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Hot Compressed Water Pretreatment and Surfactant Effect on Enzymatic Hydrolysis Using Agave Bagasse

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Abstract: Agave bagasse is a residual biomass in the production of the alcoholic beverage tequila, and therefore, it is a promising raw material in the development of biorefineries using hot compressed water pretreatment (hydrothermal processing). Surfactants application has been frequently reported as an alternative to enhance monomeric sugars production efficiency and as a possibility to reduce the enzyme loading required. Nevertheless, the surfactant's action mechanisms in the enzymatic hydrolysis is still not elucidated. In this work, hot compressed water pretreatment was applied on agave bagasse for biomass fractionation at 194 °C in isothermal regime for 30 min, and the effect of non-ionic surfactants (Tween 20, Tween 80, Span 80, and Polyethylene glycol (PEG 400)) was studied as a potential enhancer of enzymatic saccharification of hydrothermally pretreated solids of agave bagasse (AGB). It was found that non-ionic surfactants show an improvement in the conversion yield of cellulose to glucose (100%) and production of glucose (79.76 g/L) at 15 FPU/g glucan, the highest enhancement obtained being 7% regarding the control (no surfactant addition), using PEG 400 as an additive. The use of surfactants allows improving the production of fermentable sugars for the development of second-generation biorefineries.

Keywords: lignocellulosic materials; biomass; biofuels; hydrothermal processing; biorefinery

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1. Introduction

Worldwide, lignocellulosic biomass is one of the most abundant renewable sources and can be transformed into biofuels through physical, chemical, and biological processes [1,2]. Lately, bioethanol has been considered a key alternative to overcome fossil fuels dependence. However, bioethanol commercialization is still unfeasible due to high production costs related mainly to enzymatic hydrolysis of the cellulose contained in the cell wall of the plants into soluble sugars for subsequent fermentation [3,4]. Many factors preclude cellulosic enzymatic hydrolysis for large-scale bioethanol production from lignocellulosic materials [5]. The saccharification efficiency is low due to the hydrolysis rate's fast decrease over time, which produces long process times; in addition, the process requires high enzyme loadings, which leads to high production costs because cellulase enzymes are expensive [6–8]. Additionally, cellulase enzymes tend to deactivate and lose activity during the hydrolysis process by the presence of several compounds, such as xylan, cellobiose, pretreatment degradation products, and lignin. Specifically, lignin can act as a physical barrier that prevents the enzyme access to the cellulose surface. Lignin is a hydrophobic aromatic polymer. Cellulase enzymes have a great affinity for lignin, mainly caused by hydrophobic, electrostatic, or hydrogen-bonding interactions, which causes the enzyme to adsorb onto lignin's surface, producing cellulases' non-productive binding that reduces their activity. Hydrophobic interactions have been reported as the most influential

on non-specific binding of cellulases. Proteins are more adsorbed on the hydrophobic surfaces of substrates; hence, cellulases have more affinity for lignin than for cellulose, which reduces the efficiency of the enzymatic hydrolysis [9–13].

Consequently, to overcome the mentioned drawbacks, several alternatives have been studied to enhance the efficiency of enzymatic hydrolysis of cellulose into soluble sugars. One of them consists of the application of additives, such as surfactants and polymers, which can improve the enzymatic efficiency and decrease the amount of enzyme needed in the process. Surfactants are amphiphilic molecules that contain both hydrophilic and lipophilic groups that have the ability to reduce surface tension and help to remove hydrophobic molecules and modify the structure and surface of biomass [14]. Different mechanisms have been proposed to explain the action of surfactants in the enzymatic saccharification process: (1) surfactants modify the structure of the biomass and allow greater accessibility to cellulose, facilitating the adsorption and desorption of the enzyme; (2) surfactants increase the stability of the cellulase enzyme by decreasing the denaturation of the protein by thermal factors or shear forces; and (3) it has been reported that surfactants reduce the non-productive adsorption of the cellulase enzyme on lignin by adsorbing the additives onto the exposed surface of the lignin [6,13,15,16]. Moreover, surfactants addition aims to reduce enzyme quantity with saccharification-yield improvement.

Different types of surfactants have been studied in the field of enzymatic hydrolysis enhancement; however, non-ionic surfactants have been found to be the most suitable for cellulosic saccharification [6]. Ooshima et al. [17] evaluated surfactants with different compositions of head-group polarity (anionic, cationic, amphoteric, and non-ionic) for the enzymatic saccharification using four pure cellulose substrates. They found out that non-ionic surfactants showed the best cellulose-conversion yields.

On the other hand, the agave bagasse is an important residual biomass of the tequila alcoholic beverage industry in Mexico, and about of 40% of the total *Agave Tequilana* Weber blue variety is bagasse. In addition, the chemical composition composed of cellulose, hemicellulose, and lignin makes this biomass a promising raw material in the development of second-generation biorefineries [18–24]. Additionally, hot compressed water pretreatment is an important hydrothermal process where the water is pressurized in the liquid and vapor phase due to the thermodynamic equilibrium, causing an autoionization of the water and the production of acetic acid from the hemicellulosic fraction, both acting as catalysts in the process and fractionating the biomass in a liquid phase of hemicellulosic fraction and a solid fraction rich in cellulose and lignin [25–29]. Figure 1 shows the schematic representation of agave bagasse processing using hot compressed water pretreatment.

The main objective of this work was to evaluate non-ionic surfactants (Tween 20, Tween 80, Span 80, and Polyethylene glycol (PEG 400)) to enhance enzymatic hydrolysis using hot compressed water pretreatment on agave bagasse in the production of fermentable sugars (glucose) and higher cellulose conversion into glucose.

