# Enhancement of alkaline-oxidative delignification of wheat straw by semi-batch operation in a stirred tank reactor

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GRAPHICAL ABSTRACT
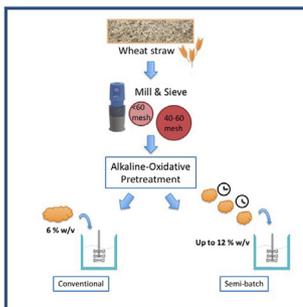
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ABSTRACT

This paper compares a semi-batch operation and a conventional one of an alkaline oxidative pretreatment of wheat straw carried out in a stirred tank reactor. For the pretreatment, different concentrations of biomass (6% up to 12% w/v) and two different particle sizes (mesh #40–60 and # > 60) were experimented. The performance of processes was evaluated through the analysis of lignocellulosic composition of the biomass, and the enzymatic hydrolysis of pretreated biomass using the Cellic® CTec2 enzyme complex by Novozymes®. The process time of semi-batch operation is significantly lower than the batch one and enables a higher load of biomass, showing a delignification yield between 55 and 60%. In the first 5 h of reaction time, the enzymatic hydrolysis experiments reached their maximum yields of 72 and 66% according to reducing sugars conversion when using the mesh # > 60 mesh and #40–60, respectively.

1. Introduction

In this century, the interest to harness the lignocellulosic biomass to produce biofuels and biomaterials has gained vital attention, becoming a promising solution to mitigate the greenhouse gas emissions (Singh et al., 2019). Lignocellulosic biomass is the largest source of renewable energy with the potential to make up > 10% of global energy supply (Wei et al., 2017). Lignocellulose is mainly composed by three main biopolymers such as cellulose (a long chain of D-glucose monomers), hemicellulose (polysaccharide of xyloglucans, xylans, mannans,

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glucomannans, and β-1,3 and β-1,4 glucans) and lignin (phenolic compounds such as p-coumaryl, coniferyl, and sinapyl alcohol) (Kim et al., 2019) and can be obtained from different sources including energy crops, waste materials (industrial, food and municipal solids), agricultural residues and grasses (Falls et al., 2019).

Ethanol should be produced from lignocellulosic biomass (second generation biofuel), because it represents a promising renewable energy source that is sustainable and non-polluting (Mohapatra et al., 2017). In particular, overall wood and agricultural residues may be more suitable from the economic and environmental points of view (Sadhukhan et al., 2019). However, second generation bioethanol is still not economically competitive compared to fuel ethanol from sugar and starch-based feedstocks (first generation biofuel) and neither with transportation fuels derived from petroleum. Thus, making a top priority the cost reduction of the production process through the improvement of the different steps involved (Liu et al., 2019).

Agricultural residues, mainly the ones derived from wheat, corn, sugarcane, sorghum and barley (Appiah-Nkansah et al., 2019), are considered to play an important role in future energy production, with estimates ranging from 23 to 35% of total lignocellulosic biomass consumption between 2017 and 2040 (Fitria et al., 2019). The wheat straw represents and agro-residue to pay attention due to its annual worldwide production is 850 million metric tons (Swain et al., 2019).

The stages to produce second-generation bioethanol include: (1) pretreatment, which focuses on modifying and rupturing structural characteristics of lignocellulosic biomass, maximizing sugar monomers production; (2) enzymatic hydrolysis, the process in which the sugar monomers such as pentoses and hexoses are liberated from cellulose and hemicellulose by enzymes action; (3) fermentation, in this step the released reducing sugars are converted to bioethanol by yeast or bacterium; (4) bioethanol purification, using separation processes like distillation and dehydration methods, and finally (5) waste treatment (Carrillo-Nieves et al., 2019).

