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Oxygen Transfer in High Solids Loading and Highly Viscous Lignocellulose Hydrolysates

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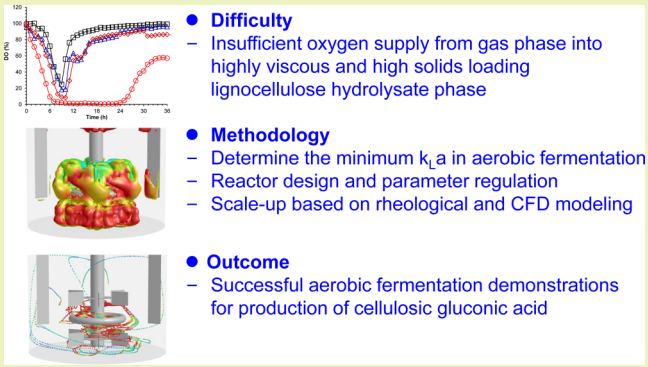
ABSTRACT: High product concentration in aerobic biorefining fermentation requires high lignocellulose feedstock loading. However, the high solids content and the consequent high viscosity of the lignocellulose hydrolysate severely limit oxygen transfer and thus lead to low aerobic fermentation rate. This study first investigates the experimental measurement and the computational fluid dynamics (CFD)-based simulation of oxygen transfer properties in the high solids loading and highly viscous lignocellulose hydrolysates. The oxygen mass transfer coefficients k_La values vary with various biorefining processing parameters. A minimum threshold k_La value was required for aerobic fermentation of glucose oxidation into gluconic acid. The rheological properties of the slurry were experimentally determined, and a CFD model was established for the design of aerobic fermentation bioreactors. The k_La values between the CFD calculation and the experimental determination were in good agreement for the high solids loading and highly viscous hydrolysate slurry. The study provided important information and useful tools for achieving efficient aerobic fermentations in high solids loading and highly viscous lignocellulose hydrolysates. The measured k_La value could be applied to general aerobic fermentation using lignocellulose feedstock.



KEYWORDS: *Oxygen transfer rate, Lignocellulose, Aerobic fermentation, High solids loading, High viscosity, Computational fluid dynamics (CFD)*

INTRODUCTION

High product concentration is crucially important for reducing the downstream recovery cost of fermentation processes.[1,2] In lignocellulose biorefining, the high lignocellulose feedstock loading in enzymatic hydrolysis and fermentation steps is the prerequisite condition for achieving the high product concentration. When anaerobic fermentation is operated in the high solids loading hydrolysate for production of ethanol by *Saccharomyces cerevisiae* strains or lactic acid by lactic acid bacterium strains, a good mixing condition is required for the hydrolysate slurry of the high lignocellulose solids with the low enzyme solution and microbial seed broth.[3−5] When aerobic fermentation is operated for production of lipids by oleaginous yeasts or citric acid by *Aspergillus niger* or amino acids by *Corynebacterium glutamicum*, both the good mixing and the high oxygen transfer rate from the gas (air) phase to the liquid (hydrolysate) phase are required. When oxygen is also a substrate of oxidative conversions in aerobic fermentations such as the fermentation of glucose oxidation to gluconic acid by *Gluconobactor oxidans*[6] or *Aspergillus niger*,[7] the oxygen transfer requirement is further enhanced and oxygen supply becomes a determinant factor for achieving high product titer and yield.

In the high solids loading and highly viscous lignocellulose hydrolysates, fine air bubbles tend to aggregate into larger bubbles and quickly escape from the hydrolysate slurry.[8−10] The aggregation and escape of the bubbles decrease the overall gas holdup, the gas−liquid interfacial area, and the interface mobility of the high viscosity and solids loading hydrolysate slurry.[11] These phenomena correspondingly reduce the oxygen transfer rate and cell metabolism activity.[12,13] One way to improve the oxygen transfer property is to remove the solid particles from the hydrolysate slurry.[14,15] However, the solid/liquid separation for a highly viscous hydrolysate has been proved to be a tough operation: Centrifugation is not practical in industrial operation due to the very high solids content of the hydrolysate, and filtration is extremely difficult to perform because of the very high viscosity.[16,17] Furthermore, heavy loss of fermentable sugars and high electricity input are inevitable if solid/liquid separation is performed. In fact, at least a partial reason for the simultaneous saccharification and fermentation (SSF) operation in biorefining fermentations is due to the

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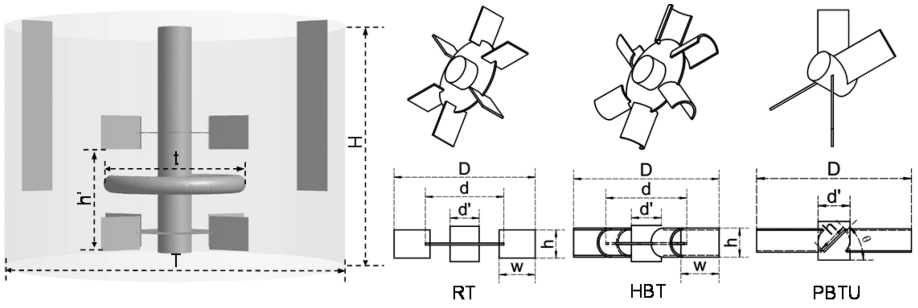

Parameters	Values	Parameters	Values
Overall size (L)	5	Impeller clearance, h' (mm)	48
Reactor height (mm)	300	Impeller diameter, D (mm)	80
Reactor diameter, T (mm)	164	Impeller width, w (mm)	21
Liquid height, H (mm)	115	Impeller height, h (mm)	16
Baffle width (mm)	13	Impeller lean angle, θ (degree)	45
Baffle height (mm)	78	Ring sparger diameter, t (mm)	69
Number of baffles	3	Sparger orifice diameter (mm)	1
Number of impellers	2	Sparger orifice count	30

Figure 1. Schematic geometry of the bioreactors equipped with different impellers including RT, HBT, and PBTU.

difficulty of and heavy loss of sugars from solid/liquid separation; therefore, the solid particles containing hydrolysate slurry directly go to fermentations without solid/liquid separation.