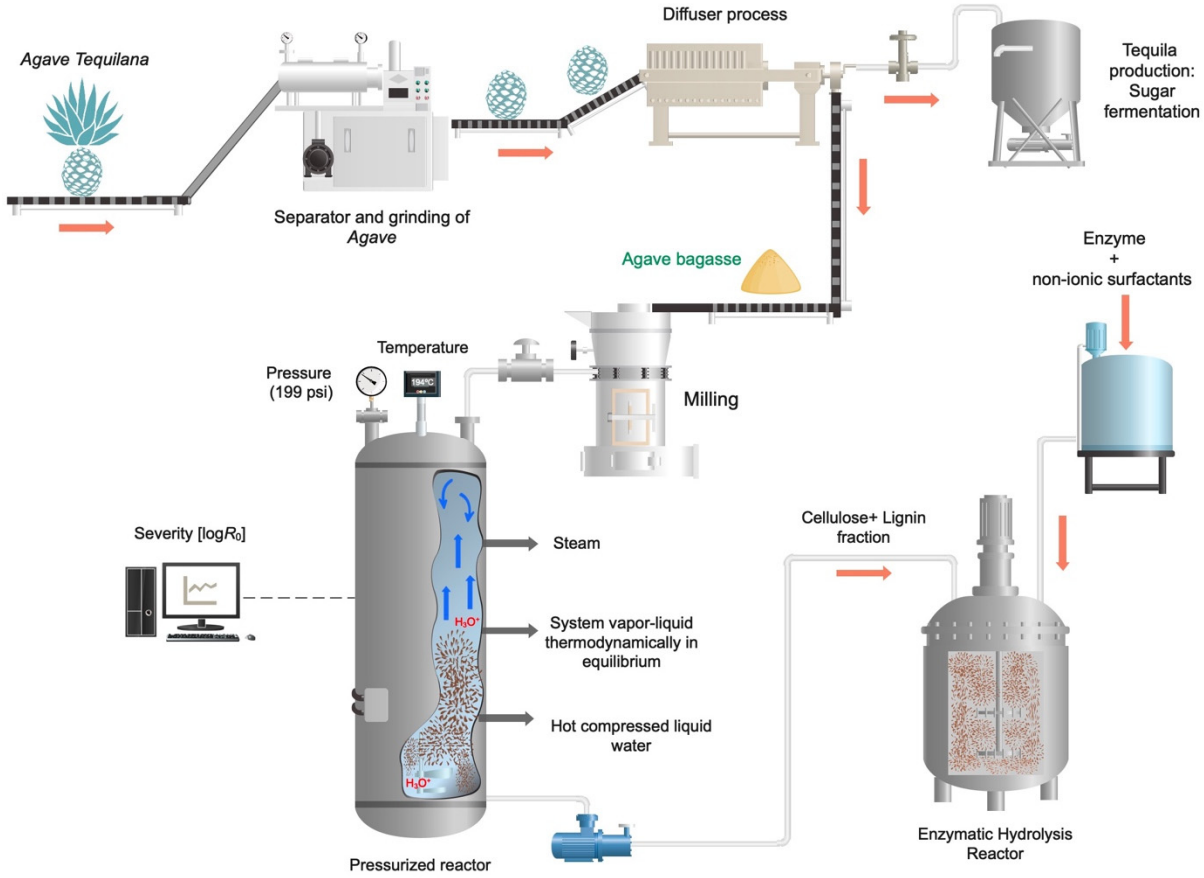

Figure 1. Schematic representation of the general process in the production of fermentable sugars using hot compressed liquid water pretreatment, agave bagasse, and non-ionic surfactants.

2. Materials and Methods

2.1. Raw Material

Agave bagasse used in this work was kindly provided by the tequila factory (Distillery Leyros, Tequila, Jalisco, Mexico). The chemical composition of agave bagasse was previously reported by Pino et al. [18]. AGB was milled, obtaining a particle size between 0.5 mm and 1.0 mm, using a blade mill.

2.2. Hot Compressed Water Pretreatment

AGB and water were mixed in a solid/liquid ratio of 1:10 (w/v) [19]. The slurry was processed under an isothermal heating regimen in a stainless-steel Parr reactor with temperature controller (2 L, Parr Instrument Company, Moline, IL, USA). The operational conditions were selected according to previous results for hot compressed water pretreatment [18]. The temperature in the reactor was 194 °C for 30 min. After the residence time, the reactor was cooled down and the slurry (liquid phase (hemicellulose) and solid phase (cellulose + lignin)) was filtrated to separate these fractions. The solid phase was washed with distilled water. The moisture content was considered as water during the pretreatment.

Subsequently, the solid fraction obtained in the pretreatment was characterized for glucan, xylan, arabinan, and Klason lignin by quantitative acid hydrolysis methodology reported by Ruiz et al. [30].

The severity index was used as a parameter to compare the operational conditions, as described in Equations (1) and (2) [31,32].

$$logR_o = [R_o\ Heating] + [R_o\ Isothermal\ process] + [R_o\ Cooling] \quad (1)$$

$$logR_o = \left[\int_0^{t_{max}} \frac{T(t) - 100}{\omega}\right] + \left[\int_{ctrl}^{ctrf} exp\left[\frac{T(t) - 100}{\omega}\right] dt\right] + \left[\int_0^{t_{max}} \frac{T(t) - 100}{\omega}\right] \quad (2)$$

where $logR_o$ is the severity factor, t_{max} (min) is the time needed to achieve the maximum autohydrolysis temperature, ctrl and *ctrf* (min) are the times needed for the whole heating-cooling period, *T(t)* (°C) are the temperature profiles in heating and cooling, respectively, and ω is an empirical parameter.

After the pretreatment, the solid phase was analyzed by HPLC. Furthermore, xylooligomers (XOS) were quantified in the liquid phase [30].

2.3. Evaluation of Surfactants Effects on Enzymatic Hydrolysis

As mentioned above, surfactants have been reported to reduce non-productive binding of cellulase enzymes onto lignin's surface. Therefore, before the study of the surfactants' effect on the saccharification process, we evaluated the effect of lignin on the enzymatic hydrolysis of microcrystalline cellulose Avicel PH 101, using commercial lignin with alkali low-sulfonate content from Sigma-Aldrich. The enzymatic digestion was carried out at 10% (*w*/*v*) solid loading on a working volume of 10 mL on 25 mL shake flasks at 50 °C with a shaking speed of 150 rpm for 72 h. A total of 50 mM citrate buffer was used to reach a pH of 4.8 in the reaction mixture. Cellulase enzyme Cellic Ctec2 with an initial activity of 123 FPU/mL was used. The enzyme loading employed was 15 FPU/g glucan. To monitor the reaction advance, samples were taken at 0, 6, 12, 24, 48, and 72 h. The substrate mixture for the saccharification assay was established according to the composition achieved on the hydrothermally pretreated AGB with the purpose of simulating the effect of lignin on the enzymatic hydrolysis of the pretreated AGB by using the same concentration of the treated biomass. Hence, the substrate consisted of 0.5365 g of Avicel and 0.3539 g of lignin. The assays were performed in duplicate. Additionally, a control was run using microcrystalline cellulose without the addition of lignin.