Deconstruction of the highly recalcitrant lignocellulosic matrix into intermediates presents the principal hurdle to attain the economic viability essential to commercialize biomass-based technologies (Jiménez et al., 2020; Lin et al., 2019); therefore, several pretreatment methods have been widely studied and developed (Hernández-Beltrán et al., 2019). The performance of most pretreatment methods will differ in the optimal conditions when applied to different feedstocks (Nielsen et al., 2019); nevertheless, a notable pretreatment strategy should provide a number of key advantages such as size reduction of biomass (Hernández-Beltrán et al., 2020), enhancement of the yield of reducing sugars through a quick enzymatic hydrolysis process (Hernández-Beltrán and Hernández-Escoto, 2018), should limit the formation of inhibitory compounds, and minimize energy demands and operational cost requirement (Lin et al., 2020). In this sense, it is worthy to highlight that the pretreatment step constitutes > 40% of the total processing cost in a bioethanol production process (Bhutto et al., 2017).

Among numerous pretreatments of lignocellulosic biomass, the mechanical method enhances the surface area of feedstock, such operation is a prerequisite step for further chemical, physicochemical or biological method (Wang et al., 2019). During mechanical pretreatment, a decrease of particle size involves higher hydrolysis yield of the lignocellulose, however size reduction further than 0.4 mm (mesh #40) has little effect on hydrolysate rate (Bhutto et al., 2017). It has been found by De la Rubia et al. (2011) that excessive size reduction of biomass may reduce the performance of further steps. In this way, the optimization of lignocellulosic particle size is essential for achieving high sugars conversion and low production cost (Kapoor et al., 2019).

The Alkaline Oxidative Pretreatment (AHP) has gained attention due to its effectiveness on the delignification step using relatively mild process conditions; this leads to the low formation of inhibitors that negatively affect downstream steps of enzymatic hydrolysis and fermentation (Meléndez-Hernández et al., 2019). Additionally, hydrogen peroxide (H_2O_2) used for AHP is commercially available and

environmentally friendly (Yuan et al., 2018), facing up to the studies that point out the need of using high concentration loadings of alkali and oxidant reagent for significant changes on the chemical composition of the biomass, which allow an increase in the theoretical sugar recoveries in enzymatic hydrolysis (Fernández-Delgado et al., 2019; Shimizu et al., 2018); e.g. Del Carmen Fong Lopez et al. (2019) found the optimal concentration loading of sodium hydroxide (NaOH) at 7% on sweet corn biomass with an internal temperature of 130 °C; Martínez-Patiño et al. (2017) achieved 80% of delignification on olive tree biomass using 7% of H_2O_2 with 90 min of pretreatment process at 80 °C. Otherwise, balancing alkaline and peroxide reagents at low loadings is a key to optimize AHP based on the improvement of enzymatic hydrolysability and to lead economic viability (Alvarez-Vasco and Zhang, 2017).

Conventionally, pretreatment methods are performed between 2 and 10% w/v of solids due to the impossibility homogenization that the reaction mixture presents with higher concentrations (Aung et al., 2018; Camargo et al., 2019; Hernández-Beltrán and Hernández-Escoto, 2018). Thereby, the tendency is to develop pretreatment technologies to operate under high solids content, as in Hou et al. (2016) where the aim was to reduce waste water generation and steam consumption obtaining more pretreated

Lately, semi-batch process effect was investigated in the enzymatic hydrolysis process (Fockink et al., 2016), and Terán Hilares et al. (2019) compared the batch and semi-continuous operations on hydrodynamic cavitation pretreatment on sugarcane bagasse, but there is a lack of studies applied to pretreatments. The semi-batch pretreatment operates under semi-continuous regime, it means that the reacting system flow allows the continuous input or output of one or more species. In the approach of this work, the semi-batch pretreatment is focused to allow small inputs of substrate to the AHP medium. Once the biomass is reacting with the pretreatment solution, it quickly allows the input of more substrate. Therefore, semi-batch process represents a solution for mixing problems and to increase the solids concentration during the pretreatment in view of costs reduction (da Conceição Gomes et al., 2018).

The aim of this work is to increase the amount of wheat straw through semi-batch operation strategy in an AHP pretreatment carried out in a stirred tank reactor. Semi-batch strategy is taken as a dynamic process in which periodic loadings of small amounts of raw wheat straw are added with the goal to avoid operational problems related to mixing. The comparison with conventional batch pretreatment is discussed to show the advantages.