Although the hydrolysate slurry without solid/liquid separation is almost the only feedstock option for practical fermentations, the oxygen transfer properties of the high solids loading and highly viscous hydrolysates had not been well characterized to date. Mussatto and Roberto[17] reported an oxygen transfer result of rice straw hydrolysate, but the all solids particles were removed and the hydrolysate slurry became a clear supernatant. Sreenath et al.[18] reported the oxygen transfer properties of alfalfa fiber hydrolysate slurry, but the solids content was only 10% (w/w). Liu et al.[19] reported the varying dissolved oxygen level of corn stover hydrolysate and the consequent lipid fermentation, but the solids content was also as low as 10% (w/w).

This study first investigates the oxygen transfer property of the high solids loading and highly viscous lignocellulose hydrolysate in a typical aerobic fermentation of gluconic acid. In this aerobic fermentation, oxygen is required by both the cell growth by the fermenting strain *Gluconobacter oxidans* and the oxidation reaction of glucose into gluconic acid. The gas−liquid oxygen transfer coefficients k_La values of the high solids loading and highly viscous hydrolysate slurry were experimentally measured. The oxygen transfer rate could be adjusted by regulating the hydrolysate time and agitation rate to meet the needs of gluconic acid fermentation. The rheological properties of the slurry were also experimentally determined, and the computational fluid dynamics (CFD) mode was established to calculate the oxygen transfer rate and air holdup. A validated CFD model was applied to design the aerobic fermentation bioreactor. The study provided important information and useful tools for achieving efficient aerobic fermentation in highly viscous lignocellulose hydrolysate slurry.

MATERIALS AND METHODS

Feedstocks. Corn stover (CS) was harvested in Nanyang, Henan, China in fall 2014. After collection, the biomass feedstock was milled coarsely using a hammer crusher and screened through a mesh with a circle diameter of 10 mm. The milled CS was washed to remove field dirt, sands, and metal pieces and then dried to avoid decaying in the one year storage period. The raw CS contained 32.1% cellulose, 20.6% hemicellulose, 26.5% lignin, and 4.4% ash on dry weight base (w/w) measured by a Cellulose Analyzer 220 (Ankom Technology, Macedon, NY, USA).

Enzymes and Chemicals. Cellulase enzyme LLC was purchased from Vland Biotech, Qingdao, China. The filter paper activity was 199 FPU/mL, and the cellobiase activity was 5514 CBU/mL determined according to the NREL protocol LAP-006[20] and Ghose,[21] respectively. The protein concentration was 76 mg/mL of cellulase solution determined by the Bradford method[22] using BSA as the protein standard.

Pure sodium gluconate was purchased from Sigma-Aldrich, St. Louis, MO, USA. D-(+)-Glucono-δ-lactone was purchased from Aladdin Reagents Co., Shanghai, China. Yeast extract was purchased from Angel Yeast Co., Yichang, Hubei, China. Agar was purchased from Biosharp Co., Shanghai, China. All other chemicals were purchased from the local supplier Linfeng Chemical Reagent Co., Shanghai, China.

Strains and Medium. *Gluconobacter oxidans* DSM 2003 was obtained from German Collection of Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany. The culture medium used for *G. oxidans* DSM 2003 included (1) an activation medium containing 40 g of sorbitol, 10 g of yeast extract, 1.5 g of KH_2PO_4, 1.5 g of $(NH_4)_2SO_4$, 0.5 g of $MgSO_4{\cdot}7H_2O$, and 20 g of agar in 1 L of deionized water and (2) a seed medium containing 80 g of sorbitol, 10 g of yeast extract, 1.5 g of KH_2PO_4, 1.5 g of $(NH_4)_2SO_4$, and 0.5 g of $MgSO_4{\cdot}7H_2O$ in 1 L of deionized water.

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Biodetoxification fungus *Amorphotheca resinae* ZN1 was isolated in our previous work[23] and stored in the China General Microorganism Collection Center (CGMCC), Beijing, China with the registration number 7452. *A. resinae* ZN1 was stored at 4 °C on potato dextrose agar (PDA) slants (200 g/L of potato juice, 20 g/L of glucose, 20 g/L of agar). The PDA medium was prepared by boiling cleaned, peeled, and sliced potatoes in 1 L of deionized water for 30 min.

Pretreatment, Biodetoxification, and Hydrolysis Operations. CS was pretreated by a dry sulfuric acid pretreatment method according to Zhang et al.[24] and He et al.[25] Briefly, dry CS and a dilute sulfuric acid solution at 5% (w/w) were cocurrently fed into the reactor at a solid/liquid ratio of 2:1 (w/w) with helically stirring mixing and then pretreated at 175 °C for 5 min. The pretreated CS contained approximately 50% (w/w) of dry solid matter, and no free wastewater stream was generated. The pretreated CS contained 35.7% cellulose and 2.4% hemicellulose measured by a two-step acid hydrolysis method according to NREL protocols.[26,27]

The pretreated CS slurry was biodetoxified using *A. resinae* ZN1 according to Zhang et al.[23] Briefly, the pretreated CS was neutralized with 20% (w/w) $Ca(OH)_2$ to a pH value of 5−6 and then disk milled to remove the residual long cellulose fibers to avoid the blockage of pipelines and valves. The *A. resinae* ZN1 spore suspension with a spore number of 4−5 × 10^6 spores per mL was inoculated onto the pretreated CS and cultured at 28 °C. The solids with full growth of *A. resinae* ZN1 spores were inoculated at a 10% (w/w) ratio onto the pretreated CS solids and incubated at 28 °C until furfural and 5-hydromethylfurfural (HMF) were completely removed. No any fresh water and nutrients were added, and no wastewater stream was generated during biodetoxification. The detoxified CS contained approximately 50% (w/w) of dry solids.

The pretreated and detoxified CS was hydrolyzed in the hydrolysis bioreactor (5L) equipped with a helical ribbon impeller as described by Zhang et al.[4] using the cellulase dosage of 6 mg total protein per gram of dry solid matter (DM) at 50 °C for 12 or 48 h depending on the individual experimental design. The pH value was maintained at 4.8 with 5 M NaOH solution.

Gluconic Acid Fermentation. CS hydrolysate slurry was rapidly transferred from the hydrolysis bioreactor using a sterilized beaker to the fermentation bioreactor for gluconic acid fermentation. Three types of impellers were used in the fermentation bioreactors including Rushton turbines (RT), hollow blade turbines (HBT), and pitched-blade turbines up-pumping (PBTU). The schematic geometry of the bioreactors is shown in Figure 1. The RT impellers were used in the practical fermentation bioreactor, and the HBT and PBTU were only used in CFD calculation.