Once we determined the effect of lignin on the enzymatic reaction, the evaluation of the surfactant effect on the enzymatic hydrolysis process was developed in 3 stages, represented in Figure 2. It is important to mention that each of the stages was established based on the results obtained in the previous stage. The assays were carried out using a solid loading of 10% (*w*/*v*) with an enzyme loading of 15 FPU/g glucan, using a commercial cellulase cocktail (Cellic Ctec2) from *Trichoderma reesei*, generously provided by Novozymes, with a cellulase activity of 123 FPU/mL. The working volume was fixed to 10 mL on 25 mL flasks, and sodium citrate buffer with a pH of 4.8 was added. The reaction was developed with a stirring speed of 150 rpm in a CERTOMAT® incubator at 50 °C for 72 h. In each of the stages, a control was run that consisted of an assay at the same conditions but without surfactant addition. The tests were carried out in duplicate using sodium azide as antimicrobial. The enzymatic hydrolysis reaction was monitored over time at 0, 6, 12, 24, 48, and 72 h, where aliquots of 300 µL were taken to analyze sugar production; samples were centrifuged at 140 rpm for 10 min, and the supernatant recovered was analyzed by HPLC with a MetaCarb 87H (300 × 7.8 mm) column at 45 °C using a Jasco chromatograph; the eluent was sulfuric acid 0.005 mol/L at a flow rate of 0.6 mL/min. The samples were analyzed for monomeric sugars (glucose, cellobiose, xylose, and arabinose).

2.3.1. Surfactant Screening

The first stage consisted of a surfactant screening to determine the most appropriate for the enzymatic hydrolysis of hydrothermally pretreated agave bagasse. Four different surfactants were preselected: Tween 20, Tween 80, Span 80, and Polyethylene glycol (PEG 400), displayed in Figure 3. It has been reported that non-ionic surfactants are the most efficient additives for enzymatic hydrolysis enhancement, with PEG and Tween as the most commonly used ones [3,6,16].

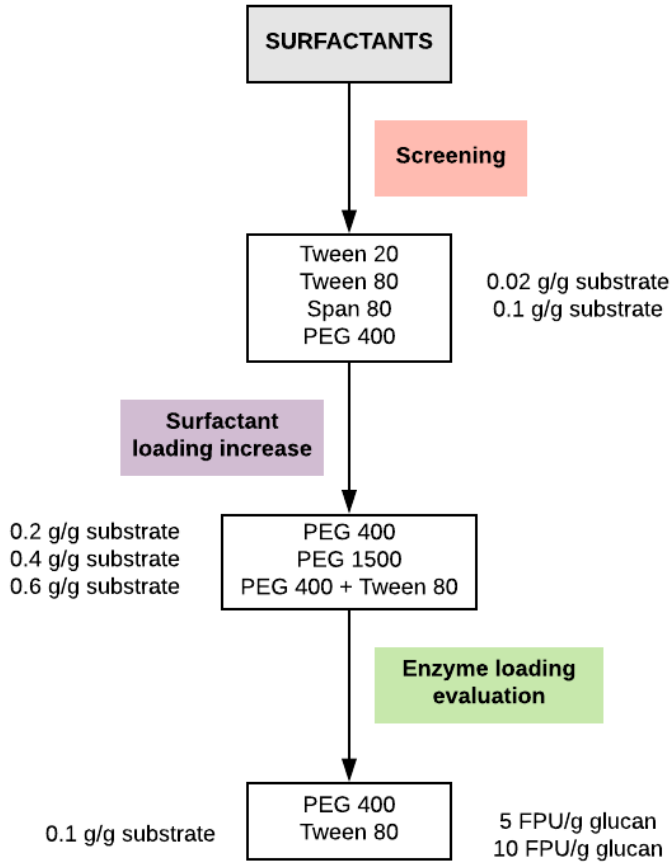

Figure 2. Methodology diagram for evaluation of surfactants' effect on enzymatic hydrolysis.

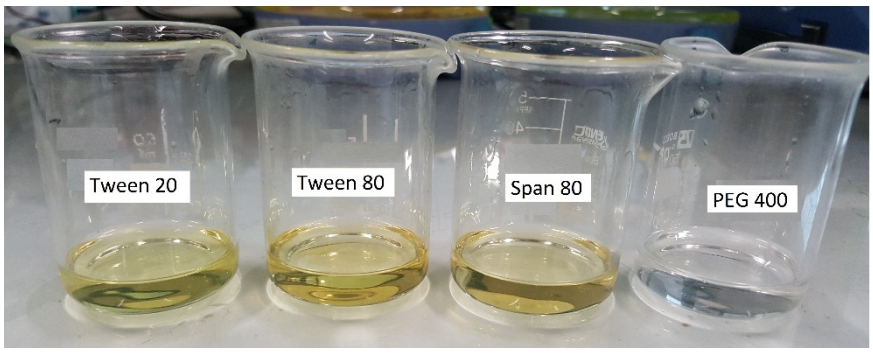

Figure 3. Surfactants evaluated for the enhancement of enzymatic saccharification.

Additionally, as proposed by Eriksson et al. [16], the hydrophile–lipophile balance (HLB) numbers were considered for the pre-selection of the surfactants, which consists of an empirical expression that relates hydrophilic and lipophilic groups on the surfactant's molecules so that a surfactant with a higher HLB number has a stronger hydrophilic property [1]. Table 1 summarizes HLB values for the non-ionic surfactants pre-selected for stage 1 [16,33]. Span 80 was evaluated as an alternative due to its lower HLB value. The surfactant concentrations evaluated in this stage were 0.02 and 0.1 g/g substrate since these are usual surfactant loads for enzymatic saccharification of cellulose [34,35].

Table 1. HLB number for pre-selected surfactants for screening assay.

Surfactant Type	Hydrophile–Lipophile Balance (HLB)
Tween 20	16.7
Tween 80	15
PEG 400	11.6
Span 80	4.3

2.3.2. Surfactant Loading

The second stage consisted of increasing the surfactant concentrations to evaluate if there was an improvement on the enzymatic conversion when working with higher surfactant loadings. Surfactant concentrations of 0.2, 0.4, and 0.6 g/g substrate were evaluated for PEG 400 since it was the surfactant that showed the best glucose production after 72 h of enzymatic saccharification in stage 1, followed by Tween 80. In addition, the effect of PEG 1500 surfactant was studied, which is another polyethylene glycol with a higher molecular weight and a HLB value of 16.1 [36]. Additionally, the mixture of PEG 400 with Tween 80 in a ratio of 1:1 was studied to determine its effect on the bioprocess.

2.3.3. Enzyme Loading Evaluation

Finally, the third stage consisted of the study of enzyme loading effect on the saccharification process with the aim to reduce the enzyme quantity. In this stage, PEG 400 and Tween 80 were used because they showed the best results in the first stage. Cellulase enzyme loadings of 5 and 10 FPU/g glucan with a surfactant concentration of 0.1 g/g substrate were evaluated.

3. Results

The hydrothermal pretreatment carried out at 194 °C for 30 min in 2 L reactor, with a heating rate (8.13 °C/min), produced a severity factor of 3.93. The chemical composition of the solid fraction obtained during the pretreatment is summarized in Table 2.

Table 2. Chemical composition of autohydrolyzed agave bagasse at optimal conditions (expressed as percentage by dry material weight).

