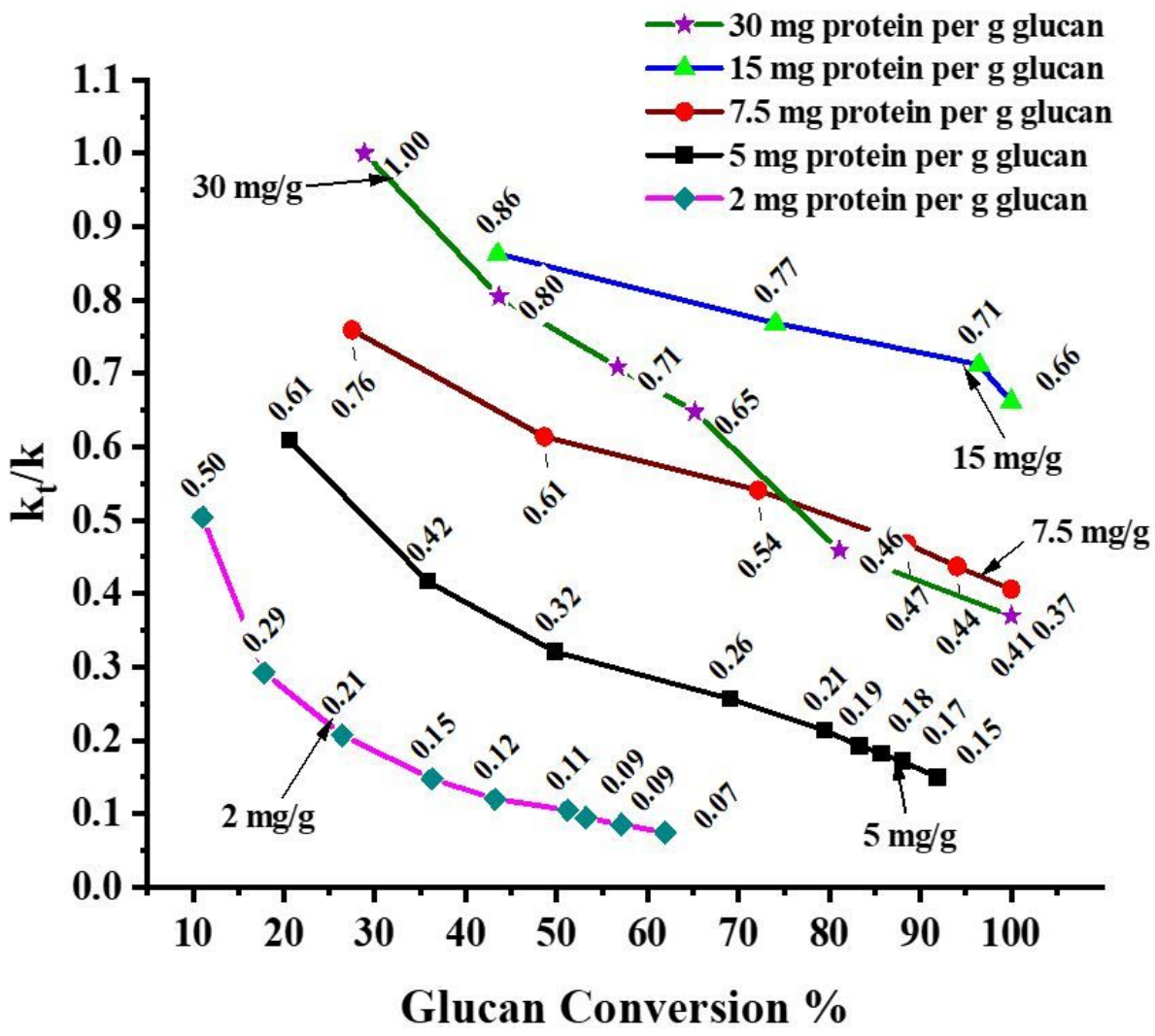

Figure 3

Change in fractal kinetic kt/k correlation value with glucan conversion for enzymatic saccharification of CELF pretreated Poplar at enzyme loadings of 2, 5, 7.5, 15, and 30 mg protein/g glucan in raw poplar.