Two milliliters of the *G. oxidans* stock vial was directly inoculated into 20 mL of the seed medium and cultured at 30 °C for 15 h. Then, the seed broth was cocurrently inoculated at a 2% (v/v) inoculation ratio into the fermentation bioreactor containing 2.5 L of the hydrolysate slurry after 48 h hydrolysis (without solids removal) and fermented at 35 °C, pH 4.8, and 1.0 vvm of aeration rate for 36 h. The samples were withdrawn at regular intervals and centrifuged at 14,500 × g for 5 min.

Analysis of Sugars, Gluconic Acid, and Inhibitors. Glucose was analyzed using a SBA-40D biosensor (Shandong Academy of Sciences, Jinan, China). Gluconic acid was analyzed using HPLC (LC-20AT, UV/vis detector SPD-20A, Shimadzu, Kyoto, Japan) with an Aminex HPX-87H column (Biorad, Hercules, CA, USA) at 55 °C using a mobile phase of 5 mM H_2SO_4 at a rate of 0.4 mL/min. Xylose, furfural, 5-hydroxyfurfural, and acetic acid were analyzed using HPLC (LC-20AD, refractive index detector RID-10A, Shimadzu, Kyoto, Japan) with an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C using a mobile phase of 5 mM H_2SO_4 at a rate of 0.6 mL/min.

Measurement of Volumetric Oxygen Mass Transfer Coefficient k_La. The oxygen mass transfer rate in the CS hydrolysate was measured using the dynamic oxygen desorption method.[28] An oxygen electrode (InPro 6800, Mettler Toledo, Schwerzenbach, Switzerland) was used for measurement of the dissolved oxygen concentration (DO). The oxygen saturation point (100%) was set to the value at the fixed agitation rate and the given solids loading at the conditions of 35 °C and 1.0 vvm of aeration rate. Nitrogen was used to purge the oxygen from the hydrolysate below 5% of the saturation before the k_La measurement. Then, the air flow was re-established, and the dissolved oxygen concentration rapidly increased to saturation. The oxygen concentration was recorded every 10 s and lasted for 600−800 s. The measurement was duplicated at each solids loading from the high agitation rate decreasing and from the low agitation rate increasing.

The k_La value was calculated based on the oxygen mass balance in the hydrolysate slurry as shown in eq 1:

$$\frac{dC}{dt} = k_La(C^* - C) \tag{1}$$

where C^* is the saturation oxygen concentration (mol/L), and C is the oxygen concentration at the measurement point (mol/L).

Rheological Properties Measurement. The rheology of hydrolysate slurry was measured by a rotational viscometer (DV2T, model LV, spindle SC4−16, Brookfield Engineering Laboratories Inc., Middleboro, MA, USA). The apparent viscosity was obtained at a shear rate (γ) from 11.6 to 46.7 s^{-1} at 25 °C. The rheological properties were fitted into a power law model $\eta_a = K_p \times \gamma^{n-1}$, where K_p is the consistency coefficient (Pa s^n), and n is the dimensionless power law index. The linearized power law model is shown in eq 2, and the K_p and the n values were calculated by measuring the intercept and the slope of eq 2:

$$\log_{10}\eta_a = \log_{10}K_P + (n-1)\log_{10}\gamma \tag{2}$$

CFD Simulation. The commercial CFD software ICEM and Fluent (Version 14.5, Ansys, Inc., PA, USA) were used to generate the 3D grids of the reactor model and run calculations, respectively. The Eulerian multiphase model was employed for simulating gas−liquid mass transfer in an air−water−CS multiphase stirred tank bioreactor. The population balance model (PBM) was used to calculate the air bubble size distribution. The PBM model was solved using the discrete method with eight bubble groups from 0.5 to 13.0 mm for simulating bubble coalescence and breakup. The air sparger was taken as the inlet boundary conditions of gas velocity. The upper surface of the liquid was defined as the degassing boundary. Impellers, walls, and baffles were defined as the no-slip boundaries with the standard wall functions. The impeller rotation was characterized with the moving mesh method. The model was solved as implicit and pressure-based. Chemical reaction was negligent. The unsteady solve method was used, and the time step was set as 0.02 s. The volumetric mass transfer coefficient was calculated as the product of interfacial area α and liquid side mass transfer coefficient k_L, in which k_L was calculated using Bird's penetration equation:[29,30]

$$k_L = \frac{2}{\sqrt{3\pi}}\sqrt{\frac{D_L U_{slip}}{d_{32}}} \tag{3}$$

where D_L is the molecular diffusivity (m^2/s) of gas in liquid, and U_{slip} is the slip velocity (m/s) between gas and liquid.

The interfacial area α was the function of the Sauter mean diameter d_{32} and the local gas volume fraction α_G as shown in eq 4:

$$\alpha = \frac{6\alpha_G}{d_{32}} \tag{4}$$

RESULTS AND DISCUSSION

Measurement of Oxygen Transfer in High Solids Loading Lignocellulose Hydrolysate. The oxygen transfer coefficient k_La in the high CS loading hydrolysate was experimentally determined under different operation conditions (Figure 2). The prolonged hydrolysis time reduced the apparent viscosity, and the k_La values were increased correspondingly. The increased agitation rate enhanced the turbulence intensity, and the k_La values increased. The increased solids loading of the hydrolysate slurry led to the

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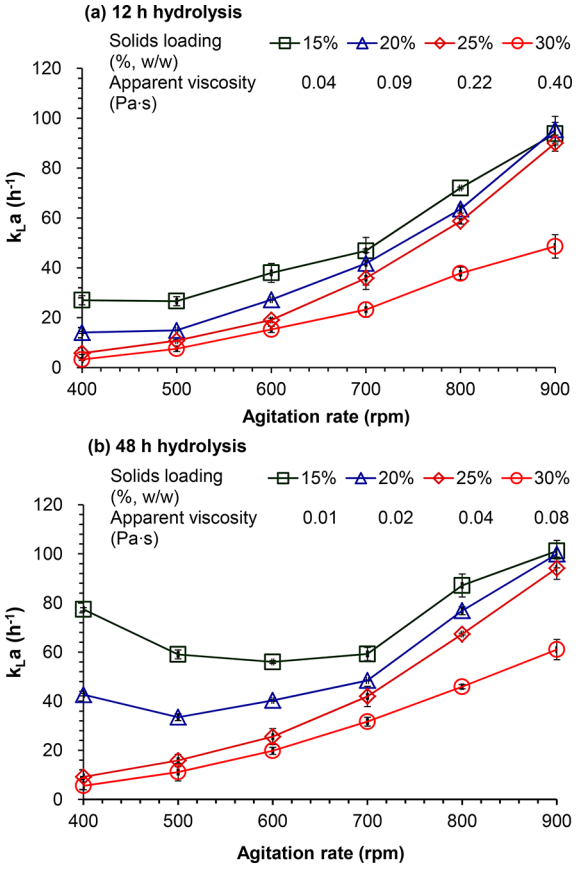