2. Materials and methods

2.1. Study approach

The study mainly consisted of carrying out an alkaline oxidative pretreatment in a stirred tank reactor fed with a frequent and small quantity of lignocellulosic material up to reach a load equivalent to the mass charged in a batch process. Fig. 1 shows the general outline followed, considering the mesh #40–60 and # > 60, both subjected to semi-batch operation evaluating different wheat straw loadings at 6, 8, 10 and 12% w/v, as well as the comparison with 6% w/v within batch operation as control experiment is carried out.

2.2. Alkaline-Oxidative Pretreatment

Raw wheat straw (RWS) was collected from El Salto, Jalisco (México) and it was milled and used in mesh sizes of #40–60 and # > 60. AHP pretreatment was carried out using a medium prepared with 2% v/v of NaOH and 2% v/v of H_2O_2. The process conditions were set at 300 rpm, 60 °C, and pH 11.5 for 5 h in a bioreactor of 1 L of reaction volume, as previously reported by Hernández-Beltrán and Hernández-Escoto (2018). A solid load equivalent to from 6% up to
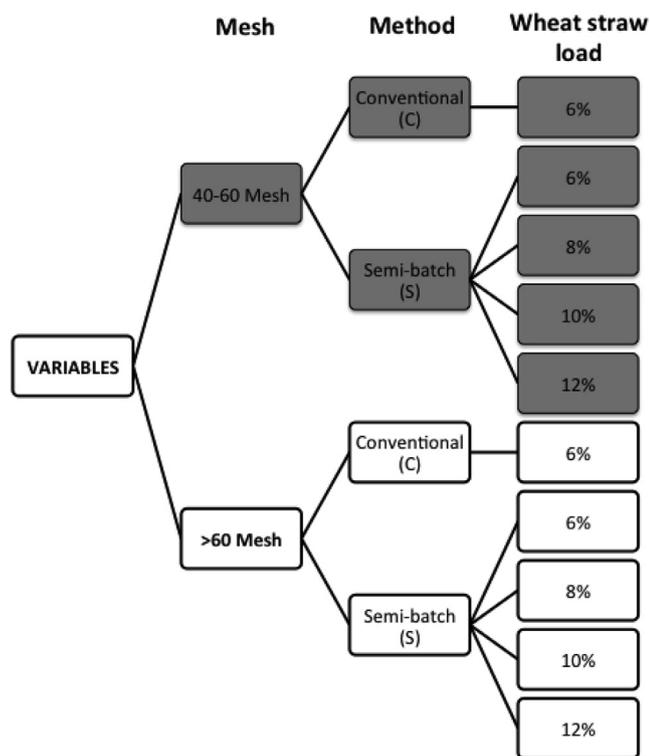
Fig. 1. Variables considered in the alkaline-oxidative pretreatment (particle size, method, and initial wheat straw load). A conventional method with 6% w/v wheat straw load was used as control.

12% w/v was directly used in the pretreatment. The use of a citrate buffer to regulate the pH was needed to reduce the variability of the experiments.

After pretreatment, the pretreated wheat straw (PWS) was filtered, washed with 1 L of distilled water for impurity removal, and stored at 4 °C.

The chemical composition analysis of RWS and PWS was carried out according to AOAC International methods (Goering and Van Soest, 1970). The RWS showed the following fractions (% dry weight basis): cellulose, 40.52 ± 1.01; hemicellulose, 28.92 ± 1.82; Klason lignin, 7.20 ± 1.16 and extractives, 23.37 ± 1.67.

2.2.1. Batch operation

The corresponding amount of RWS was added in one load to 920 mL of distilled water and heated at 50 °C, maintaining a low mechanical stirring of 100 rpm. Once the temperature of 50 °C was reached, NaOH and H_2O_2 were alternately added within 10 mL each time up to achieve the final concentrations: 30–40 mL of NaOH corresponds to 1.65–2% v/v and 40 mL of H_2O_2 corresponds to 2% v/v. The operation conditions were manually maintained along the process.