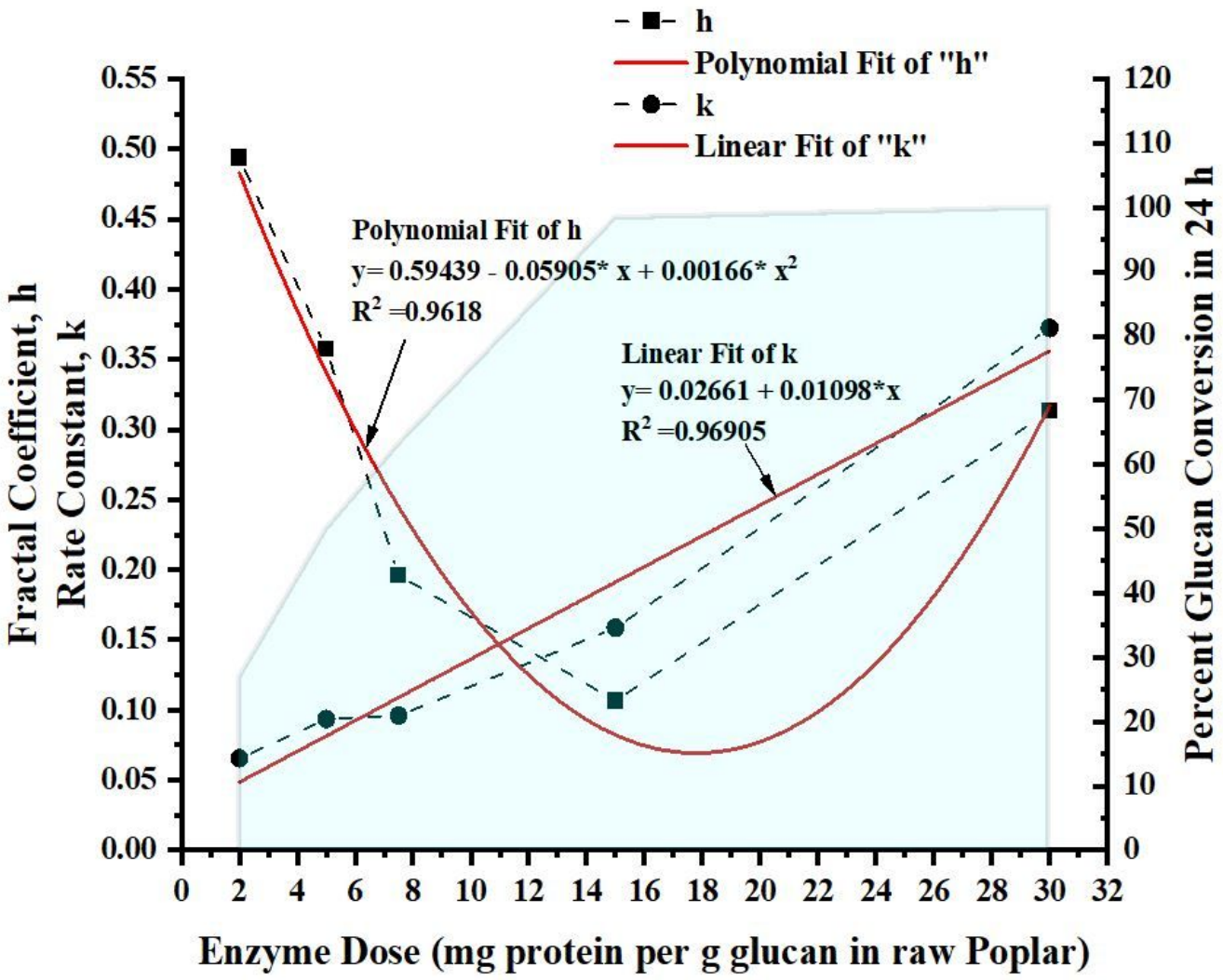

Figure 4

Effect of enzyme loadings on fractal exponent h and rate constant k for enzymatic hydrolysis of CELF poplar solids at a glucan loading of 1 wt%. The shaded area indicates the percent glucan conversion achieved in 24 h.