Figure 2. Measurement of oxygen transfer rate k_La in the CS hydrolysate slurry: (a) hydrolyzed for 12 h and (b) hydrolyzed for 48 h. The apparent viscosity was calculated at the shear rate of 70 s^{-1} using the rheological properties K_p and n measured by a rotational viscometer. CS was dry acid pretreated using the solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g DM at pH 4.8 and 50 °C for 12 or 48 h. Oxygen transfer rate in the hydrolysate slurry was measured at 35 °C and aeration rate of 1 vvm.

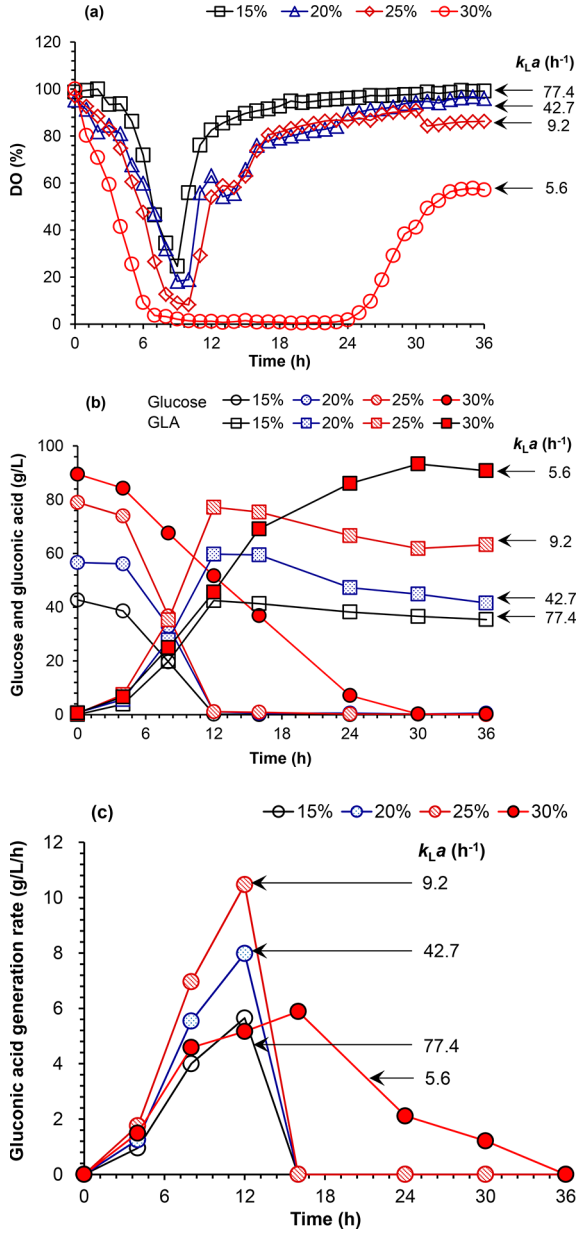

Figure 3. Cellulosic gluconic acid fermentation under different oxygen transfer rates created by varying solids content in hydrolysate slurry: (a) dissolved oxygen, (b) gluconic acid generation, and (c) gluconic acid generation rate. CS was dry acid pretreated using the solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g DM at pH 4.8 and 50 °C for 48 h. Cellulosic gluconic acid fermentation was conducted at an agitation rate of 400 rpm, inoculum size of 2%, and aeration rate of 1 vvm at pH 4.8 and 35 °C for 36 h.

increased apparent viscosity and reduced k_La values. The only exception is the k_La values at the lower solids content after longer hydrolysis time (Figure 2b), in which a severe foam was formed and the DO sensor reading was disturbed, leading to higher but inaccurate k_La values. The accuracy of the k_La measurement was improved at a higher agitation rate by the enhanced turbulence level and the reduced foam generation.

Aerobic fermentation requires a sufficient oxygen supply to achieve satisfying cell growth and conversion yield. Few examples include the k_La value at 22 h^{-1} for glutamic acid fermentation by *Corynebacterium glutamicum*,[31] 30 h^{-1} for citric acid fermentation by *Yarrowia lipolytica*,[32] and 42 h^{-1} for gluconic acid fermentation by *Gluconobacter oxidans*.[33,34] Although the oxygen transfer rate in the lignocellulose hydrolysate was generally lower than that of the conventional liquid medium or the clear lignocellulose hydrolysate,[8] Figure 2 shows that the k_La value in the high solids loading hydrolysate is still sufficiently high enough to meet the oxygen transfer need at moderate operation conditions. The results indicate that general aerobic fermentations could be conducted in highly viscous and high solids loading lignocellulose slurry.

Evaluation of Aerobic Fermentation Based on Oxygen Transfer Coefficient. To evaluate the oxygen transfer coefficient of the high solids containing hydrolysates in aerobic fermentation, gluconic acid fermentation by *G. oxidans* was selected as the model fermentation for its high oxygen requirement for both cell growth and conversion needs. In this aerobic fermentation, cellulose is converted into glucose and then converted to glucono-γ-lactone, followed by a spontaneous hydrolysis of glucono-γ-lactone into gluconic acid.[35,36] Since the intermediate glucono-γ-lactone is a strong inhibitor to cellulase enzyme,[37,38] the enzymatic hydrolysis is completed before fermentation. Briefly, the pretreated and