2.2.2. Semi-Batch operation

Semi-batch operation was performed by dosing an RWS handful of 3 g every 3 min up to complete the loadings presented in Fig. 1; e.g. the experiment of 6% w/v reached the final amount of 60 g of RWS until 1 h of reaction time, and the experiment of 12% w/v reached the amount of 120 g of RWS until 2 h of reaction time. The mechanical stirring was slow at the beginning of RWS dosing, and it was increased step by step until reaching 300 rpm; this depended on the homogeneity observed in the suspension after each wheat straw dose.

2.3. Lignin quantification and characterization

The lignin present in the liquid phase was analyzed using a separation procedure according to Sun et al., (2000). The effective removal of lignin makes the polysaccharides more reachable to digestion using hydrolytic enzymes, therefore, the quantification of the solubilized lignin indicates the efficiency of AHP pretreatment.

It has been previously determined the use of UV–Vis spectrophotometry at 280 nm to quantify the presence of lignin. Additional to the peak at 280 nm, the UV spectrum of lignin shows another maximum absorption at 230 nm and a small shoulder out around 315 nm, presented in grass lignin (Hatfield and Fukushima, 2005). The noise level at 280 nm is sufficiently low to allow sensitive determination and thus it was used to quantify the lignin.

2.4. Likely effectiveness evaluation of PWS in enzymatic hydrolysis

In order to corroborate that remaining lignin does not limit the saccharification and enables an effective conversion of PWS by enzyme complex, the enzymatic hydrolysis of PWS was evaluated by following a microreaction approach (Hernández-Beltrán et al., 2018).

The approach consisted into considering the yield of reducing sugars as response variable that is obtained from the enzymatic hydrolysis of PWS carried out in 1.5 mL microreactors, with an enzymatic load of 5 μL of the enzyme complex CTec2, varying the temperature at 45, 50 and 55 °C, pH of 4, 4.5 and 5, and mesh size of #40–60 and # > 60, and if the pretreatment was made in a batch or semi-batch operation. pH was regulated using citrate buffer in microreaction system of 1.5 mL. These factors were evaluated by a full factorial design in triplicate. Optimal conditions were found applying the response surface methodology (RSM) employing statistical software MiniTab-v 17.1 (Minitab Inc., PA, USA), where a quadratic model (1) was used.

$$Y = \beta_0 + \beta_1 * T + \beta_2 * pH + \beta_3 * Ms + \beta_4 * Me + \beta_{11} * T^2 + \beta_{22} * pH^2$$
$$+ \beta_{44} * Me^2$$
$$+ \beta_{12} * T * pH + \beta_{13} * T * Ms + \beta_{14} * T * Me + \beta_{23} * pH * Ms$$
$$+ \beta_{24} * pH * Me$$
$$+ \beta_{34} * Ms * Me \tag{1}$$

where Y is the yield of reducing sugars with respect to the amount of PWS, T is the temperature, Ms indicates the biomass mesh size, and Me, the delignification process operation, either batch or semi-batch.

The enzyme complex used was Cellic® CTec2 kindly provided by Novozymes® Latin America Ltda. (Rua Profesor Francisco Ribeiro, Araucária - Paraná, Brazil) with an enzymatic activity of 124 FPU/mL, determined by the NREL technique (Adney and Baker, 2008).

2.5. Enzymatic saccharification in a stirred-tank bioreactor

Using the predicted optimal conditions from microreaction system approach, enzymatic hydrolysis experiments were carried out in a stirred-tank bioreactor system in duplicate. The system consisted of a Corning® spinner flask of 0.5 L with mechanical stirring and a temperature controller device. pH was adjusted from the beginning using 100 mM citrate buffer at the determined optimal pH. Reactors were loaded with 5% w/v of PWS and 1.25% v/v of enzyme complex CTec2. Reducing sugars were determined using the DNS method established by Miller (1959). The processing time was 5 h due previously was demonstrated that enzymatic hydrolysis process at this time begins a stationary phase. Samples were taken every hour to construct the kinetic trajectory.