Component	Composition (%)
Cellulose	53.65 ± 0.51
Hemicellulose	2.89 ± 0.16
Lignin	35.39 ± 0.57

3.1. Evaluation of Surfactants Effect on Enzymatic Hydrolysis

The effect of lignin addition on enzymatic hydrolysis is illustrated in the glucose-production kinetic shown in Figure 4, where lignin presence clearly demonstrated to render a negative effect on the glucose concentration in the enzymatic hydrolysis process. The glucose production underwent a reduction at 72 h of reaction from 72.92 g/L to 49.77 g/L with the lignin addition, which corresponded to a decrease of 31.75%. These results are consistent with previous reports. Rahikainen et al. [11] studied the effect of two lignin-rich residues on the enzymatic hydrolysis of Avicel and demonstrated that both lignin preparations decreased the saccharification efficiency, for which the adverse effect increased with the increment of lignin's concentration. Moreover, Ko et al. [37] isolated lignin from mixed hardwoods to study its effect on cellulases activity. The researchers found a higher inhibition for β-glucosidase, with enzyme activity recoveries ranging from 2 to 18% after reaction with lignin, while endoglucanases and exoglucanases showed a lower inhibition, with 50 to 60% remaining activity.

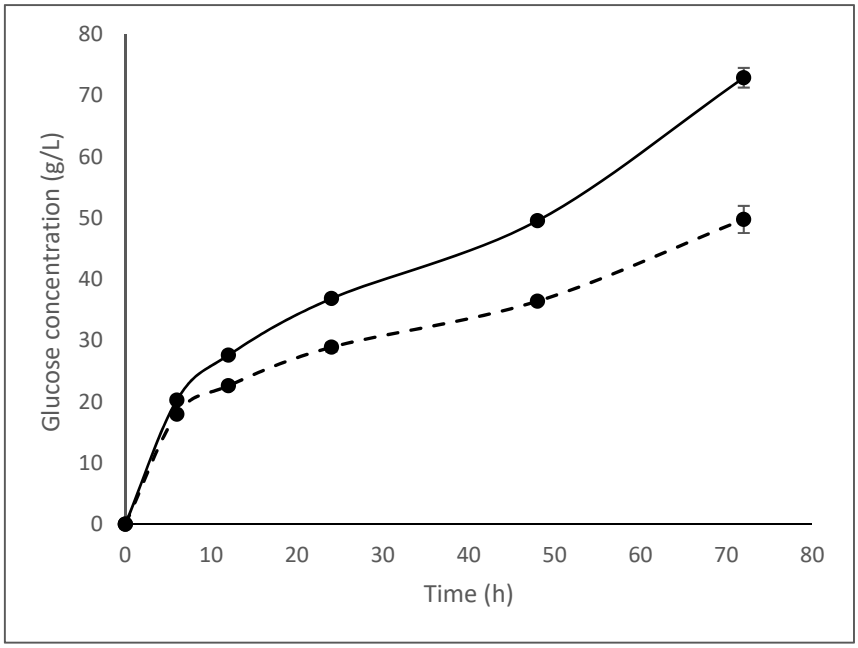

Figure 4. Glucose-concentration kinetics of lignin effect on enzymatic hydrolysis of Avicel at 10% solid loading. (—) Control; (- -) 35.39% Lignin.

3.1.1. Surfactant Screening

Figure 5A–D presents the kinetics of glucose production by the cellulase enzyme in the presence of Tween 20, Tween 80, Span 80, and PEG 400. It can be observed that, regarding the control, additives Tween 20, Tween 80, and PEG 400 showed some improvement in the glucose concentration produced at 72 h; however, in the case of the Span 80 surfactant, both evaluated surfactant concentrations, 0.02 and the 0.1 g/g substrate, were below the glucose production of the control. The inefficiency of Span 80 can be attributed to its low HLB number, related to a low hydrophilic property, which probably indicates a lower capacity to stimulate the desorption of the enzyme from the binding site on the surface of the substrate after the hydrolysis was carried out on that site [1]. Bardant et al. [35] studied the enzymatic hydrolysis of empty palm fruit bunches' pulp using Tween 20 and Span 85 and reached higher cellulose-conversion yields for Tween 20 than the ones obtained for Span 85; the authors attributed that behavior to the lowest HLB of Span 85. Moreover, Oliva-Taravilla et al. [38] studied the effect of several biosurfactants on the enzymatic saccharification of pretreated spruce. The authors argued that the differences on cellulose conversion between saponins and rhamnolipid was related to the chemical structure of their aglycones. However, rhamnolipid contains shorter hydrophilic moieties compared to saponins, whose hydrophilic moieties are longer. Therefore, rhamnolipid presents lower HLB than saponin, and the results are in concordance with the current study.

The highest glucose concentration achieved in this stage was 79.76 g/L with PEG 400 and a surfactant concentration of 0.1 g/g substrate; however, it should be noted that, regarding the control, it only represents an enhancement of 7% in the glucose production, while the other conditions evaluated were below this percentage. Zhou et al. [3] found similar results to the ones reached in this work; they studied the effect of Tween 20 and Tween 80 using filter paper and microcrystalline cellulose as substrate, where cellulose conversions enhancements lower than 5% were achieved. In addition, the authors investigated the effect of shaking speed, pH, cellulose crystallinity, and structural features of the substrate, concluding that the positive effect of the surfactants was restricted by several factors, including surfactant type and substrate features, as well as saccharification operational conditions. Moreover, Alencar et al. [39] found no enhancement with the addition of Tween 80 in the enzymatic hydrolysis of cactus pear.

Non-ionic surfactants have been highly reported as reducing sugars-production enhancement in enzymatic hydrolysis of the cellulose process; nevertheless, most of the investigation in this matter was developed using cellulose model substrate (pure cellulose) [13,15,39]. Lignocellulosic biomass has different hydrophobic properties than pure cellulose, mainly due to the significant lignin content in its structure, which may interfere in the surfactants' ideal action [3].