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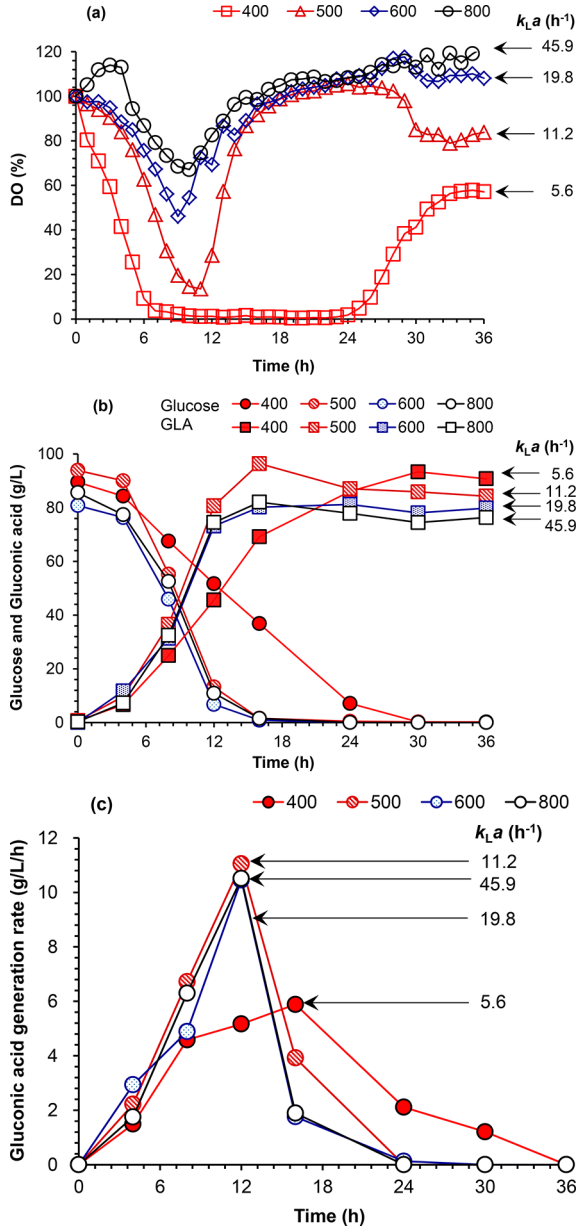

Figure 4. Cellulosic gluconic acid fermentation under different oxygen transfer rates created by varying agitation rate: (a) dissolved oxygen, (b) gluconic acid generation, and (c) gluconic acid generation rate. CS was dry acid pretreated using a solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g DM at pH 4.8 and 50 °C for 48 h. Cellulosic gluconic acid fermentation was conducted at solids loading of 30% (w/w), inoculum size of 2%, and aeration rate of 1 vvm at pH 4.8 and 35 °C for 36 h.

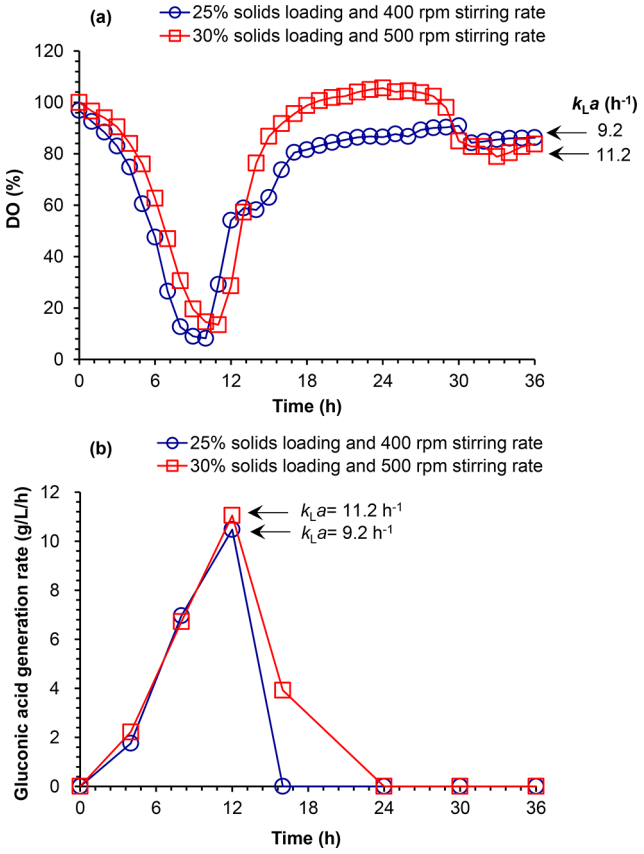

Figure 5. Cellulosic gluconic acid fermentation under the similar oxygen transfer rate created by different operation approaches: (a) dissolved oxygen and (b) gluconic acid generation rate. CS was dry acid pretreated using the solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g DM at pH 4.8 and 50 °C for 48 h. Gluconic acid fermentation was conducted at an inoculum size of 2% and aeration rate of 1 vvm, pH 4.8, and 35 °C for 36 h. Blue lines represent the solids loading of 25% and agitation rate of 400 rpm. Red lines represent the solids loading of 30% (w/w) and agitation rate of 500 rpm.

Table 1. Rheological Properties of CS Hydrolysate Slurry at Different Solids Loadings[a]

CS solids loading (%, w/w)	12 h hydrolysis			48 h hydrolysis		
	K_p (Pa·s^n)	n (−)	η_a (Pa·s)	K_p (Pa·s^n)	n (−)	η_a (Pa·s)
15	0.53	0.37	0.04	0.04	0.51	0.01
20	1.51	0.35	0.09	0.21	0.43	0.02
25	4.98	0.27	0.22	0.58	0.37	0.04
30	8.98	0.27	0.40	1.42	0.31	0.08

[a]The consistency coefficient K_p and the dimensionless power law index n of hydrolysate slurry were measured by a rotational viscometer. The apparent viscosity η_a was calculated at the shear rate of 70 s^{-1}. CS was dry acid pretreated using the solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g dry solids matter (DM) at pH 4.8 and 50 °C for 12 or 48 h.

biodetoxified CS solids were hydrolyzed into oligomer sugars for 48 h in the first reactor, then the whole CS hydrolysate slurry was moved to the second bioreactor for aerobic gluconic acid fermentation.

The oxygen transfer performance in cellulosic gluconic acid fermentation was measured under changing CS solids loading (Figure 3). The dissolved oxygen (DO) level was approximately the same in the k_La range of 9.2−77.4 h^{-1} (Figure 3a), and glucose was completely converted into gluconic acid within 12 h (Figure 3b). When k_La was reduced to 5.6 h^{-1} with 30% solids loading hydrolysate, DO was completely exhausted during the conversion of glucose oxidation, and the conversion rate was significantly reduced (Figure 3c).