3. Results and discussion

3.1. Chemical composition analysis

Table 1 shows the fractions of cellulose, hemicellulose, lignin, and extractives of RWS, and PWS obtained from the different pretreatment

Table 1
Chemical composition of wheat straw before and after alkaline-oxidative pretreatment.

	Component	RWS	Batch6% load	Semi-batch 6% load	Semi-batch 8% load	Semi-batch 10% load	Semi-batch 12% load
40–60 mesh	Cellulose	40.52 ± 1.01	64.26 ± 2.38	64.89 ± 3.16	63.68 ± 0.54	62.45 ± 1.42	64.70 ± 2.36
	Hemicellulose	28.92 ± 1.82	19.76 ± 2.12	16.59 ± 2.77	15.35 ± 1.73	19.35 ± 2.16	15.94 ± 1.40
	Lignin	7.20 ± 1.16	2.97 ± 0.17	3.60 ± 0.11	3.44 ± 0.57	2.80 ± 0.04	2.81 ± 1.12
	Extractive	23.37 ± 1.67	13.00 ± 0.08	14.92 ± 0.27	17.54 ± 1.70	15.39 ± 0.70	16.56 ± 0.15
> 60 mesh	Cellulose	40.52 ± 1.01	66.92 ± 0.69	65.52 ± 0.80	59.95 ± 0.95	60.66 ± 0.75	59.61 ± 0.76
	Hemicellulose	28.92 ± 1.82	17.12 ± 1.90	16.82 ± 1.31	17.68 ± 1.78	17.73 ± 0.78	19.19 ± 1.37
	Lignin	7.20 ± 1.16	3.30 ± 0.09	3.25 ± 0.08	2.87 ± 0.94	3.37 ± 0.66	3.37 ± 0.51
	Extractive	23.37 ± 1.67	12.66 ± 1.13	14.41 ± 0.43	19.49 ± 1.77	18.24 ± 2.19	17.84 ± 1.12

Table 2
Enzymatic saccharification yields obtained by microreaction of pretreated wheat straw at optimal conditions.

	Method	Initial wheat straw load(%)	Optimal temperature (°C)	Optimal pH	Saccharification yield RS/PWS (%)	Saccharification yield RS/Ho (%)
Mesh #40–60	Conventional	6	45	5	28.31 ± 0.003	37.18 ± 0.005
	Semi-batch	6	45	4	28.53 ± 0.004	36.72 ± 0.006
		8	51	4	28.30 ± 0.007	36.40 ± 0.002
		10	55	4.76	26.38 ± 0.012	33.89 ± 0.001
		12	45	4.18	28.34 ± 0.012	36.50 ± 0.011

	Method	Initial wheat straw load(%)	Optimal temperature (°C)	Optimal pH	Saccharification yield RS/PWS (%)	Saccharification yield RS/Ho (%)
Mesh # > 60	Conventional	6	52	5	33.96 ± 0.005	49.53 ± 0.003
	Semi-batch	6	55	5	34.56 ± 0.005	55.60 ± 0.006
		8	54	4.72	34.08 ± 0.003	49.71 ± 0.004
		10	45	5	34.18 ± 0.006	49.88 ± 0.011
		12	48	4	33.76 ± 0.013	49.28 ± 0.013

RS .- Reducing Sugars, PWS .- Pretreated Wheat Straw, Ho .- PWS Holocellulose.

experiments. Compared with the RWS composition, PWS got a greater cellulose fraction, between 19 and 26% more, and with respect to hemicellulose and extractives, PWS got lower fractions, between 9 and 12% less, and 4 and 11% less, respectively. These outcomes go along with the fact that the structure of hemicellulose is mainly amorphous which makes it susceptible to be easily crumbled during pretreatment by the alkali reagents; in the meanwhile, the cellulose is mainly crystalline, which makes it hard to suffer a structural change. So, it is typically observed after pretreatment that cellulose fraction in biomass increases and the hemicellulose one decreases (Kim et al., 2016).