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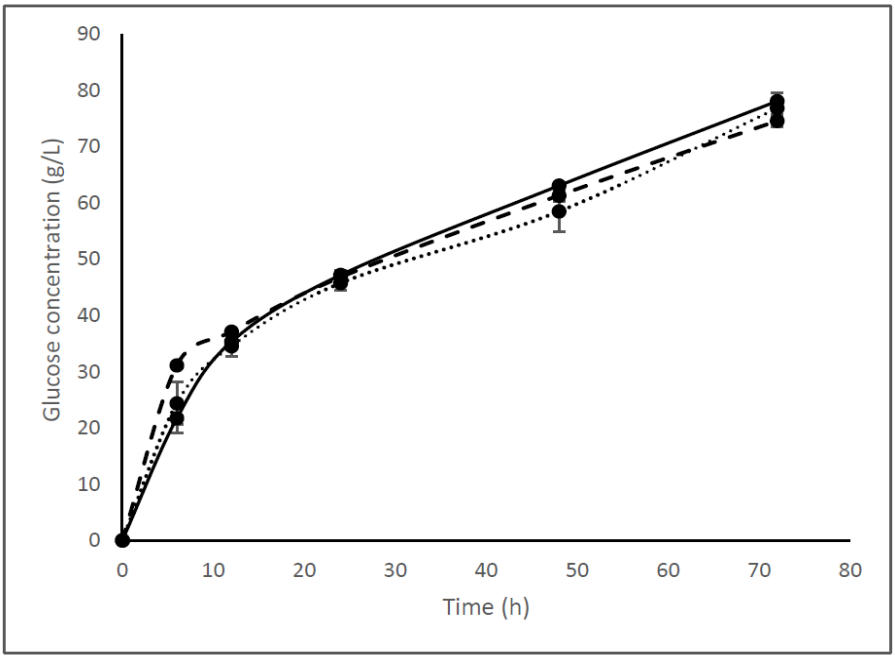

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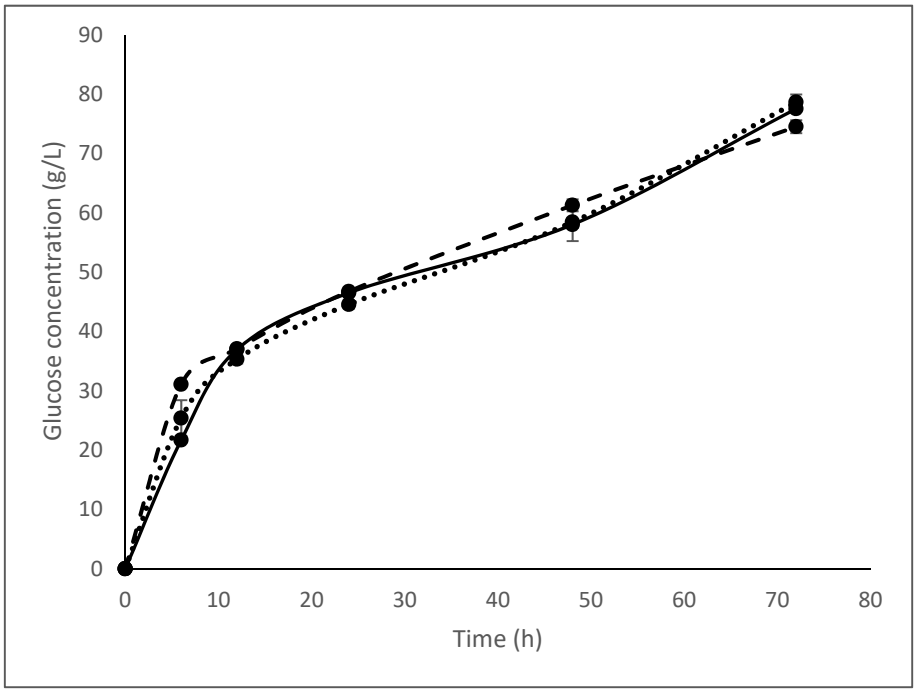

Figure 5. *Cont*.

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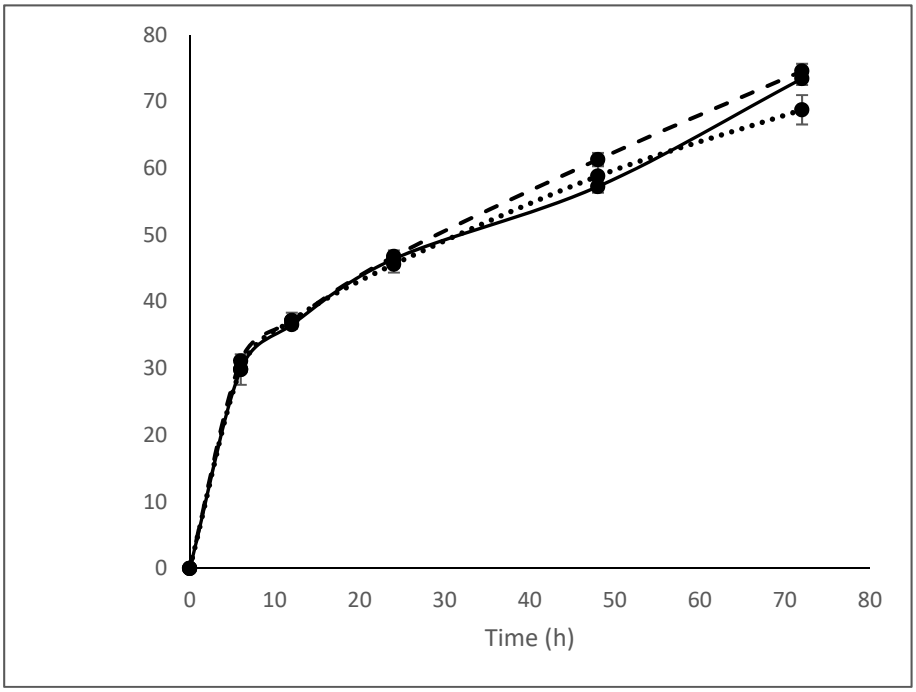

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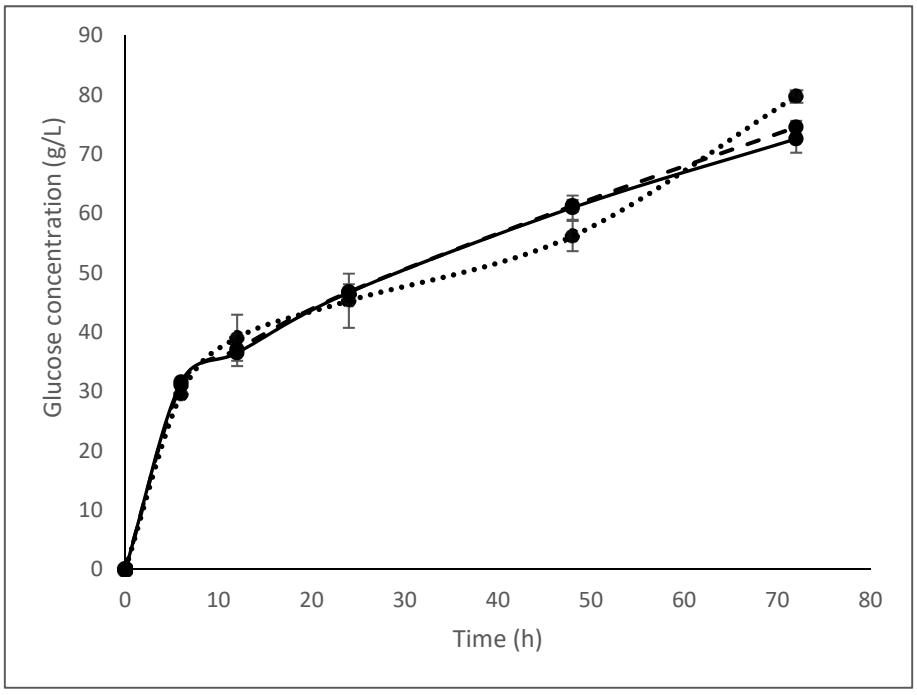

Figure 5. Enzymatic hydrolysis kinetics for surfactant screening. (**A**) Tween 20; (**B**) Tween 80; (**C**) Span 80; (**D**) PEG 400. (—) 0.02 g surfactant/g substrate; (···) 0.1 g surfactant/g substrate; (- -) Control.