To achieve the higher glucose titer in the hydrolysis step and the higher gluconic acid titer in fermentation step, the k_La

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Table 2. Comparison of Experimentally Measured k_La Values with CFD Calculated Values in Highly Viscous Lignocellulose Hydrolysate Slurry[a]

Agitation rate (rpm)	Experimental k_La (h^{-1})	Simulated k_La (h^{-1})	Relative error (%)
400	5.6 ± 1.6	4.8	−14.3
500	11.2 ± 3.6	9.4	−16.1
600	19.8 ± 1.4	16.9	−14.6
800	45.9 ± 0.9	50.0	+9.0

[a]CS was dry acid pretreated using the solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g DM and solids loading of 30% (w/w) at pH 4.8 and 50 °C for 48 h. Oxygen transfer rates in the hydrolysate slurry were measured at 35 °C and aeration rate of 1 vvm.

values were increased by accelerating the agitation rate of the two 6-blade Rushton impellers in the practical range (Figure 4). When the agitation rate increased from 400 to 500 rpm, the k_La value increased by one fold from 5.6 to 11.2 h^{-1} and the glucose conversion rate reached to the optimum (Figure 4a and b). When the agitation rate increased to 600 and 800 rpm, the k_La values further increased to 19.8 and 45.9 h^{-1}, but the conversion rate of glucose to gluconic acid stayed similar (Figure 4c). The results indicate that the oxygen supply of the aerobic gluconic acid fermentations under the high solids loading and highly viscous hydrolysates could be met by adjusting operation conditions such as agitation rate acceleration.

The gluconic acid fermentation performance at the similar k_La values (9.2 and 11.2 h^{-1}) was compared in Figure 5. Although similar k_La values were obtained by adjusting different operation parameters (25% solids loading at 400 rpm and 30% solids loading at 500 rpm, respectively), the gluconic acid productivity stayed almost the same. The result indicates that the oxygen transfer rate is the determinant factor for cellulosic aerobic fermentation, and a minimum threshold value of k_La for cellulosic gluconic acid fermentation of *G. oxidans* (around 10 h^{-1}) was required. This study extended the biorefining fermentation from anaerobic type such as ethanol fermentation into the aerobic type such as gluconic acid, citric acid, and glutamic acid fermentations by confirming that the oxygen transfer rate in highly viscous lignocellulose hydrolysate was sufficiently high enough for aerobic fermentation.

CFD Simulation. The CFD model of aerobic fermentors in the high solids loading and highly viscous lignocellulose hydrolysate was established based on the rheological property measurement (Table 1). The CFD calculated oxygen transfer rate was compared with that of the experimentally measured data (Table 2). The calculated and experimentally measured k_La values were in good agreement with the acceptable error range of ±13.5%. The result indicates the validity of the CFD model for calculation of oxygen transfer property in the high lignocellulose solids loading hydrolysate.

The oxygen transfer coefficient under different agitation rates in high solids loading hydrolysate was calculated using the established CFD model (Figure 6). The volumetric average gas holdup significantly increases from 0.4% to 1.1% and 2.3% with increasing agitation rate from 400 to 600 and 800 rpm (Figure 6a). The gas (air) pathline chart indicates that air flow tends to accumulate into the center of the bioreactor with increasing agitation rate (Figure 6b). The k_La increases with increased agitation rate perhaps due to the increased liquid flow velocity and turbulence intensity.

The oxygen transfer coefficient under different impeller configurations (RT, HBT, and PBTU) was also calculated using the CFD model (Figure 7). Under the same agitation power input, the volumetric average gas holdup of the RT combination is relatively greater (Figure 7a), and the k_La value is 1.3 and 4.4 times greater than that of the PBTU and the HBT combinations (Figure 7b), respectively. The result shows that the commonly used Rushton impeller is the efficient impeller configuration for supplying sufficient oxygen to the

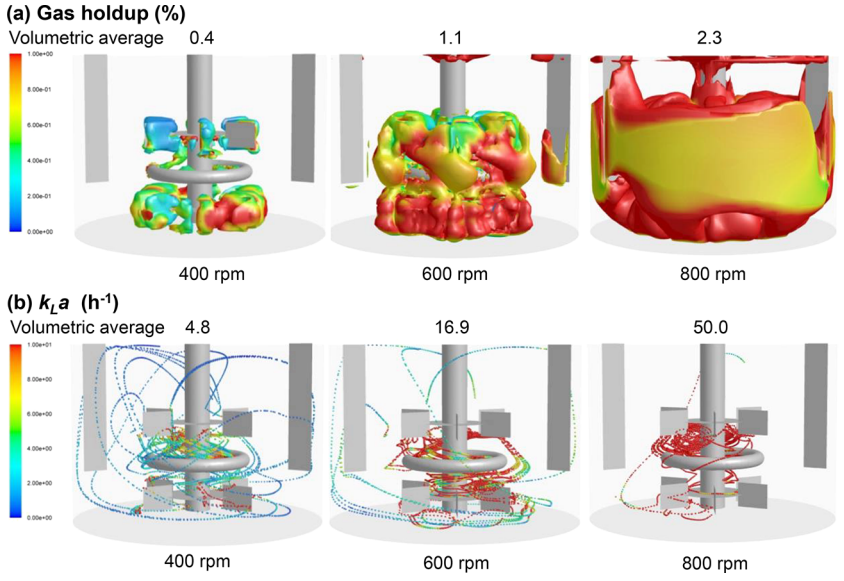

Figure 6. CFD simulation of gas holdup and oxygen transfer rate of the high solids loading hydrolysate slurry at different agitation rates. (a) Gas holdup on the isosurface of k_La value at 10 h^{-1}. (b) Oxygen transfer rate distribution along with gas pathlines within 600 steps at the step size of 0.01 m. CS was dry acid pretreated using the solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g DM and solids loading of 30% (w/w) at pH 4.8 and 50 °C for 48 h.

