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CONTINUOUS AND SEMICONTINUOUS REACTION SYSTEMS FOR HIGH-SOLIDS ENZYMATIC HYDROLYSIS OF LIGNOCELLULOSICS

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Abstract - An attractive operation strategy for the enzymatic hydrolysis of lignocellulosics results from dividing the process into three stages with complementary goals: continuous enzyme adsorption at low-solids loading (5% w/w) with recycling of the liquid phase; continuous liquefaction at high-solids content (up to 20% w/w); and, finally, continuous or semicontinuous hydrolysis with supplementation of fresh enzymes. This paper presents a detailed modeling and simulation framework for the aforementioned operation strategies. The limiting micromixing situations of macrofluid and microfluid are used to predict conversions. The adsorption and liquefaction stages are modeled as a continuous stirred tank and a plug flow reactor, respectively. Two alternatives for the third stage are studied: a train of five cascading stirred tanks and a battery of batch reactors in parallel. Simulation results show that glucose concentrations greater than 100 g L^{-1} could be reached with both of the alternatives for the third stage.
Keywords: Reactor, High-solids; Continuous; Semicontinuous; Micromixing; Recycling.

INTRODUCTION

Modeling and simulation of reaction systems for chemicals and fuels production from lignocellulosics is a useful approach for exploring process configurations. Previous studies have highlighted the biochemical conversion of lignocellulosics as one of the routes for obtaining fermentable sugars that can be converted to ethanol or other chemical products (Jørgensen *et al*., 2007; Humbird *et al*., 2011; Mora *et al*., 2013; Modenbach and Nokes, 2013; Idrees *et al*., 2014). In this conversion route, lignocellulosic biomass is first

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pretreated to increase the enzyme accessibility and improve the digestibility of cellulose. The pretreated material is then hydrolyzed in the presence of enzymes to produce sugars, mainly glucose and xylose. Despite some success at the pilot and demonstration scales, many questions must be resolved before the full potential of the technology will be realized (Modenbach and Nokes, 2013). Given the availability of validated kinetic models for the enzymatic hydrolysis of lignocellulosics (Kadam *et al*., 2004; Zheng *et al*., 2009), it is convenient to model and simulate large-scale operation strategies to provide feedback on further experimental work and economical assessment. This paper focuses on the enzymatic hydrolysis step and proposes continuous and semicontinuous systems which address two current concerns: increasing solids loading while maintaining conversion and reducing enzyme loading. These are central issues to develop an economically feasible process.

Enzymatic hydrolysis of lignocellulosics has been typically carried out at 5-8% w/w solids to ensure a proper contact with enzymes. However, solids loadings higher than 10% w/w are required to obtain cost-effective concentrations of sugars (Wingren *et al*., 2003). Due to the high water-holding capacity of solids, the reaction medium cannot be efficiently sheared and mixed at solids loadings higher than 10% w/w (Viamajala *et al*., 2009). In addition, conversion at increasing solids loadings exhibits a general decreasing trend (Kristensen *et al*., 2009). On the other hand, enzymes cost has been pointed out as one of the major costs of the biochemical conversion (Newman *et al*., 2013). As a solid-liquid enzyme-catalyzed reaction, the reaction rate is directly related to adsorbed enzyme. Again, a negative correlation between solids loading and adsorbed enzyme has been observed (Kristensen *et al*., 2009).

An alternative to increase solids loading has been the fed-batch operation, where solids and/or enzymes are added at different times. The fed-batch operation enables operatation at solids loadings higher than 10% w/w while overcoming mixing constrains (Rosgaard *et al*., 2007; Wanderley *et al*., 2013; Zhao *et al*., 2013) and allows sugar concentrations up to four times greater than those of the equivalent batch reaction (Gupta *et al*., 2012). Two to four additions of solids have been reported and enzymes are added either all at the beginning of the reaction or at each solids feeding event (Gonzalez Quiroga *et al*., 2010-1). Operating in the fed-batch mode offers additional advantages such as lower instantaneous solids concentration and lower apparent viscosities, which could be beneficial for enzyme adsorption. Hodge *et al*. (2009) developed and validated a model-based fed-batch strategy to maintain the solids concentration at manageable levels during the course of the reaction and reached cumulative substrate concentrations of 25%. Following the work of Hodge *et al*. (2009), Morales-Rodriguez *et al*. (2010) and Cavalcanti-Montaño *et al*. (2013) presented optimized feedback control strategies that further improve the fed-batch operation by reducing the consumption of enzymes.

Several studies have focused on recycling strategies of free (not adsorbed) and adsorbed enzyme to reduce the consumption of the enzymes of the hydrolysis step. Results have shown a fresh substrate conversion of 67% after three rounds of recycle of solid residue and ultrafiltration retentate of the supernatant without extra fresh enzyme, provided that the lignin content of solids on a dry basis is lower than 5% w/w and the cumulative solids concentration is lower than 3% w/w (Qi *et al*., 2011; Rahikainen *et al*., 2013). However, optimal process conditions are not necessarily identical in processes with and without enzyme recycling (Lindedam *et al*., 2013). Ouyang *et al*. (2013) employed the enzyme bound on residual substrate as a more effective method for recovery, although another experimental study pointed out that there is no actual accumulation of enzyme activity with solids recycling (Pihlajaniemi *et al*., 2014). Rodrigues *et al.* (2014) found that ultrafiltration on a lab scale allowed the recovery of free enzyme with a recovery of 80% and highlighted the thermal stability of enzymes as essential for recycling.

A recent experimental study on the laboratory scale demonstrated that adding enzymes at low-solids loading (5% w/w), followed by filtering the mixture after short retention times (10 min) and supplementing the thickened pulp (solids after thickening 20% w/w) with fresh enzymes, results in conversions comparable to those with low-solids loading and final sugar concentrations higher than 100 g L^{-1}(Xue *et al*., 2012). This operation strategy proved to be effective in aspects directly related to the economic feasibility of the biochemical route: high solids loading, high conversion and efficient enzyme utilization. When solids loading increased from 5% to 20% w/w under a conventional operation, final conversions decreased by 30% (Xue *et al*., 2012), as has been pointed out in previous research (Kristensen *et al*., 2009).

Some proposals of continuous and semicontinuous reaction systems for large-scale enzymatic hydrolysis of lignocellulosics are summarized in Table 1. As a common feature the reaction system has been divided into two subsystems with complementary goals: (1) liquefaction at high-solids loading and (2) long-time retention/reaction, where the final conver-

sion of cellulose and hemicelluloses to reducing sugars is achieved. During the liquefaction stage, a significant drop in the apparent viscosity of the reaction medium occurs. Sjoede *et al*. (2013) reported that the apparent viscosity dropped from 100,000 to 2 Pa.s when 10% w/w of alkaline sulfited bagasse was hydrolyzed at 50 °C during 2 h (the enzyme used was *acellerase,* but the enzymatic activity was not reported). The liquefaction stage has been represented by a train of 2 or 3 cascading *CSTR*s (Continuous Stirred Tank Reactors) with distributed feeding of substrate and enzyme, and either a downward flow or an upward flow tower-type *PFR* (Plug Flow Reactor). On the other hand, the long-time retention/reaction stage has been represented by a battery of batch in parallel, a train of cascading *CSTR*s and a *PFR* (See Table 1). A train of cascading *CSTR*s with distributed feeding of substrate and enzyme seeks to translate the fed-batch operation into a continuous basis. A simulation study by Gonzalez Quiroga *et al*. (2010a) pointed out that continuous distributed feeding has the potential to increase solids loading from 5% to 20% w/w with a decline in conversion of 15% and an increase in final glucose concentration from 27 to 92 g L^{-1}.