The completely randomized design statistical analysis with four factors (surfactant type), two levels (surfactant concentration), and two repetitions allowed to reject the null hypothesis, which means that there is a significant difference among the treatments evaluated, with 95% confidence level. The analysis of variance (ANOVA) is summarized in Table 3. Furthermore, due to the difference found on the effect of the type of surfactant on the glucose produced in the enzymatic hydrolysis, a multiple means comparison test was performed according to Tukey's criteria (presented in Table 4), where it was determined that the two best treatments were PEG 400 and Tween 80, with a concentration of 0.1 g/g substrate, since they produced the highest sugars concentration at 72 h of saccharification.

Table 3. ANOVA for surfactant screening.

	DF	SS	MS	F	*p*-Value
Treatments	7	197.489	28.213	6.02	0.011000
Error	8	37.493	4.687		
Total	15	234.983			

Table 4. Tukey multiple comparison test for surfactant screening.

Treatment		Glucose Concentration (g/L)	0.05
Surfactant Type	Surfactant Concentration (g/g Substrate)		
PEG 400	0.1	79.760	a
Tween 80	0.1	78.675	a
Tween 20	0.02	78.055	ab
Tween 80	0.02	77.570	abc
Tween 20	0.1	76.800	abc
Span 80	0.02	73.415	bcd
PEG 400	0.02	72.585	cd
Span 80	0.1	68.755	d

The treatments that do not have the same letters are significantly different.

3.1.2. Surfactant Loading

According to the results obtained in stage 1, PEG 400 was used for further analysis. Additionally, a mixture of PEG 400 and Tween 80 and a polyethylene glycol with higher molecular weight (PEG 1500) were tested at increased surfactant concentrations to evaluate their performance at greater loads. No improvement was achieved for glucose production regarding the control with surfactant concentrations of 0.2, 0.4, and 0.6 g/g substrate, as displayed in Figure 6A–C for PEG 400, PEG 400 + Tween 80, and PEG 1500, respectively, where the kinetics of each of the surfactant assays were below the glucose-production kinetics of the control. Different authors have stated that the surfactant concentration increase is not proportional to a rise in the enzymatic saccharification efficiency. Ouyang et al. [40] did not find improvement in cellulose conversion with the increase of PEG 4000 concentration from 0.08 to 0.14 g surfactant/g substrate in Avicel hydrolysis. The highest sugars concentration addressed by the researchers was achieved at a surfactant concentration of 0.05 g/g glucan. In addition, Zhou et al. [3] found an inhibitory effect of surfactants Tween 20 and Tween 80 using filter paper and microcrystalline cellulose, which was accentuated with the increase in the concentration of the surfactant. Likewise, the authors demonstrated that the additive PEG 4000 did not contribute to a significant enhancement in the conversion of cellulose to monomeric sugars. Withal, Park et al. [1] reported a negative effect of Tween 80 using newspaper as substrate when the concentration of the surfactant was above 0.25 g/g newspaper. On the other hand, Eriksson et al. [16] ascertained that the increments of PEG 4000 concentration on the enzymatic hydrolysis of steam-pretreated spruce from 0.5 g/L to 5 g/L corresponded to greater cellulose-conversion yields, reaching the highest conversion with the highest concentration of surfactant. However, it should be noted that 5 g/L is equivalent to 0.05 g/g substrate, a concentration below the ones evaluated in stage 2 of the present study. Therefore, it can be stated that high surfactant concentrations have an inhibitory effect on the enzymatic saccharification.

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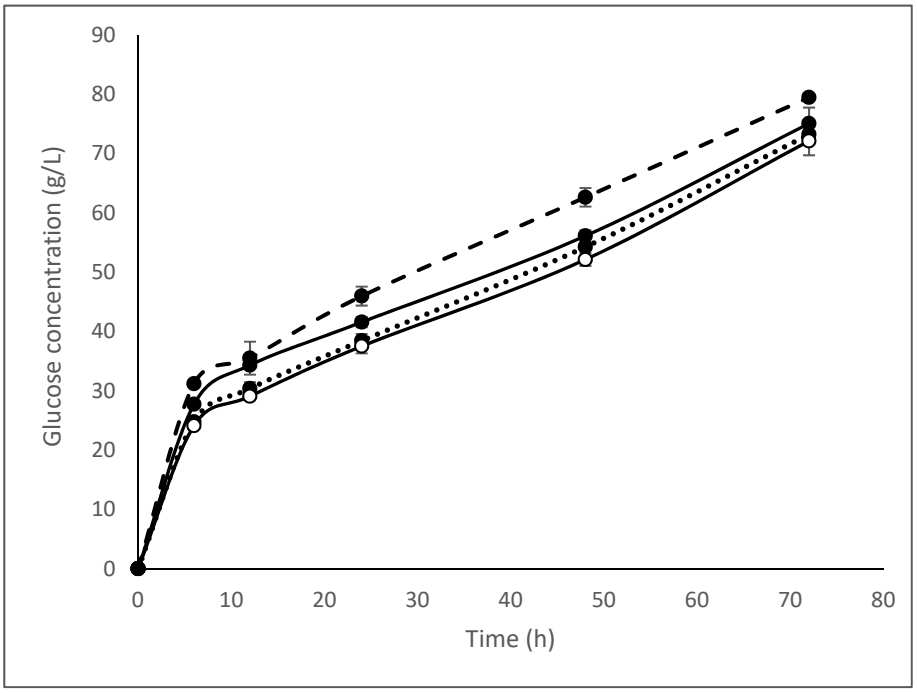