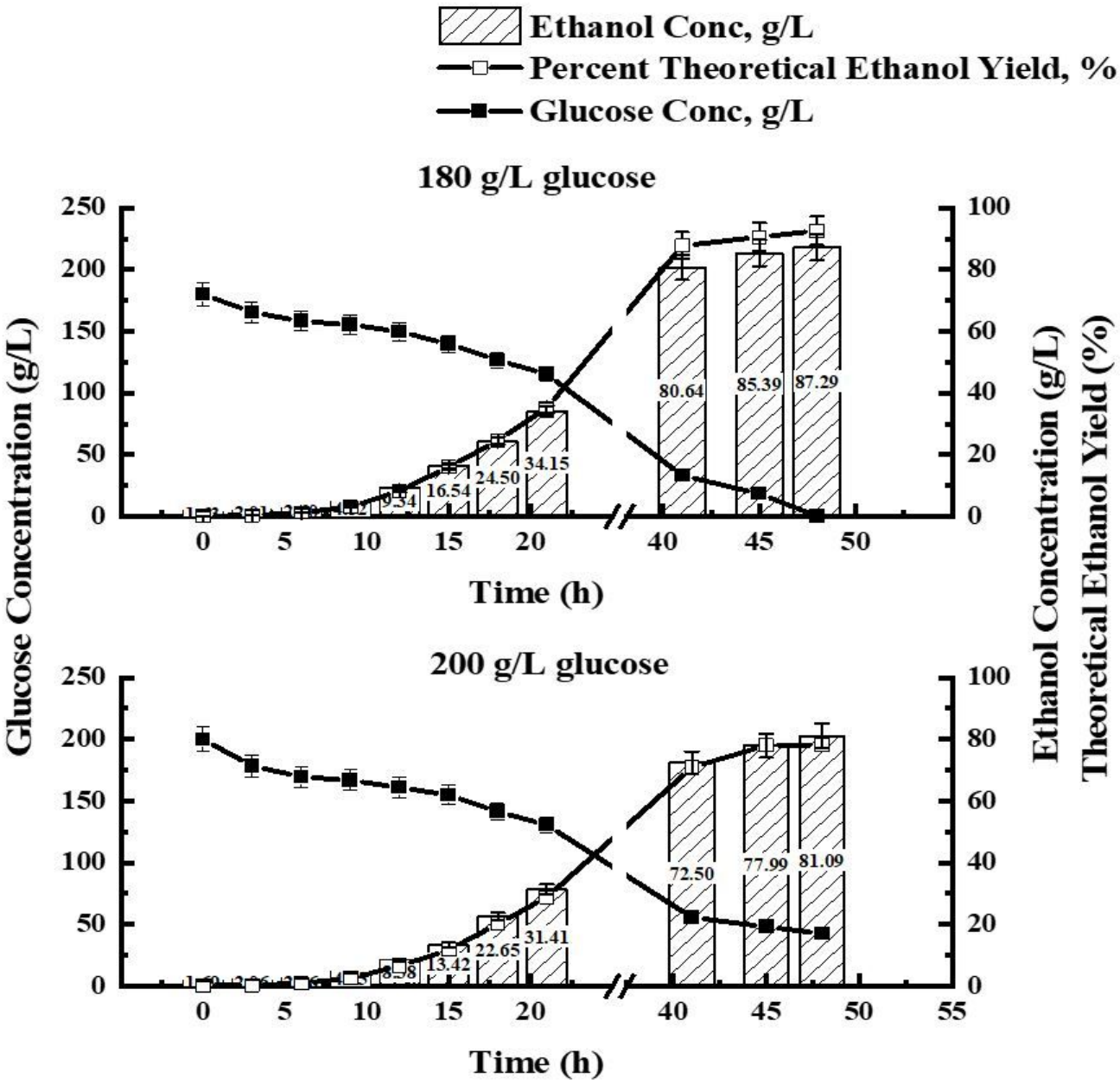

Figure 5

Glucose consumption, ethanol titers, and theoretical ethanol yields for fermentations of 180 g/L and 200 g/L glucose by S. cerevisiae variant D5A at 37 °C.

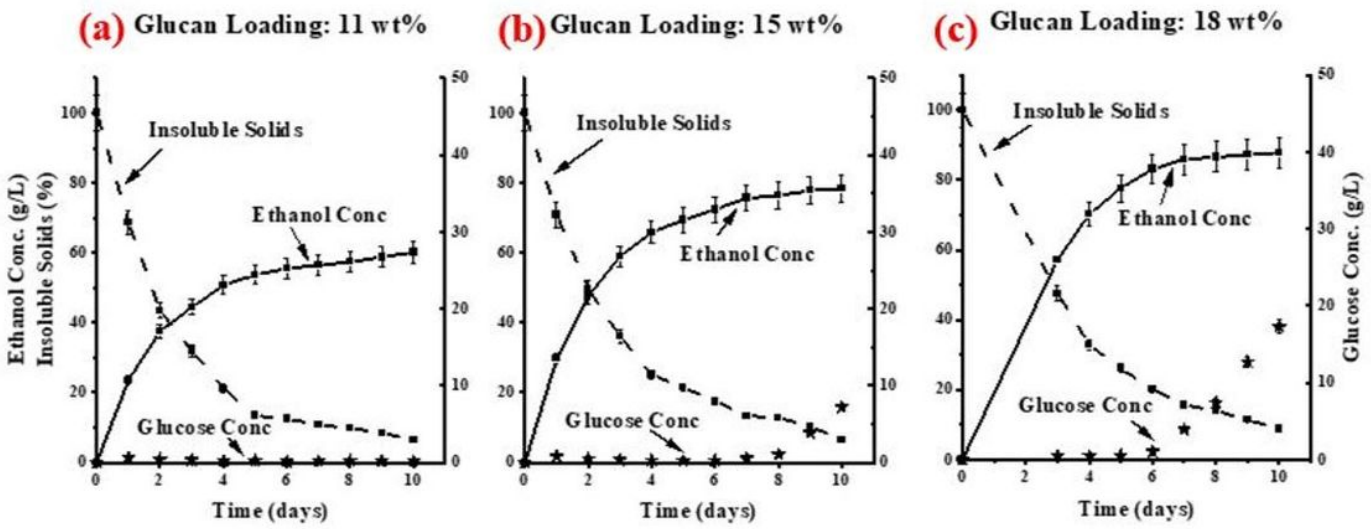

Figure 6

Time profiles of ethanol concentration, glucose concentration, and percent insoluble solids remaining for SSF of 13, 17, and 20 wt% CELF pretreated Poplar solids loadings corresponding to 11, 15, and 18 wt% glucan loadings, respectively. SSFs were conducted at a Cellic® CTec2 enzyme loading of 15 mg protein per g glucan in raw Poplar with fermentations by the D5A strain of S. cerevisiae yeast.