In this paper a previously developed and validated kinetic model (Zheng *et al*., 2009) and the limiting micromixing situations of microfluid and macrofluid (Gonzalez Quiroga *et al*., 2010a, 2010b) are used to explore the capabilities of continuous and semicontinuous reaction systems. The kinetic model of Zheng *et al*. (2009) is a semi-mechanistic three-reaction kinetic model intended for optimization, economical evaluation and process design. There are more kinetic models particularly useful either for developing and testing understanding at the level of substrate features and multiple enzyme activities (Zhang and Lynd, 2004) or for scale-up, design and process optimization (Sousa *et al*., 2011; Carvalho *et al*., 2013). On the other hand, micromixing refers to the contact among fluid elements at the microscopic or molecular scales and is characterized by the dynamic environment renovation around each molecule. The state of microfluid prevails if the incoming material immediately comes into intimate contact with other fluid elements of all ages at the molecular level as in an ideal *CSTR*. Conversely, the state of macrofluid is kept if the incoming material is broken up into discrete clumps in which elements of different ages do not intermix at all while in the reaction system, and reactions proceed independently in each fluid element. The following section presents the modeling framework, which includes a summary of the kinetic model, a detailed explanation of the operation strategies and the description of the mathematical models.

Table 1: Summary of proposed continuous and semicontinuous reaction systems for enzymatic hydrolysis of lignocellulosics.

PROCESS	REACTORS CONFIGURATION	REF
SHF	*PFR* followed by a train of three to twelve cascading *CSTR*s *PFR* followed by two trains of three to twelve cascading *CSTR*s Two *PFR* in parallel followed by a train of three to twelve cascading *CSTR*s	Harlick and Zheng, 2011
SHF	A train of three cascading *CSTR*s A train of three cascading *CSTR*s with liquid phase recycling from the third to the first reactor, enzyme supplementation at the second reactor and liquid phase withdrawing at the outlet of the first reactor A train of three cascading *CSTR*s with distributed feeding of enzyme and distributed withdrawing of liquid phase	Sjoede *et al*., 2013
SHF	A train of ten cascading *CSTR*s or a *PFR*, both with and without recycle A train of ten cascading *CSTR*s with distributed feeding of substrate and enzyme at the first two or three reactors A train of three cascading *CSTR*s with distributed feeding of substrate and enzyme at the first two or three reactors, followed by a PFR	Gonzalez Quiroga, 2009; Gonzalez Quiroga *et al*., 2010a, 2010b
SSF	Three trains of six cascading *CSTR*s	Wooley *et al*., 1999
SHF	Train of five cascading *CSTR*s	Aden *et al*., 2002
SHF	Tower type *PFR* (downward flow) followed by twelve batch in parallel	Humbird *et al*., 2011
SSF	Train of four *STR*s with intermittent feeding	Shao *et al*., 2009

MODELING FRAMEWORK

Kinetic Model

The model is made up of two heterogeneous reactions for the breakdown of cellulose into cellobiose (G_2) and glucose (G), and a homogeneous reaction for the breakdown of G_2 into G. The multi-enzyme system is quantitatively represented by two enzyme concentrations, *endoglucanase/cellobiohydrolase* (*EG/CBH*), which catalyze the heterogeneous reactions, and *β-glucosidase* (*BG*), which catalyze the homogeneous reaction. The model incorporates *EG/CBH* adsorption on *C* and lignin (*L*), and *BG* adsorption on *L*. Due to the adsorption of *EG/CBH* on both cellulose and lignin, the amount of *EG/CBH* adsorbed on cellulose is calculated as "*EG/CBH* adsorbed on substrate – *EG/CBH* adsorbed on lignin". In addition, the kinetic model takes into account competitive inhibition of *EG/CBH* and *BG* by G_2 and G, and substrate reactivity (Zheng *et al*., 2009). Substrate reactivity is a parameter that lumps the change of substrate structural features like crystallinity, degree of polymerization, accessibility to enzymes, etc.

The pretreated substrate (creeping wild ryegrass pretreated with dilute sulfuric acid) was composed of 53% w/w *C* and 38% w/w *L* on a dry basis. The model was fitted and validated under solids loadings from 4 to 12% w/w, *EG/CBH* loadings from 15 to 150 *FPU*(g *C*)$^{-1}$, *BG* loadings from 15 to 150 *CBU*(g *C*)$^{-1}$, background G_2 of 10 g L^{-1} and background G of 30 and 60 g L^{-1}.

Mass balances on *C*, G_2, *G*, *EG/CBH* and *BG* were established as follow:

$$\frac{dC}{dt} = -r_1 - r_2 \tag{1}$$

$$\frac{dG_2}{dt} = 1.056r_1 - r_3 \tag{2}$$

$$\frac{dG}{dt} = 1.1116r_2 + 1.053r_3 \tag{3}$$

$$E_{1T} = E_{1f} + E_{1b} \tag{4}$$

$$E_{2T} = E_{2f} + E_{2b} \tag{5}$$

where:

r_1: heterogeneous reaction rate (*C* to G_2)
r_2: heterogeneous reaction rate (*C* to *G*)
r_3: homogeneous reaction rate (G_2 to *G*)
t: elapsed time
E_1: *EG/CBH*
E_2: *BG*
f: free enzyme in solution
b: bound enzyme

The kinetic rate equations r_1, r_2 and r_3 have been reported by Zheng *et al*. (2009). The quantities 1.056, 1.1116 and 1.053 stem from the differences in molecular weights between glucose, cellobiose and the equivalent monomer of cellulose.

Operation Strategies

The operation strategies are based on the experimental work of Xue *et al*. (2012). In that study *cellulose* enzymes (mainly *EG/CBH*) were added to 5% w/w solids (Pulp obtained by pretreatment of hardwood chips with green liquor; see Xue *et al*. (2012) for pretreatment conditions) and mixed. After 10 minutes of retention the pulp was thickened to 20% w/w solids by vacuum filtration. After various time intervals, supplementary *xylanase* and *BG* enzymes, with and without supplementary *EG/CBH* enzymes, were added to the thinned mixture and incubated for 48 h (Figure 1). When the aforementioned operation was compared with an operation where all solids and enzymes are added at the beginning of the reaction, the results summarized in Table 2 were obtained.