Observing Table 1, the semi-batch pretreatments at different biomass concentrations with the particle size of mesh #40–60 yielded a PWS of similar cellulose fraction to the one obtained through corresponding batch pretreatment at 6% w/v, and with respect to the other components, the fractions are not significantly different. This shows that for medium particle size, the semi-batch process has the same effect on biomass than the batch one; but it is worthy to recall that the amount of biomass pretreated is greater (up to twice). Unlike, batch and semi-batch experiments with biomass of mesh # > 60 were a little different each other; in comparison with PWS from batch experiments, the cellulose fraction of PWS from semi-batch ones is around 7% less (except for the experiment of 6% load), the fraction of hemicellulose is similar, and the fraction of extractives is a little greater. It was expected that as smaller the size of biomass as greater the cellulose fraction of PWS, but this effect did not occur in semi-batch pretreatments. With respect to lignin fraction, the values are in the same magnitude order for all the pretreatments.

On the other hand, Table 1 evidenced that the use of AHP pretreatment on smaller particle sizes and the increase of substrate concentration, the cellulose content recovered in the biomass is lower by 6%. Therefore, AHP is more able to use with a particle size of mesh #40–60 than a smaller one.

The efficiencies of lignin removal were 57% ± 3.74 and 60% ± 2.16 using mesh #40–60 and # > 60, respectively. Rego et al. (2018) showed a lignin removal of around 60% using an AHP pretreatment with 3% of H_2O_2 on sugarcane bagasse. Dong et al. (2019)

reached a lignin removal of 62% through alkaline pretreatment on 10% w/v of rice straw solids as well. Huang et al. (2019) suggested an alkaline extraction method by using urea after pretreatment to improve the lignin removal in 30% and as well the yield of enzymatic hydrolysis process in a 45% w/w. Therefore, the efficiency of lignin removal did not decrease with semi-batch pretreatment.

On the application and adjustment of analysis technique, the typical UV spectrum of lignin showed two absorption maxima at 230 and 280 nm. The wavelength value of 280 nm of the spectra represents peak associated with lignin, being this one the same for all pretreatments and being compared with pure lignin from Sigma-Aldrich®. Considering the spectra trajectories for the different loads evaluated in AHP pretreatment, it can be pointed out, as the greater the loads, the greater the absorbance; meaning that removed lignin increases with the substrate load, as expected.

3.2. Likely effectiveness evaluation of PWS in enzymatic hydrolysis

Table 2 shows the higher yields that were reached in the enzymatic hydrolysis carried out in microreactors, of the PWS obtained from the batch and semi-batch pretreatments; i.e., the better process conditions (pH and temperature) were chosen among the experiment sets for every particular biomass mesh size.

As it can be observed, the optimal conditions of every case are different from each other; however, the yields are in the same magnitude order within each biomass mesh size. Microhydrolysis experiments carried out with PWS of mesh #40–60 reached a similar yield of around 28% (g-reducing sugar/g-PWS). The same effect was found with PWS of mesh # > 60, reaching a yield between 33 and 34% (g-reducing sugar/g-PWS). These outcomes evidenced that AHP pretreatment on semi-batch operation had the same delignification effectiveness as the conventional one but processing up to twice the amount of RWS.