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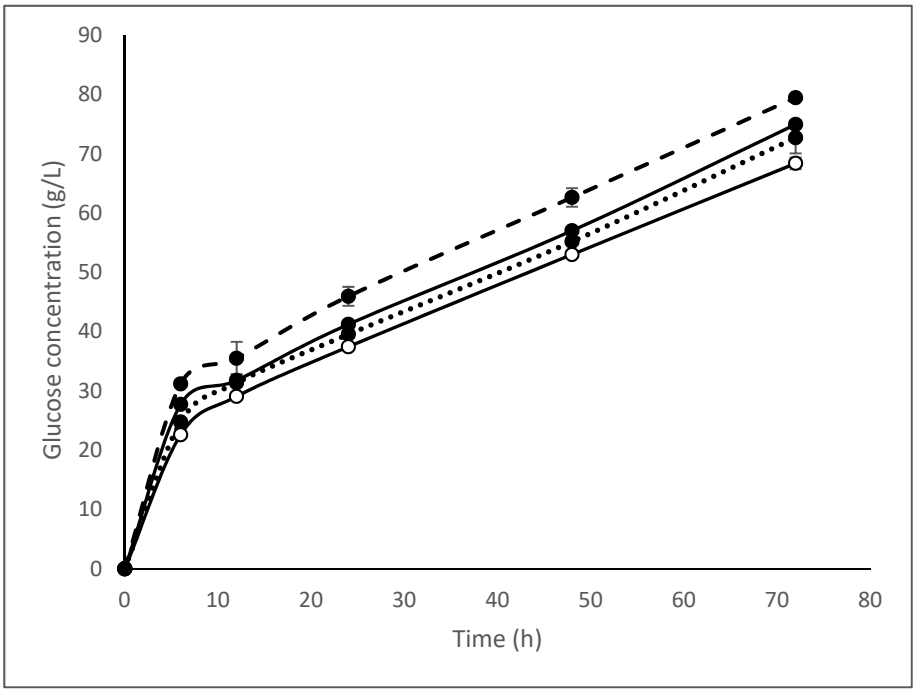

Figure 6. *Cont*.

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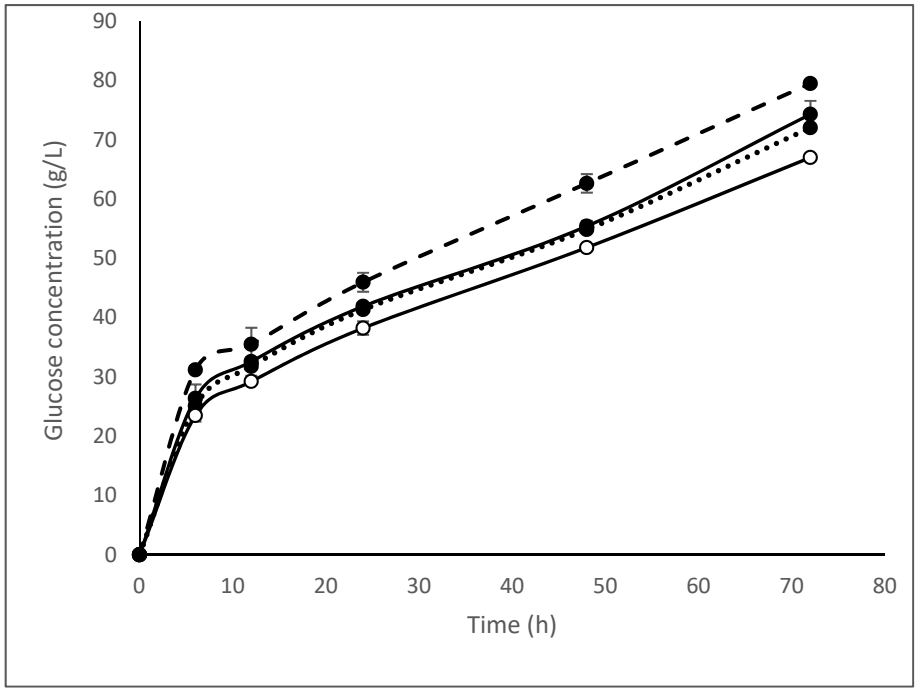


Figure 6. Enzymatic hydrolysis kinetics for surfactant loading increase. (**A**) PEG 400; (**B**) PEG 400 + Tween 80 mixture; (**C**) PEG 1500. (—) 0.2 g surfactant/g substrate; (···) 0.4 g surfactant/g substrate; (○) 0.6 g surfactant/g substrate; (- -) Control.

3.2. Enzyme Loading Evaluation

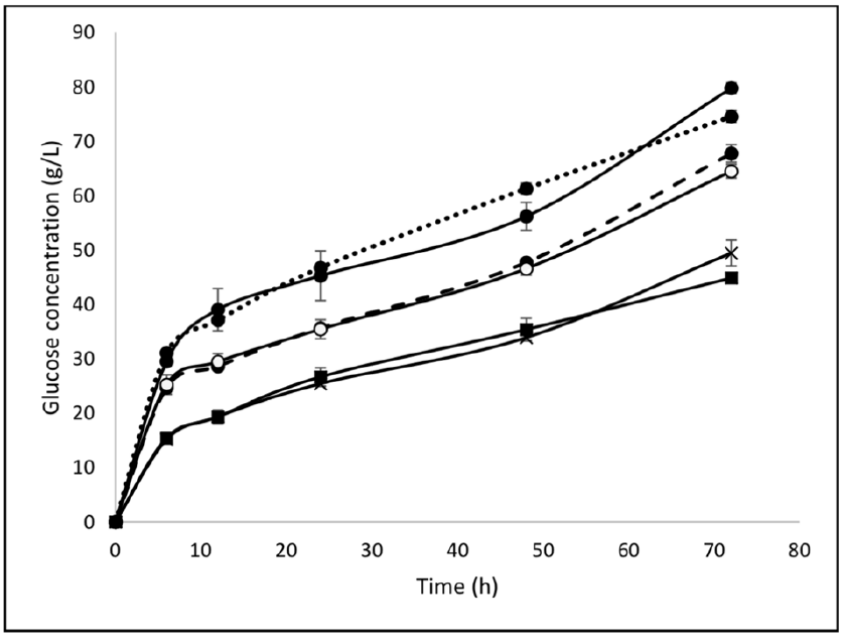
The study of the effect of the enzyme loading on the saccharification of cellulose with the addition of surfactants is presented on Figure 7A,B for PEG 400 and Tween 80, respectively, and for cellulase enzyme loadings of 5, 10, and 15 FPU/g glucan and a fixed surfactant concentration of 0.1 g/g substrate. The results showed that higher enzyme loadings produce greater glucose concentrations; however, regarding the control, the improvement with the application of the surfactant was limited, reaching a maximum increase of 7% and 5.6% with respect to the control for the enzyme loading of 15 FPU/g glucan, where 5 and 10 FPU/g glucan enzyme loadings were below these percentages. In a recent work, Aguirre-Fierro [22] reported 110.5 g/L of fermentable sugars from agave bagasse using 20% (*w*/*v*) of high solid loading. Perez-Pimienta et al. [41] studied the recalcitrance of agave bagasse applying different pretreatments. They reported 42.5, 39.7, and 26.9 kg (glucose and xylose) per 100 kg of biomass as yield conversion in the enzymatic hydrolysis for ammonia fiber-expansion pretreatment, ionic liquid, and autohydrolysis, respectively.