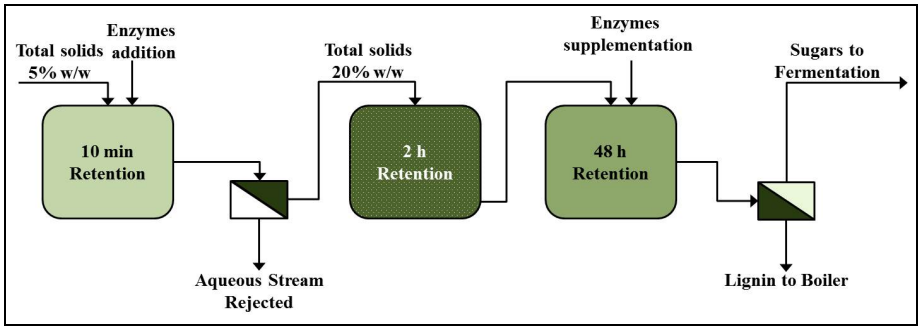


Figure 1: Simplified scheme of the operation procedure tested on a laboratory scale by Xue *et al*. (2012); EG/CBH loadings ranged from 10 to 40 CBU/g substrate.

Table 2: Sugar yields and sugar concentrations for the schemes evaluated by Xue *et al.* (2012). *Cellulase* loading 20 *FPU*(g-substrate*)$^{-1}$ and total reaction time 50 h.

Operation procedure	Yield %	Sugars concentration [g L^{-1}]
1) 5% w/w solids and all the enzymes added at the beginning	64	26
2) 20% w/w solids and all the enzymes added at the beginning	44	84
3) All substrate and *cellulase* added at the beginning. Slurry thickened to 20% w/w after 10 min. *Xylanase* and *BG* supplemented after 8 h	59	114
4) All substrate and part of the *cellulase* added at the beginning. Slurry thickened to 20% w/w after 10 min. *Cellulase*, *xylanase* and *BG* supplemented after 2 h	63	121

*Glucan 61.1%; Xylan 15.0%; Acid insoluble *L* 20.0% and Acid soluble *L* 2.9%. % w/w on a dry basis

According to the study of Xue *et al*. (2012) the key for increasing solids content while maintaining conversion is to mix a fraction of *EG/CBH* enzymes at low-solids loading, thicken to high-solids content, and allow the adsorbed *EG/CBH* enzymes to perform the liquefaction. After the liquefaction process there is an abundant continuous liquid phase, which enables a thorough mixing of supplementary enzymes in the heterogeneous system. Instead of operating under the two subsystems concept summarized in Table 1 (liquefaction at high-solids loading followed by long-time retention/reaction), the results of Xue *et al*. (2012) suggest a three subsystems approach: (1) adsorption at low-solids loading, (2) liquefaction and (3) long-time retention/reaction.

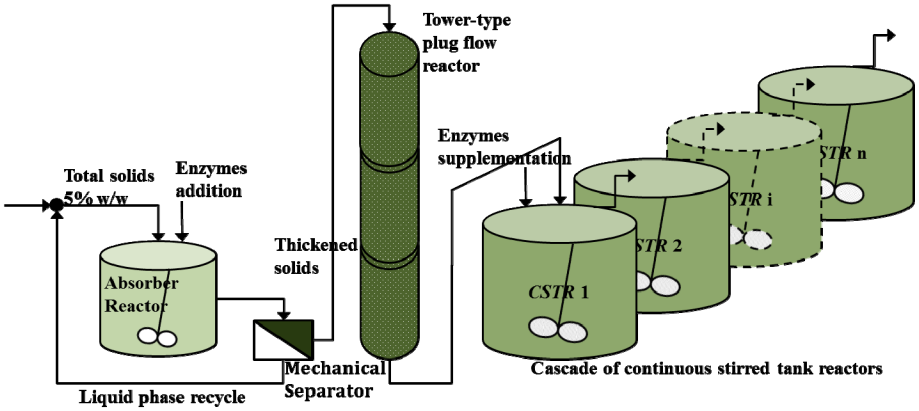
The current operation strategies seek to translate the Xue *et al*. (2012) bench-scale experiment into a continuous or semicontinuous operation at a modeling and simulation level. Additional considerations are the recycling of the liquid phase and an alternative for enzyme supplementation (Figure 2). The simulation assumes that pretreated solids and enzymes (*EG/CBH* or *EG/CBH+BG*) are continuously mixed at low-solids loading (5% w/w) in a well-stirred tank called Adsorber-Reactor (*AR*) with a mean retention time (τ_{AR}) of 0.2 h. The purpose of the *AR* is to provide an environment with abundant free aqueous phase favorable to enzyme adsorption. The stream leaving the *AR* contains water-swollen partially depolymerized solids with adsorbed enzyme and liquid phase with dissolved G_2, *G* and free enzymes. After pressing in a Mechanical Separator (*MS*), the liquid phase with dissolved G_2, *G* and enzymes is recycled to the *AR* and the thickened pulp is liquefied in a tower-type plug flow reactor (*PFR*) with a mean retention time (τ_P) of 2 h. The recycle ratio (*RR*) relates the volumetric flow sent back to the *AR* and the volumetric flow sent to the *PFR*. Finally, the liquefied slurry is conveyed to a train of cascading continuous stirred tank reactors (*CSTR*s) where additional enzymes (*EG/CBH* or *EG/CBH+BG*) are supplemented in the first reactor (Figure 2(a)), or a battery of batch reactors in parallel where additional enzymes are supplemented in each reactor (Figure 2(b)). The battery of batch in parallel was proposed in the last *NREL* (National Renewable Energy Laboratory) technical report (Humbird *et al*., 2011).

Reactor Modeling

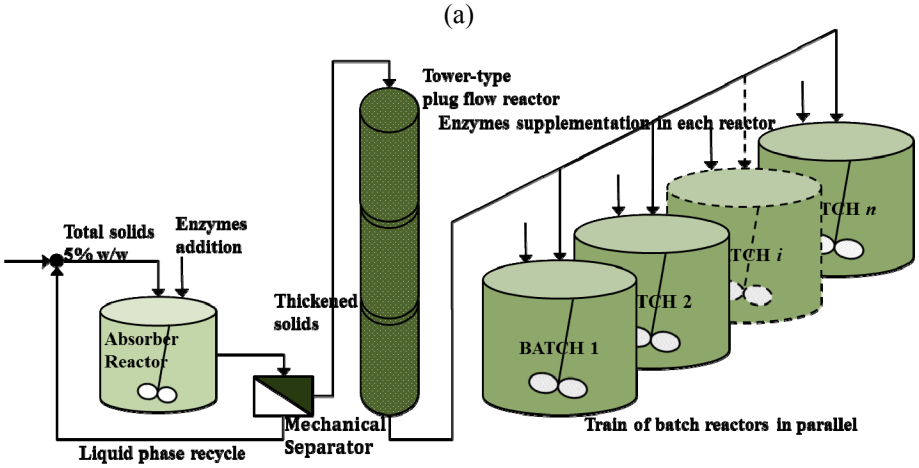
The following general assumptions were necessary for modeling: isothermal reactors, steady state, well mixed tanks in the macroscopic sense, plug flow in the tower-type reactor and simultaneous adsorption/reaction in the *AR*. To study the effect of thickening after enzyme adsorption, the concentration of solids after thickening (S_{AT}) ranged from 5 to 20% w/w. A train of five cascading *CSTR*s was considered to explore the performance of the continuous system. Residence times per reactor (τ_R) along the cascade of equal size *CSTR*s ranged from 10 h to 50 h. Incubation times up to 180 h were simulated for the battery of batch in parallel.