Table 2 data followed a test of analysis of variance (ANOVA) for the different PWS loads, which was considered as independent variable with 5% of significance ($p < 0.05$). ANOVA test found that PWS concentrations did not influence the enzymatic hydrolysis carried out at
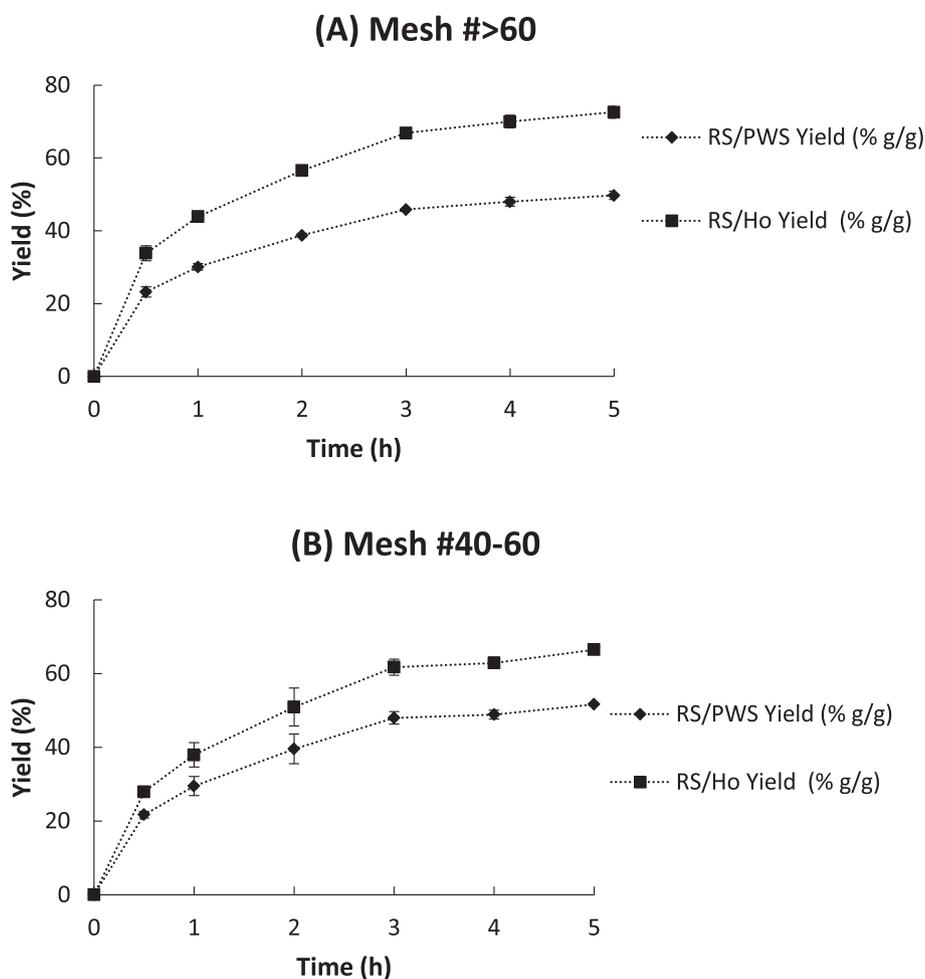
(A) Mesh #>60

(B) Mesh #40-60

Fig. 2. Sugar yields from enzymatic hydrolysis of wheat straw after alkaline-oxidative pretreatment using different particle sizes: mesh # > 60 (A) and #40–60 (B). RS = Reducing Sugars, PWS = Pretreated Wheat Straw, Ho = PWS Holocellulose. Data represent the mean of two independent experiments; error bars indicate the SD.

the microreaction scale. Conversely, the smaller particle mesh size allowed a significant interaction between the substrate and the enzymatic complex, increasing the saccharification yields. Table 2 displays the difference of yields between the two particle sizes compared (40–60 mesh vs > 60 mesh). Approximately 6% difference in the yield of reducing sugars according to PWS and 14% of difference in the yield of reducing sugars according to holocellulose content. This effect has been previously reported in different studies (Han et al., 2012), although some other works have shown that reduction of biomass particle below 0.4 mm has no significant effect on rate and yield of hydrolysis (Kumar and Sharma, 2017).

All the outcomes of the experiments of enzymatic hydrolysis in microreactors at different process conditions, following the Response Surface Methodology, are enclosed in the following quadratic model (2):

$$Y = 11 - 2.44 * T + 9 * pH + 35.3 * Ms + 6.28 * Me + 0.0327 * T^2$$
$$- 0.32 * pH^2 - 0.015 * Me^2$$
$$+ 0.037 * T * pH - 0.327 * T * Ms - 0.0638 * T * Me$$
$$- 3.06 * pH * Ms - 0.33 * pH * Me$$
$$- 0.578 * Ms * Me \tag{2}$$

Commonly, the related works in the scientific literature report the optimization of operation variables as temperature, pH or enzyme load but they do not consider the relation with operation variables from pretreatment process that quadratic model (2) provides such as the

mesh sizes, operation mode (batch and semi-batch methods) and load of biomass in the pretreatment process. The standard deviation of the quadratic model (2) for the adjustment to the experimental data was 4.3%, which means that the error is within the limit according to a confidence of 95%.