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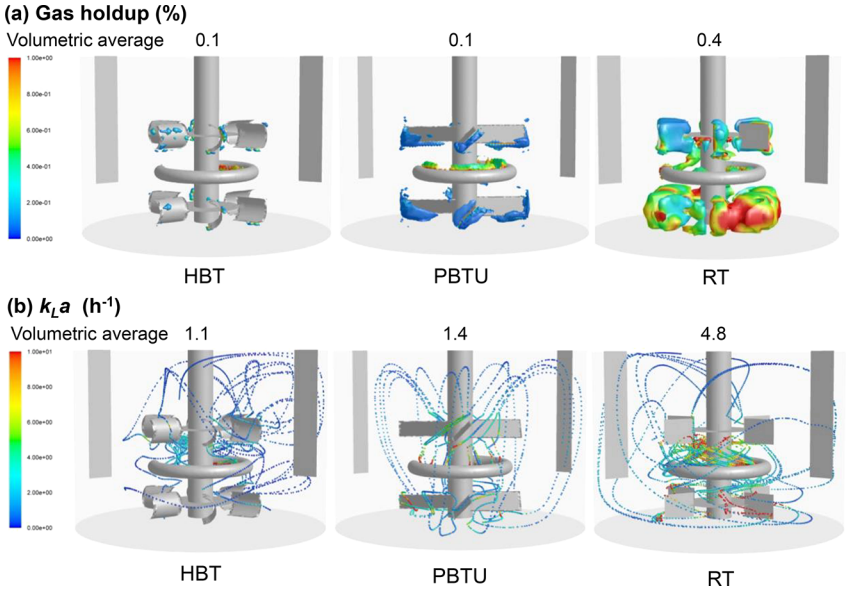

Figure 7. CFD simulation of gas holdup and oxygen transfer rate of the high solids loading hydrolysate slurry using different impellers at the same power input (2.1 kW/m^3). (a) Gas holdup on the isosurface of k_La value at 10 h^{-1}. (b) Oxygen transfer rate distribution along with gas pathlines within 600 steps at the step size of 0.01 m. CS was dry acid pretreated using the solid/liquid ratio of 2:1 and 2.5% sulfuric acid usage per dry CS at 175 °C for 5 min. Enzymatic hydrolysis was conducted with 6 mg cellulase proteins/g DM and solids loading of 30% (w/w) at pH 4.8 and 50 °C for 48 h.

aerobic fermentation in the high solids loading and highly viscous lignocellulose hydrolysates.

This study confirmed that the oxygen transfer is sufficiently high enough for aerobic fermentation even in highly viscous and high solids loading lignocellulose hydrolysate slurry by regulating process parameters and reactor design. The results of the solid foundation provides for industrial application of oxidative conversions of biofuels and biochemicals with high economic potentials. This study also provided a CFD method for calculating oxygen transfer coefficients at changing process parameters based on the accurate rheology model of the highly viscous and high solids loading hydrolysate. The CFD model also paved the way for bioreactor scale-up in industrial applications.

CONCLUSION

The oxygen transfer properties of high solids loading and highly viscous lignocellulose hydrolysates were first investigated. The oxygen transfer rate decreased with increased solids loading and increased with prolonged hydrolysate time and increased agitation rate. Aerobic fermentation indicated that the oxygen transfer rate was a determination factor for efficient fermentation conversion. For the typical aerobic fermentation of glucose oxidation into gluconic acid, a minimum threshold k_La value of around 10 h^{-1} was required for maintaining the aerobic fermentation efficiency. The rheological properties of the slurry were experimentally determined, and a CFD model was established. The k_La values between the CFD calculation and the experimental determination were in good agreement with the acceptable error range of ±13.5% for the highly viscous hydrolysate slurry. The validated CFD model was successfully used for aerobic bioreactor design. This study extended the biorefining fermentation from anaerobic type into aerobic type by confirming that the oxygen transfer rate in highly viscous lignocellulose hydrolysate was sufficiently high enough for aerobic fermentation.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Galbe, M.; Sassner, P.; Wingren, A.; Zacchi, G. Process engineering economics of bioethanol production. *Adv. Biochem. Eng/Biotechnol.* **2007**, *108*, 303−327.

(2) Larsen, J.; Petersen, M. O.; Thirup, L.; Li, H. W.; Iversen, F. K. The IBUS process-lignocellulosic bioethanol close to a commercial reality. *Chem. Eng. Technol.* **2008**, *31*, 765−772.

(3) Jorgensen, H.; Vibe-pedersen, J.; Larsen, J.; Felby, C. Liquefaction of lignocellulose at high-solids concentration. *Biotechnol. Bioeng.* **2007**, *96*, 862−870.

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

(5) Modenbach, A. A.; Nokes, S. E. Enzymatic hydrolysis of biomass at high-solids loadings - A review. *Biomass Bioenergy* **2013**, *56*, 526−544.

(6) Zhang, H. S.; Liu, G.; Zhang, J.; Bao, J. Fermentative production of high titer gluconic and xylonic acids from corn stover feedstock by *Gluconobacter oxydans* and techno-economic analysis. *Bioresour. Technol.* **2016**, *219*, 123−131.

(7) Zhang, H. S.; Zhang, J.; Bao, J. High titer gluconic acid fermentation by *Aspergillus niger* from dry dilute acid pretreated corn stover without detoxification. *Bioresour. Technol.* **2016**, *203*, 211−219.

DOI: 10.1021/acssuschemeng.7b02503
ACS Sustainable Chem. Eng. 2017, 5, 11395−11402