The limiting micromixing situations of microfluid and macrofluid were used for predicting conversion (Gonzalez Quiroga *et al*., 2010a, 2010b**)**. For the enzymatic hydrolysis of lignocellulosics, a micromixing behavior close to macrofluid was found in a *CSTR* for the residence times required to achieve cellulose conversions from 0.50 to 0.85 (South *et al*., 1995). A gradual evolution from macrofluid to microfluid may be expected as the solids structure collapses and the apparent viscosity of the slurry decreases.

An *EG/CBH* loading of 15 *FPU*(g-substrate)$^{-1}$ and a *BG* loading of 15 *CBU*(g-substrate)$^{-1}$ was used in this study. The split addition of *EG/CBH* with and without *BG* addition in the *AR* was modeled and simulated. The bound concentration of *EG/CBH* and the free (in solution) concentration of *BG* were calculated by means of the Langmuir adsorption isotherms reported as part of the kinetic model (Zheng *et al*., 2009**)**.



(a)



(b)

Figure 2: Schemes of the reaction systems proposed. Enzyme adsorption, thickening and liquid phase recycling, followed by a tower-type plug flow reactor and: (a) a train of cascading continuous stirred reactors, or (b) a battery of batch reactors in parallel.

Macrofluid Model

For a well-mixed train of cascading *CSTR*s, the residence time distribution (*RTD*) function E is given by (Levenspiel, 1999):

$$E = \frac{t^{(nr-1)}}{(nr-1)!\,\tau^{nr}} \exp\left(\frac{-t}{\tau}\right) \tag{6}$$

where t is the reaction time, nr the number of reactors and τ the residence time per reactor. The *RTD* function at the outlet of the *AR* (E_{AR}) is given by:

$$E_{AR} = \frac{1}{\tau_{AR}} \exp\left(\frac{-t}{\tau_{AR}}\right) \tag{7}$$

The *RTD* function at the outlet of *CSTR* i of the cascade (E_i) is given by:

$$E_i = \frac{t^{(nr_i-1)}}{(nr_i-1)!\,\tau_R^{nr_i}} \exp\frac{-t}{\tau_R} \tag{8}$$

where nr_i is the *CSTR* i of the cascade.

C, G_2 and G concentrations at the outlet of reactor i (C_i, G_{2i} and G_i, respectively) are expressed in terms of the kinetic model and the corresponding *RTD* functions as follows:

$$C_i = \int_0^{t\to\infty} CE_i dt \tag{9}$$

$$G_{2i} = \int_0^{t\to\infty} G_2 E_i dt \tag{10}$$

$$G_i = \int_0^{t\to\infty} GE_i dt \tag{11}$$

Note that C, G_2 and G on the right side of Equations (9), (10), and (11) are the time-dependent concentrations of cellulose, cellobiose and glucose that are predicted by the kinetic model. On the other hand, C_i, G_{2i} and G_i are the predicted concentrations of cellulose, cellobiose and glucose at the outlet of the reactor i, which are discrete values and depend on both the kinetic model and the corresponding *RTD*.

Each *RTD* function was numerically evaluated, and the maximal value of Δt ($\Delta t \approx dt$) that guarantees a minimal value of 0.9999 for each *RTD* time integral was used for the numerical evaluation of Equations (9) to (11). The numerical values of C, G_2 and G for evaluating Equations (9) to (11) were obtained by numerical integration of the linear differential equation system represented by Equations (1) to (3).

Microfluid Model

For *CSTR* i in the cascade, mass balances on C, G_2 and G are expressed respectively as follows:

$$C_{i-1} - C_i - \tau_R(r_1 + r_2) = 0 \tag{12}$$

$$G_{2i-1} - G_{2i} - \tau_R(1.056r_1 - r_3) = 0 \tag{13}$$

$$G_{i-1} - G_i - \tau_R(1.1116r_2 + 1.053r_3) = 0 \tag{14}$$

Batch Model

For a batch reactor, the mass balances of C, G_2 and G are expressed in the integral form as follows:

$$C = \int_0^t (-r_1 - r_2)dt \tag{15}$$

$$G_2 = \int_0^t (1.056r_1 - r_3)dt \tag{16}$$

$$G = \int_0^t (1.1116r_2 + 1.053r_3)dt \tag{17}$$

PFR Model

Roche *et al*. (2009) found that the intrinsic solids density throughout the enzymatic hydrolysis of lignocellulosics is constant at 1400 g L^{-1}. Hodge *et al*. (2009) reported a correlation for the density of the liquid phase which includes the concentration of sugars. According with these findings the calculated density of the slurry at the beginning of the liquefaction stage is 1080 g L^{-1} and the density of the slurry at the end of the liquefaction results in 1074 g L^{-1}. Constant density of the slurry in the *PFR* was assumed so the reactor is described by the same set of equations of the batch model (Equations (15)-(17)), where t in the batch is equivalent to τ_P in the *PFR*. The numerical solution of the three models was obtained by means of Compaq Visual Fortran® 6.6.

RESULTS AND DISCUSSION

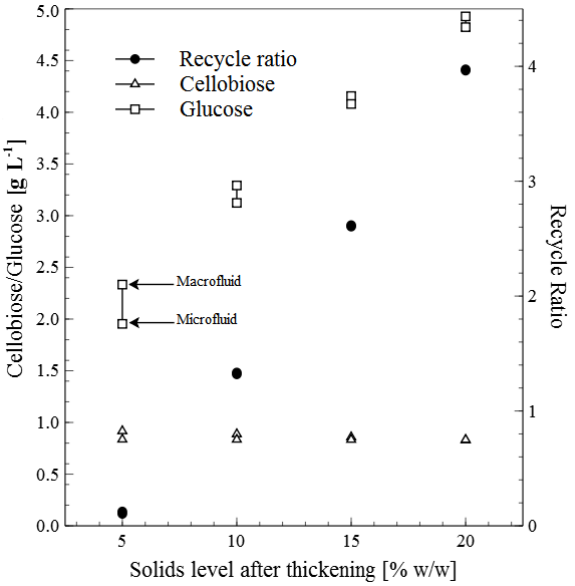
Results in Figures 3-7 show that, for a given residence time, cellulose conversion (X_C) and G concentrations predicted by the macrofluid model are greater than those predicted by the microfluid model. As expected, the predictions of both micromixing models, macrofluid and microfluid, get closer to each other as the residence time increases. The differences between the predictions are significant along the train of cascading *CSTR*s. In the first reactor of the cascade, experimental evidence suggests a micromixing behavior close to macrofluid (South *et al*., 1995). For intermediate reactors, a gradual evolution from macrofluid to microfluid may be expected as the solids structure collapses, releasing liquid phase, and the apparent viscosity of the slurry decreases. The current study assumes ideal flow patterns; however, to take further advantage of the micromixing models the real *RTD* of the reaction systems must be obtained.