3.3. Enzymatic Saccharification in stirred-tank bioreactor

Considering PWS coming from a semi-batch pretreatment process, the quadratic model (2) estimates as best enzymatic hydrolysis conditions the following: temperature of 45 °C, pH of 4.18 and substrate loading of 12% w/v for the particle size #40–60, whereas for particle size # > 60, temperature of 48 °C, pH of 4 and substrate loading of 12% w/v. It should be noted that for both particle sizes the highest yield can likely be achieved with PWS coming from the pretreatment of highest load, which means a positive effect in view of the use of high solids loadings in the pretreatment process through semi-batch operation. Besides, it indicates that even with the processing of the same substrate and pretreatment for both particle sizes, the best process conditions are different. This motivates and justifies performing a previous optimization task if one of the variables involves changes for the enzymatic hydrolysis.

With the optimal process conditions mentioned above, enzymatic hydrolysis experiments in a stirred tank reactor were carried out, whose evolutions are shown in Fig. 2. The kinetics were monitored during 5 h of reaction time, since the optimal conditions enabled a quick

conversion of holocellulose-to-sugars. Fig. 2A shows the trajectories for the experiments carried out with a particle size of mesh > #60. Regarding holocellulose content, the reducing sugars yield achieved was 72.55% ± 1.67; and considering a more practical performance index by regarding the total amount of PWS loaded into the bioreactor, the reducing sugars yield reached 49.74% ± 1.14. The standard deviation for every sample fit in the 5% of error.

On the other hand, with respect to the enzymatic hydrolysis of PWS of the size of mesh #40–60, Fig. 2B shows the evolution in which the highest reducing sugars yield was of 66.44% ± 0.5, according to holocellulose content. The yield regarding the total amount of PWS was 51.64% ± 0.40, which got a similar value to the one of the PWS of the size of mesh # > 60. The standard deviation for every sample was no > 5%, except for the second hour of reaction, with an error of 5.16%.

It was evidenced a positive effect related to the use of the smallest particle size with an increment of sugars yield according to the holocellulose content. The milling process can decrease the degree of crystallinity of cellulose and thereby an eventual increase in the accessible surface area and pore size of the RWS (Ponnusamy et al., 2019). Thus, it favors the interaction between the enzyme complex and PWS (Sun et al., 2016). However, there is no universal optimal particle size for lignocellulosic substrates, but it needs to be found for every case considering the nature of raw material and the pretreatment method (Hernández-Beltrán et al., 2019; Zhang et al., 2019). Although Fig. 2A and Fig. 2B show a higher yield (around 6% w/w) with the use of particle size of mesh # > 60, obtaining a small particle size could lead to a high energy requirement.

4. Conclusions

This work demonstrated that, in a single stirred tank reactor, high amounts of wheat straw (up to an equivalent load of 12% w/v-dry basis) can be easily pretreated with an alkaline-oxidative medium at mild process conditions through a semi-batch operation, and that pretreated biomass keeps the composition and easiness to be converted to fermentable sugars as the one obtained from conventional batch pretreatment; even more, the biomass pretreated in semi-batch reactor enabled fast enzymatic hydrolysis processes, of time lower than 5 h, with yields above of 65% (g-reducing sugar/g-holocellulose).

Credit authorship contribution statement

Alicia Hernández-Guzmán: Methodology, Formal analysis, Software. **Ivette Michelle Navarro-Gutiérrez:** Methodology, Formal analysis. **Perla Araceli Meléndez-Hernández:** Formal analysis, Validation. **Javier Ulises Hernández-Beltrán:** Investigation, Writing - original draft, Writing - review & editing. **Héctor Hernández-Escoto:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2020.123589.

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