As mentioned previously, one of the main objectives of using surfactants in enzymatic hydrolysis is to reduce saccharification process costs by reducing the quantity of enzyme employed, the high cost of which makes the saccharification process economically unfeasible, as well as maximizing the glucose production [7]. The analysis of variance for the factorial statistical analysis with two factors (surfactant type and enzyme loading) with two repetitions is presented on Table 5. The ANOVA demonstrated that the enzyme loading as well as the interaction between surfactant type and enzyme loading were significant features in the concentration of glucose produced in the enzymatic hydrolysis, with a 95% of confidence level. Nonetheless, the surfactant type did not present significance.

Consequently, it can be deduced that the utilization of the non-ionic surfactants evaluated does not promote a reduction in the enzyme quantity employed in saccharification due to the greater cellulose digestibility at higher enzyme loadings. Finally, on the opposite side, Oliva-Taravilla et al. [38] found a positive effect of the addition of saponins through a reduction in the enzyme dosage required for achieving similar cellulose conversion on pretreated spruce, where 6 g/100 g red saponin dosage combined with 7.5 FPU/g cellulase

loading gave a conversion comparable to that achieved by using 4 g/100 g saponins and 10 FPU/g.

(**A**)



(**B**)

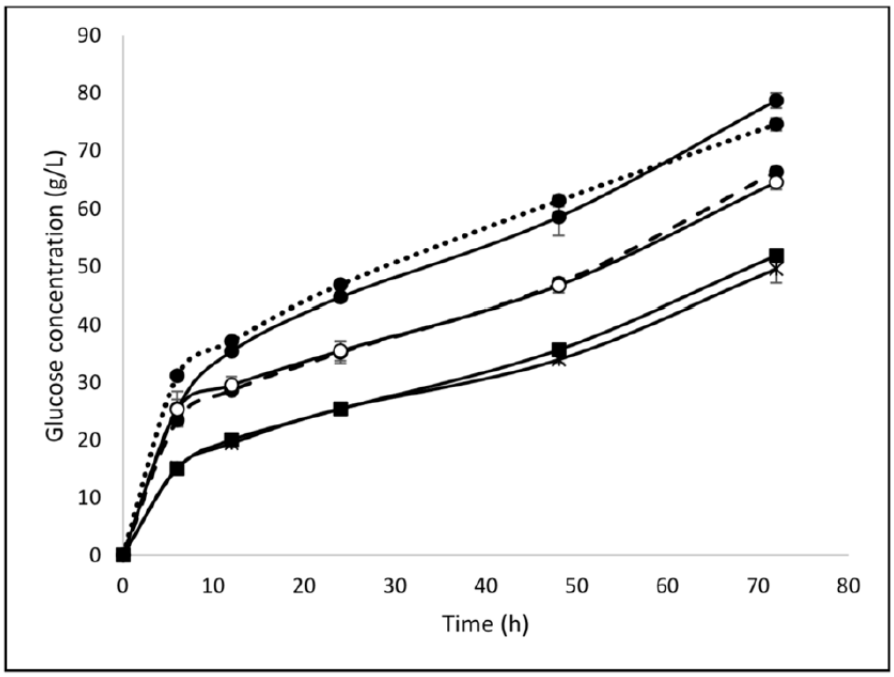


Figure 7. Enzymatic hydrolysis kinetics for enzyme loading evaluation. (**A**) PEG 400; (**B**) Tween 80. (■) 5 FPU/g glucan; (- -) 10 FPU/g glucan; (—) 15 FPU/g glucan; (X) Control 5 FPU/g glucan; (○) Control 10 FPU/g glucan; (···) Control 15 FPU/g glucan.

Table 5. ANOVA for enzyme loading evaluation on surfactant effect.

	DF	SS	MS	F	*p*-Value
Surfactant type	1	0.18	0.18	0.04	0.842733
Enzyme loading	2	1738.39	869.19	203.50	0.000003
Interaction	2	71.56	35.78	8.38	0.018335
Error	6	25.63	4.27		

The concentrations of glucose obtained in this study are higher than those achieved by Nogueira et al. [42] and Li [43] using PEG4000 as surfactant; however, the values are lower compared with the studies reported by Vignesh et al. [42] and Agrawal et al. [44]. Some of the works reported in Table 6 used high solid pretreated loading in the enzymatic hydrolysis process (20–35%, *w*/*v*); therefore, the operation of high solid loading is an important operative strategy that together with the use of surfactants can overcome the development of second-generation biorefineries producing high concentrations of fermentable sugars.

Table 6. Enzymatic hydrolysis and glucose production of different lignocellulosic biomasses using different pretreatments strategies and surfactants.

Raw Material	Pretreatment	Enzymatic Hydrolysis	Surfactant Type	Glucose Production (g/L)	References
Agave bagasse	Hot compressed water	Solid loading of 10% (*w*/*v*) and 15 FPU/g substrate	PEG 400	79.76	Present study
Cotton microdust	Two-stage alkali-acid pretreatment	Solid loading of 35% (*w*/*v*) and enzyme loading 22 FPU/g glucan	Polyethylene glycol (PEG)	134	[45]
Oil palm fruit bunch	Sodium hydroxide	Solid loading of 2% (*w*/*v*) and enzyme loading 10 FPU/g solid fiber	Tween 80	10.75	[46]
Rice straw	Pilot scale—dilute sulfuric acid	Fed batch mode, solid loading of 20% (*w*/*v*) and 3 FPU/g total solids	Ecosurf E6 (Alcohol Ethoxylate)	132	[44]
Green coconut fiber	Steam explosion	Solid loading of 5% (*w*/*v*) and 20 FPU/g substrate	PEG 4000	9.9	[42]
Poplar fibers	Vacuum drying	Solid loading of 2% (*w*/*v*) and 25 FPU/g substrate	PEG 8000	7.15	[47]
Rice straw	Nitric acid	Solid loading of 2.5% (*w*/*v*)	PEG 4000	2.345	[43]

4. Conclusions

The results from this work demonstrated that agave bagasse is a promising raw material, and hot compressed water pretreatment is an efficient process in the fractionation of lignocellulosic biomass. In addition, the addition of such non-ionic surfactants as PEG 4000 during the enzymatic hydrolysis stage is a good supplement to improve the conversion yield of cellulose into fermentable sugars (79.76 g/L), improving the process by up to 7% with respect to the non-addition of surfactants. Moreover, different studies must be carried out in the future to optimize the enzymatic hydrolysis process using a high pretreated solid loading, reduction of the amount of surfactants and enzyme loading, impacting the cost and development of second generation biorefineries.

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