An important issue is the assumption of ideal flow patterns. Solids can be efficiently mixed at an initial loading of 5% w/w. Also a drop in the apparent viscosity of the reaction medium of five orders of magnitude is expected at the outlet of the *PFR* due to the release of liquid phase confined in the solids, although the solids concentration only diminishes from 20% to 16% w/w. Under these circumstances it is technically feasible to achieve well mixed conditions along the cascade of *CSTR*s or the battery of batch. Due to the effects of solids type, pretreatment process, level of thickening and enzymes loading on the liquefaction process, further experimental studies should be done to set the residence time τ_P after which the material can be pumped to and mixed in conventional agitated tanks. On the other hand, the flow of the thickened pulp through the tower-type *PFR* can be upward or downward. Other proposed options for the *PFR* are a baffled tubular reactor (Martinez *et al*., 2009; Gonzalez Quiroga *et al*., 2013) and a screw conveyor reactor (Borchert and Buchholz, 1987). Due to the relatively high consistency of the slurry, the residence time distribution at the outlet of the *PFR* is expected to be narrow. However, some channeling or laminar flow of the liquid phase could occur as the slurry moves through the *PFR*.

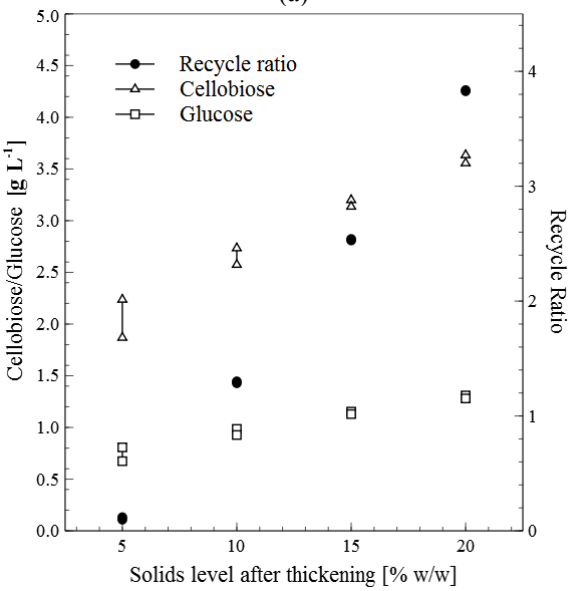
Figure 3 shows the *RR*, G_2 and *G* concentrations at the outlet of the *AR* as a function of S_{AT}. At this point, a peak of adsorption of *BG/CBH* enzymes on cellulose and lignin is expected. Without *BG* addition, the results show a fast release of G_2 and a slow release of *G* (Figure 3(a)). If *BG* is simultaneously added, a peak of adsorption of *BG* on lignin and a significant breakdown of G_2 into *G* is expected (Figure 3(b)). No significant differences were found for *RR* with and without *BG* addition in the *AR*. The adsorption model assumes that *EG/CBH* enzymes adsorb not only on cellulose, but also on lignin, and *BG* adsorbs on lignin (Zheng *et al*., 2009). Enzyme adsorbs on the surface of the substrate, occupying some of the sites available, and only enzyme adsorbed on cellulose is able to catalyze the heterogeneous reactions.

According to Xue *et al*. (2012), to prevent *BG* losses by unproductive adsorption on lignin it should be added after the liquefaction stage. However, the sites on the surface of lignin that would be occupied by *BG* are likely to be occupied by *CBH,* leading to unproductive adsorption in any case. It has also been reported that *BG* adsorption on lignin depends on the type of *BG* enzyme and that this adsorption is not always unproductive (Haven *et al*., 2013). Besides, additives such as bovine albumin and ethylenglycol can partially prevent the adsorption of *BG* on lignin (Haven *et al*., 2013). Further experimental results are needed to assess the optimal location for *BG* addition, so in this work the addition of *BG* in the *AR* is also considered.

After leaving the *AR* there is a mechanical separation from which part of the liquid phase is recycled to the *AR* and the thickened solids with part of the liquid phase are conveyed to the tower-type *PFR*. As the main residence time in the *AR* was fixed for any *RR*, its volume is not constant but varies proportionally to (*1*+*RR*). By recycling liquid hydrolysate from the reaction medium of longer reaction times, significant concentrations of enzyme inhibitors (G_2 and *G*) are recirculated. The inhibitory effect of G_2 and *G* could be partially alleviated by recycling at early retention times. In the batch laboratory experiments reported by Xue *et al*. (2012), the retention time for enzyme adsorption was set as 0.17 h and the liquid stream with dissolved sugars and *cellulose* enzymes leaving the mechanical separation was rejected. The current work suggests recycling this stream to avoid sugar or enzyme losses even at greater retention times in the *AR*. Further research should be done to set the mean residence time in the *AR* because mixing times are strongly dependent on solids loading and *AR* volume.



(a)



(b)

Figure 3: G_2, *G* and *RR* at the outlet of the *AR* as a function of the solids level after thickening. (a) *EG/CBH* 7.5 *FPU*(g-substrate)$^{-1}$ + *BG* 15 *CBU*(g-substrate)$^{-1}$. (b) *EG/CBH* 7.5 *FPU*(g-substrate)$^{-1}$.

Figure 4 illustrates the concentration of G_2 with and without *BG* addition in the *AR* for the continuous (Figure 4(a)) and the semicontinuous system (Figure 4(b)). When *BG* is not added in the *AR,* G_2 exhibits a peak at the outlet of the *PFR*. This peak is a strong function of S_{AT} and the residence time τ_{AR}. *BG* cannot be added immediately after thickening because of the high apparent viscosity of the material, which makes the homogenization difficult. G_2 is a strong inhibitor of *EG/CBH* activity and concentrations of

16 g L^{-1} could be considered too high. This result provides further support for operating with *BG* addition in the *AR*, which resulted in a G_2 peak of 3.3 g L^{-1}. By operating with split addition of *BG*, a G_2 peak between 3.3 and 16 gl^{-1} would be expected, so the G_2 peak could be a good indicator for adjusting the loading of *BG*.

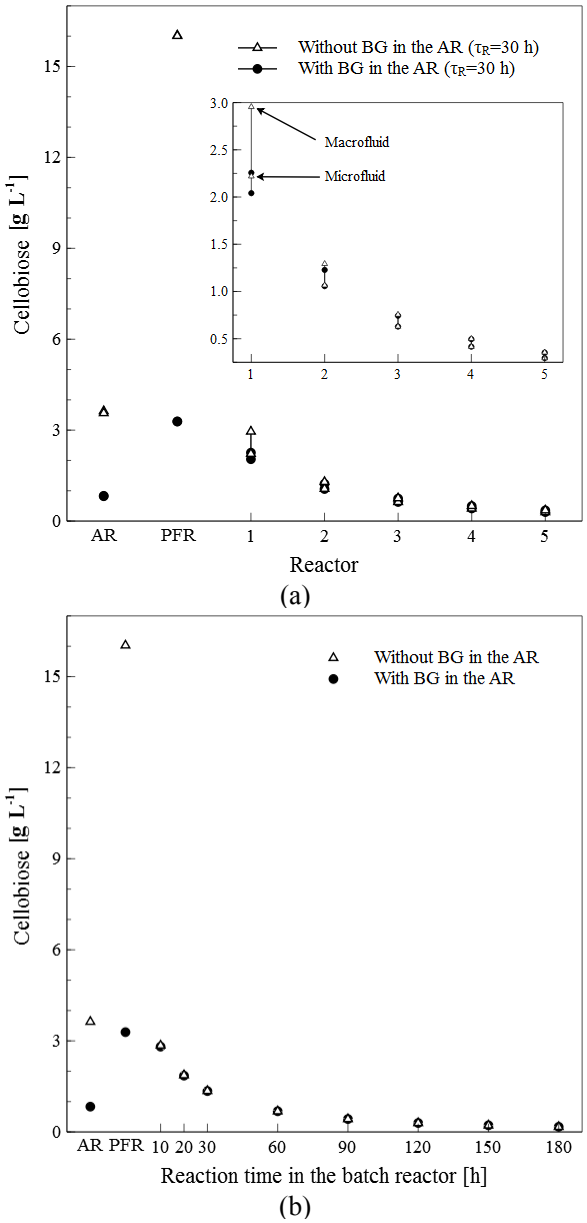


Figure 4: G_2 profiles with and without *BG* addition in the *AR* and a solids level after thickening of 20% w/w. (a) Continuous reaction system with τ_R=30 h; (b) Semicontinuous reaction system.