(8) Freitas, C.; Teixeira, J. A. Oxygen mass transfer in a high solids loading three-phase internal-loop airlift reactor. *Chem. Eng. J.* **2001**, *84*, 57−61.
(9) Sivaiah, M.; Majumder, S. K. Mass transfer and mixing in an ejector-enduced downflow slurry bubble column. *Ind. Eng. Chem. Res.* **2013**, *52*, 12661−12671.
(10) Tobajas, M.; Garcia-Calvo, E.; Siegel, M. H.; Apitz, S. E. Hydrodynamics and mass transfer prediction in a three-phase airlift reactor for marine sediment biotreatment. *Chem. Eng. Sci.* **1999**, *54*, 5347−5354.
(11) Chen, Z.; Liu, H. W.; Zhang, H. T.; Ying, W. Y.; Fang, D. Y. Oxygen mass transfer coefficient in bubble column slurry reactor with ultrafine suspended particles and neural network prediction. *Can. J. Chem. Eng.* **2013**, *91* (3), 532−541.
(12) Su, Y. K.; Willis, L. B.; Jeffries, T. W. Effects of aeration on growth, ethanol and polyol accumulation by *Spathaspora passalidarum* NRRL Y-27907 and *Scheffersomyces stipites* NRRL Y-7124. *Biotechnol. Bioeng.* **2015**, *112*, 457−469.
(13) Shu, C. H.; Liao, C. C. Optimization of L-phenylalanine production of *Corynebacterium glutamicum* under product feedback inhibition by elevated oxygen transfer rate. *Biotechnol. Bioeng.* **2002**, *77* (2), 131−141.
(14) Mussatto, S. I.; Roberto, I. C. Kinetic behavior of *Candida guilliermondii* yeast during xylitol production from highly concentrated hydrolysate. *Process Biochem.* **2004**, *39*, 1433−1439.
(15) Bellido, C.; Gonzalez-Benito, G.; Coca, M.; Lucas, S.; Garcia-Cubero, M. T. Influence of aeration on bioethanol production from ozonized wheat straw hydrolysates using Pichia stipites. *Bioresour. Technol.* **2013**, *133*, 51−58.
(16) Kinnarinen, T.; Golmaei, M.; Hakkinen, A. Use of filter aids to improve the filterability of enzymatically hydrolyzed biomass suspension. *Ind. Eng. Chem. Res.* **2013**, *52*, 14955−14964.
(17) Sievers, D. A.; Lischeske, J. J.; Biddy, M. J.; Stickel, J. J. A low-cost solid−liquid separation process for enzymatically hydrolyzed corn stover slurries. *Bioresour. Technol.* **2015**, *187*, 37−42.
(18) Sreenath, H. K.; Moldes, A. B.; Koegel, R. G.; Straub, R. J. Lactic acid production from agriculture residues. *Biotechnol. Lett.* **2001**, *23*, 179−184.
(19) Liu, W.; Wang, Y.; Yu, Z. C.; Bao, J. Simultaneous saccharification and microbial lipid fermentation of corn stover by oleaginous yeast. *Bioresour. Technol.* **2012**, *118*, 13−18.
(20) Adney, B.; Baker, J. *Measurement of Cellulase Activities, Laboratory Analytical Procedure (LAP)*; Techincal Report NREL/TP-510-42628; NREL: Golden, CO, 1996. http://purl.access.gpo.gov/GPO/LPS94126 (accessed November 2017).
(21) Ghose, T. Measurement of cellulase activities. *Pure Appl. Chem.* **1987**, *59* (2), 257−268.
(22) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72* (1−2), 248−254.
(23) Zhang, J.; Zhu, Z. N.; Wang, X. F.; Wang, N.; Wang, W.; Bao, J. Biodetoxification of toxins generated from lignocellulose pretreatment using a newly isolated fungus, *Amorphotheca resinae* ZN1, and the consequent ethanol fermentation. *Biotechnol. Biofuels* **2010**, *3*, 26.
(24) Zhang, J.; Wang, X. S.; Chu, D. Q.; He, Y. Q.; Bao, J. Dry pretreatment of lignocellulose with extremely low steam and water usage for bioethanol production. *Bioresour. Technol.* **2011**, *102*, 4480−4488.
(25) He, Y. Q.; Zhang, L. P.; Zhang, J.; Bao, J. Helically agitated mixing in dry dilute acid pretreatment enhances the bioconversion of corn stover into ethanol. *Biotechnol. Biofuels* **2014**, *7*, 1.
(26) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples, Laboratory Analytical Procedure (LAP)*; Technical Report NREL/TP-510-42623; NREL: Golden, CO, 2008. https://www.nrel.gov/docs/gen/fy08/42623.pdf (accessed November 2017).
(27) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass, Laboratory Analytical Procedure (LAP)*; Technical Report NREL/TP-510-42618; NREL: Golden, CO, 2012. http://purl.access.gpo.gov/GPO/LPS94089 (accessed November 2017).
(28) Shuler, M. L., Kargi, F. *Bioprocess Eng.*, Second ed.; Prentice-Hall, Upper Saddle River, NJ, 2002.
(29) Bird, R. B.; Stewart, W. E.; Lightfoot, E. N. *Transport Phenomena*; John Wiley & Sons, New York, 1960.
(30) Huang, Q. S.; Yang, C.; Yu, G. Z.; Mao, Z. S. CFD simulation of hydrodynamics and mass transfer in an internal airlift loop reactor using a steady two-fluid model. *Chem. Eng. Sci.* **2010**, *65*, 5527−5536.
(31) Calik, G.; Unlutabak, F.; Ozdamar, T. H. Product and by-product distributions in glutamic acid fermentation by *Brevibacterium flavum*: effects of the oxygen transfer. *Biochem. Eng. J.* **2001**, *9*, 91−101.
(32) Ferreira, P.; Lopes, M.; Mota, M.; Belo, I. Oxygen mass transfer impact on citric acid production by *Yarrowia lipolytica* from crude glycerol. *Biochem. Eng. J.* **2016**, *110*, 35−42.
(33) Luchterhand, B.; Fischoder, T.; Grimm, A. R.; Wewetzer, S.; Wunderlich, M.; Schleputz, T.; Buchs, J. Quantifying the sensitivity of *G. oxydans* ATCC 621H and DSM 3504 to osmotic stress triggered by soluble buffers. *J. Ind. Microbiol. Biotechnol.* **2015**, *42*, 585−600.
(34) Silberbach, M.; Maier, B.; Zimmermann, M.; Buchs, J. Glucose oxidation by *Gluconobacter oxydans*: characterization in shaking-flasks, scale-up and optimization of the pH profile. *Appl. Microbiol. Biotechnol.* **2003**, *62*, 92−98.
(35) Krajewski, V.; Simic, P.; Mouncey, N. J.; Bringer, S.; Sahm, H.; Bott, M. Metabolic engineering of *Gluconobacter oxydans* for improved growth rate and growth yield on glucose by elimination of gluconate formation. *Appl. Environ. Microbiol.* **2010**, *76* (13), 4369−4376.
(36) Shinagawa, E.; Ano, Y.; Yakushi, T.; Adachi, O.; Matsushita, K. Solubilization, purification and properties of membrane-bound D-glucono-δ-lactone hydrolase from. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 241−244.
(37) Cannella, D.; Hsieh, C. C.; Felby, C.; Jorgensen, H. Production and effect of aldonic acids during enzymatic hydrolysis of lignocellulose at high dry matter content. *Biotechnol. Biofuels* **2012**, *5*, 26.
(38) Kim, M.; Day, D. F. Use of cellulase inhibitors to produce cellobiose. *Appl. Biochem. Biotechnol.* **2010**, *162*, 1379−1390.

DOI: 10.1021/acssuschemeng.7b02503
ACS Sustainable Chem. Eng. 2017, 5, 11395−11402