For a train of cascading *CSTR*s with a mean residence time of 30 h, there were no significant differences in G_2 concentrations between the cases with and without *BG* from the second reactor of the cascade, and G_2 concentrations below 1 gl^{-1} were obtained at the outlet of the third reactor. Similar results were observed for the battery of batch in parallel after 10 and 60 h of reaction, respectively.

Cellulose conversions for the reaction systems are depicted in Figure 5. Note that these results apply for the pretreated substrate (creeping wild ryegrass pretreated with dilute sulfuric acid) used for fitting the kinetic model.

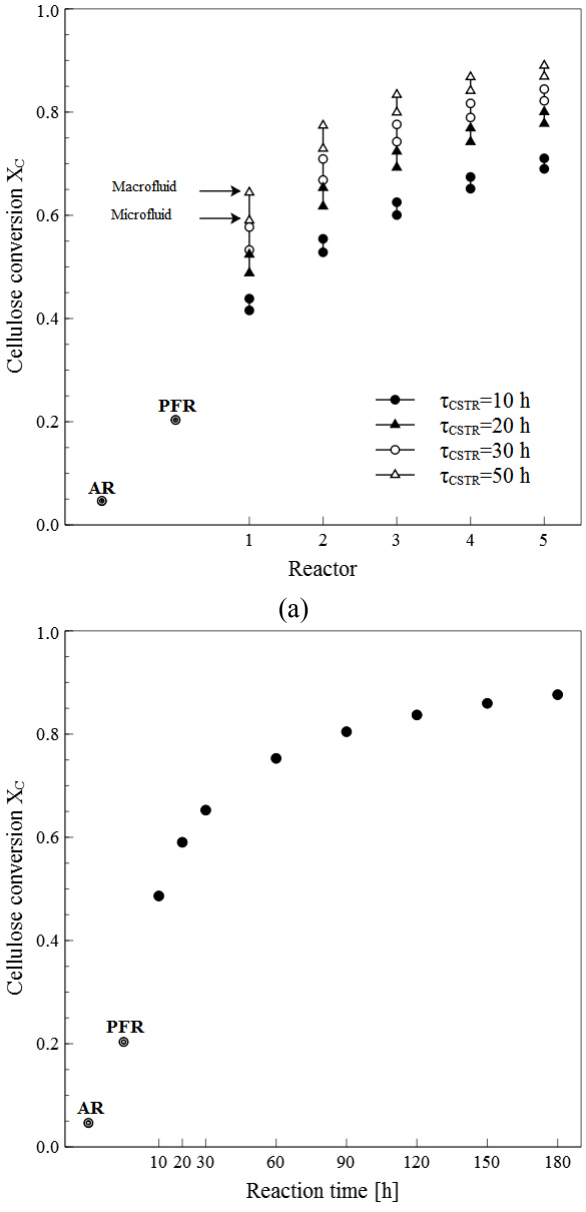


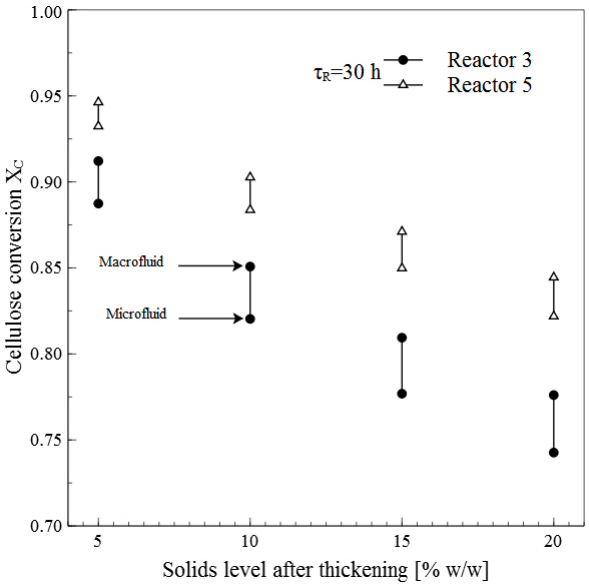
Figure 5: Cellulose conversion profiles with *BG* addition in the *AR* and a solid level after thickening 0f 20% w/w. (a) Continuous reaction system with; (b) Semicontinuous reaction system.

Differences in solids type and pretreatment process could lead to significant differences in both final conversions and conversion profiles. However, the modeling and simulation framework presented here remains useful even if a more detailed kinetic model were to be used. For the train of five cascading *CSTR*s it is manifest that a τ_R of 10 h is insufficient to reach conversions greater than 0.7. Values of τ_R between 20 and 30 h seems to offer a balance between conversion and residence time. A cellulose conversion of 0.8 could be reached after 80 h in the battery of batch in parallel; similar conversions could be attained in a train of 4 cascading *CSTR*s with a mean residence time per reactor of 30 h.

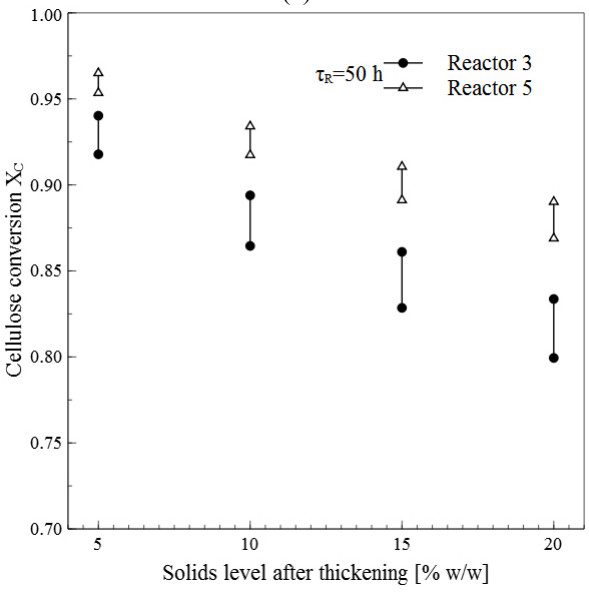
The last *NREL* technical report (Humbird *et al*., 2011) assumes a *PFR* with a residence time of 24 h followed by a battery of batch in parallel with a retention time of 60 h to reach a final cellulose conversion of 0.9. From Figure 5 it is evident that, for the current pretreated substrate and enzyme loadings, such conversion could not be attained even in a batch with reaction times greater than 150 h. Conversions greater than 0.8 could lead to prohibitive residence or reaction times, but from an economical point of view a minimal *G* concentration at the outlet of the enzymatic hydrolysis stage has to be guaranteed.

According to the results of Xue *et al*. (2012), one of the main features of the current reaction systems would be a significant increase in solids loading with final conversions similar to those achieved at low-solids loading. As an initial solids loading of 20% w/w falls outside of the experimental validation interval, it is not possible to obtain results for an operation under such initial conditions. Figure 6 shows the effect of S_{AT} on cellulose conversion for the continuous system at the outlets of the third and fifth reactors of the cascade. An operation with S_{AT}=20% w/w and τ_R=30 h showed reductions in conversion of 19% and 13% in the third and fifth reactor when compared with an operation with S_{AT}=5% w/w (Figure 6(a)). Likewise, an operation with τ_R=50 h showed reductions in conversion of 14% and 9% in the third and fifth reactor. Contrary to the expectations raised by the experimental work of Xue *et al*. (2012), there are substantial differences between conversions at increasing S_{AT}, although the differences tend to be less significant at greater retention/reaction times (> 150 h).

There are a number of reasons for the results of Figures 6 and 7. First of all, the substrate and the pretreatment process of the study of Xue *et al*. (2012) and those of the kinetic model (Zheng *et al*., 2009) used here are different. Also, the *EG/CBH* loading used for Xue *et al*. (2012) was 20 *FPU*(g-substrate)$^{-1}$, whereas the one set in the current study was 15 *FPU*(g-substrate)$^{-1}$. Enzyme loading has a significant and complex impact on process economics and a conservative value was preferred. Determining an economical enzyme loading requires the optimization of various parameters (such as temperature, solids loading, residence/retention time, etc.) which is beyond the purposes of the current study.



(a)



(b)

Figure 6: Effect of the solids level after thickening on cellulose conversion for the continuous system. (a) τ_R=30 h and (b) τ_R=50 h.

Greater enzyme loadings like the one of the study of Xue *et al.* (2012) could significantly reduce retention/reaction times and keep conversions close to

those of 5% w/w. Finally, Xue *et al*. (2012) supplemented *xylanase* enzymes after liquefaction, but their action is not included in the current kinetic model. It is important to highlight that, when a solid loading of 20% w/w was added all at once (procedure 2 of Table 1), a reduction of 43% in sugar yield was observed when compared with the operation with split addition of enzyme (Procedure 4 of Table 1). In this sense, a continuous or semicontinuous operation with split addition of enzyme as proposed here offers advantages in terms of cellulose conversion.

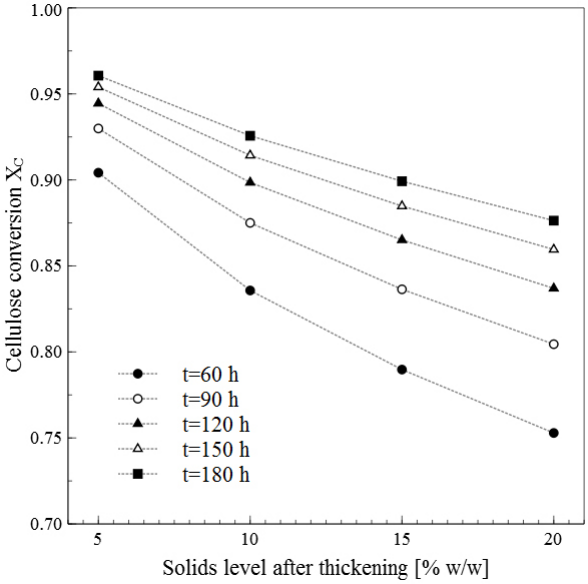


Figure 7: Effect of the solids level after thickening on cellulose conversion for the semicontinuous system at different reaction times.

A critical economical issue for the enzymatic hydrolysis of lignocellulosic biomass is the final glucose concentration. It has been stated that the ethanol concentration in the broth entering distillation should be greater than 40 g L^{-1} (Wingren *et al*., 2003). Assuming an ethanol yield of 0.48 g(g G)$^{-1}$, a G concentration of at least 83 g L^{-1} would be required to reach this target. G concentrations in Figure 8 were calculated as the average between the macrofluid and the microfluid model predictions. Figure 8(a) shows that, depending on τ_R, cascades with different numbers of *CSTR*s fit the aforementioned cutoff. For instance, with τ_R=10 h it is not possible to attain a G concentration of 83 g L^{-1}, whereas with τ_R=30 h 2 *CSTR*s are required. On the other hand, reaction times of 60 h are enough for attaining the mentioned G concentration in the semicontinuous system (Figure 8(b), see the dotted line for the abovementioned cutoff in glucose concentration).

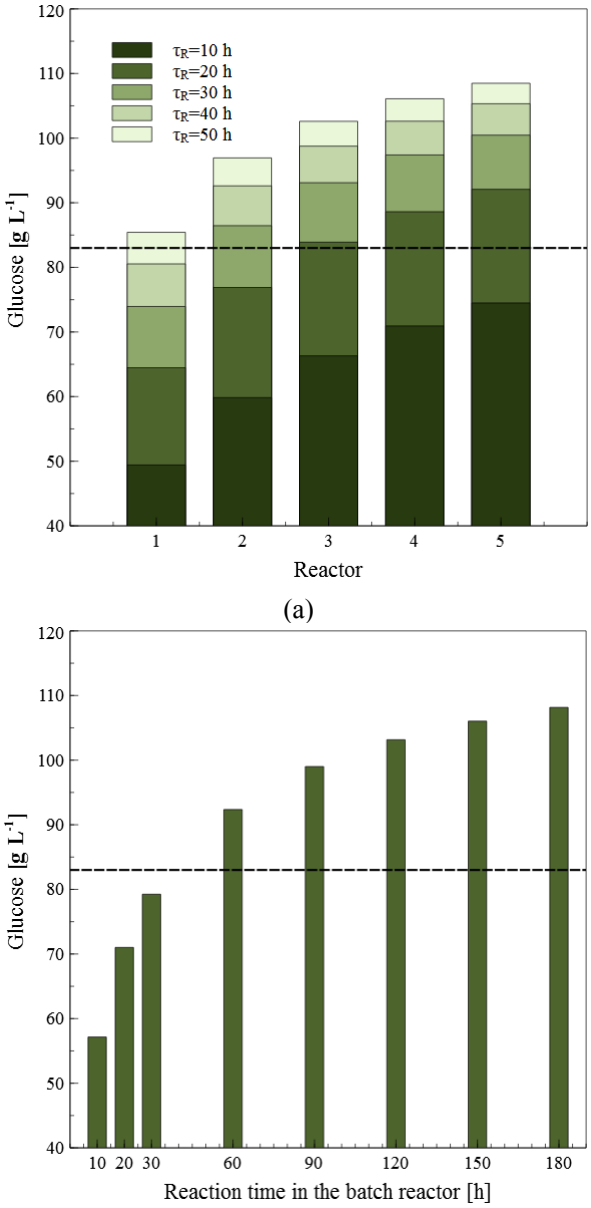


Figure 8: Glucose concentrations for a solid level after thickening of 20% w/w. (a) Continuous and (b) semicontinuous reaction system.

To strengthen the kinetic model there are suggestions related to three main aspects: enzyme adsorption, kinetic rates and long-time enzyme-substrate interactions. First of all, following the proposed scheme, recycled enzyme would be reutilized by readsorption on fresh substrate; however, experimental evidence is necessary to elucidate the potential of this alternative. Simulation results show that it is beneficial to add *BG* in the *AR*; however, Xue *et al*. (2012) added *BG* after liquefaction. The question of unproductive adsorption of *BG* should be clarified

experimentally. The split addition of enzymes implies enzyme adsorption on a partially hydrolyzed substrate in the presence of significant G and G_2 concentrations. Adsorption isotherms under these conditions should be obtained to improve the calculation of adsorbed enzyme after the *PFR*. Secondly, it would be desirable to include *xylanase* adsorption, xylan hydrolysis and enzyme inhibition by xylose in the kinetic model. Finally, the kinetic model used in this work accounts for substrate reactivity; however, enzyme deactivation is not taken into account. The kinetic model accounts for the adsorption of *BG/CBG* on *C* and *L*, which is a unique feature among the published models; a global adsorbed enzyme deactivation rate could further improve the predictions.

While the results of the present simulation study are encouraging, the experimental validation is crucial. A recent bench-scale study (Brethauer *et al*., 2014) reports the continuous simultaneous saccharification and fermentation of dilute acid-pretreated corn stover (2% w/w) in a train of three agitated vessels. Productivity at identical total residence times was 12% higher for operation with 3 *CSTR*s than for a single *CSTR*. To our best knowledge this study is the first step towards the validation of continuous operation strategies like the one presented in this paper. Brethauer *et al*. (2014) concluded that the simulation results in one of our previous papers (González Quiroga *et al*., 2010a) pointed in the same direction as their experimental results. On the other hand, the work of Xue *et al*. (2009) supports the importance of the liquefaction stage, although experimental studies on the liquefaction of the thickened slurry in a *PFR* should be carried out.

To validate the modeling framework and the proposed operation strategy, the experimental work on enzyme adsorption at low solids loading should be continuous and with recycle of hydrolysate. The substrate concentration should be kept at 5% w/w by adjusting the feeding rate of fresh substrate. For different residence times in the *AR*, which imply different recycle ratios and reaction volumes in the AR, the effect of enzyme loading, split addition of enzymes and enzyme inhibition by final product could be clarified. While the optimization of enzyme loading and enzyme feeding mode are the most important results, the feasibility of operating with recycle ratios higher than 3.5 needs to be proved. For these adsorption experiments the *EG/CBH* loading should vary between 5 to 15 $FPU(\text{g-substrate})^{-1}$, while *BG* should be either absent or at a loading of 15 $CBU(\text{g-substrate})^{-1}$. Note that enzymes would be supplemented after the liquefaction stage to final equivalent loadings between 10 to 30 $FPU(\text{g-substrate})^{-1}$ and 15 $CBU(\text{g-substrate})^{-1}$.

Experimental research is essential to set the residence time of the material in the liquefaction stage. The most important variable in this stage is the minimum apparent viscosity of the material that allows mixing in conventional tanks. The interested reader is referred to Humbird *et al*. (2011) for the technical aspects of the measurement of apparent viscosity of the slurry. As previously stated, the residence time of this stage is strongly dependent on the enzyme loading, the efficiency of the previous adsorption stage, the nature of the substrate and the pretreatment method. For these liquefaction experiments, the *EG/CBH* and BG loadings vary according to the setting of the previous adsorption stage. A semi-empirical correlation between conversion, insoluble solids concentration, yield stress and apparent viscosity in the liquefaction stage is essential to connect kinetics and rheology, which allow controlling the operation. Regarding this last aspect, the work of Hodge *et al*. (2009) constitutes a good starting point for planning the experimentation.

A question that remains is whether to use continuous or semicontinuous operation. In general, continuous processing is the preferred mode of operation for the production of commodity chemicals because of reduced labor cost, improved process control and uniform product quality. On the other hand, a semicontinuous operation provides for greater flexibility and lower investment costs, and could be preferred for pilot plant research (Peters *et al*., 2003). As reaction systems are constrained by the required reaction volumes, a compromise between solids loading, conversion and final glucose concentration of the system has to be sought. This compromise could imply that the conversions greater than 0.85 assumed in previous economic evaluations of the technology (Humbird *et al*., 2011) are not realistic.

CONCLUSIONS

- The current modeling and simulation framework remains useful even if a more detailed kinetic model is to be used. Differences in raw material and/or the pretreatment process imply the fitting of the current kinetic model with the new set of experimental data.
- Glucose concentrations higher than 100 g L^{-1} could be attained with both of the alternatives for the third stage of the proposed operations. However, a compromise between solids loading, cellulose conversion and final glucose concentration has to be sought because reaction volumes of several hundred cubic meters are required.

- Experimental work is essential to elucidate relevant aspects such as reutilization of recycle enzyme by readsorption on fresh substrate and *BG* addition in the adsorption stage. Future kinetic models should incorporate *xylanase* adsorption, xylan hydrolysis and a global adsorbed enzyme deactivation rate.
- For detailed design purposes it is imperative to link kinetics and rheology. Semi-empirical relations to connect the progress of the enzymatic hydrolysis with insoluble solids concentration and yield stress should be developed. Besides, the *RTD* of the reaction systems, especially for the *PFR*, are required to take further advantage of the micromixing models.
- The current simulation framework can be extended from *SHF* to *SSF*.

NOMENCLATURE

AR	Adsorber-Reactor
BG	β - *glucosidas*e enzyme
C	Cellulose [g L^{-1}]
CBH	*Cellobiohydrolase* enzyme
CBU	*Cellobiase* Unit
CSTR	Continuous Stirred Tank Reactor
E	Residence time distribution function
E_1	*EG*/*CBH* [g-protein.L^{-1}]
E_2	*BG* [g-protein.L^{-1}]
EG	*Endoglucanase* enzyme
FPU	Filter Paper Unit
G	Glucose [g L^{-1}]
G_2	Cellobiose [g L^{-1}]
L	Lignin [g L^{-1}]
MS	Mechanical Separator
Nr	Number of reactors
NREL	National Renewable Energy Laboratory
PFR	Plug Flow Reactor
r_1	Heterogeneous reaction rate (*C* to G_2) [g h^{-1}]
r_2	Heterogeneous reaction rate (*C* to *G*) [g h^{-1}]
r_3	Homogeneous reaction rate (G_2 to *G*) [g h^{-1}]
RR	Recycle ratio
RTD	Residence Time Distribution
S	Solids [% w/w]
SHF	Separated Hydrolysis and Fermentation
SSF	Simultaneous Saccharification and Fermentation
STR	Stirred Tank Reactor
t	Elapsed time [s]
X	Conversion

Greek Symbols

β	A variety of *glucosidase* enzyme
Δ	Change
τ	Residence time [h]

Subscripts

AT	After thickening
B	Bound
F	Free (in solution)
I	Identifier of *CSTR i* in the cascade
P	*PFR* reactor
R	*CSTR* reactor
T	Total

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