Commercial Washing Detergents-Assisted Alkaline Pretreatment for Lignocellulosic Sugars Production: A First Report

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Abstract Lignocellulosic sugars are the major renewable building blocks for green fuels and chemicals production. However, the implication of an effective pretreatment process is an inevitable process to access the biomass sugars. Alkaline pretreatment is a viable pretreatment process, causing a selective removal of lignin, with a minimum degradation of carbohydrates, increasing porosity and surface area, eventually enhancing enzymatic hydrolysis. Here, we have assessed commercial cloth washing detergents as catalytic agents, for the lignin removal from sugarcane bagasse. Three different detergents (Brilhante® (B), Omo® (O), Sabonito Flash® (F)) were tested using three different concentrations (5, 10 and 15%) with and without pH adjustment. Pretreatment with O5pH (5% Omo®, pH 12) showed the maximum lignin removal (81.14%) and retainment of cellulose (44.15%) and hemicellulose (29.71%) in the pretreated bagasse. The maximum sugars (26.62 g/L) were released from the O10pH-pretreated sugarcane bagasse. This study shows the potential of washing detergents as the new potential catalytic agents for the pretreatment of biomass for efficient sugars recovery and retaining maximum lignin in the pretreated substrate.

Keywords Sugarcane bagasse · Alkaline pretreatment · Washing detergent · Saccharification · Second-generation sugars

Introduction

The primary source of energy in the world for transportation and materials comes from fossil fuel refinery (Cherbini 2010). Currently, the world demand of fuels is approximately 84 million barrels a day (mb/d) (16 million barrels lower than 2019, because of the impact of COVID-19) and is projected to increase by 5.7 mb/d over the 2019–25 period at an average annual rate of 950 kb/d (IEA 2020; EIA 2020). Because of burgeoning oil demand, dwindling prices and geopolitical factors, biomass valorization is an important issue which could be significant in the reduction of the global dependence on fossil fuels (Ferreira 2017). Sugarcane bagasse among the lignocellulosic biomass is among the most promising feedstock in the world for alternative fuels and chemicals production (Sun et al. 2016).

Given that the native form of lignocellulosic biomass is recalcitrant, so pretreatment of biomass an indispensable step before enzymatic hydrolysis to get sugar monomers (Cantero et al. 2019; Brethauer and Studer 2015). Alkaline pretreatment disrupts the hydrogen-bonding network in cellulose and hemicellulose polymers in biomass altering crystallinity, increasing porosity and thus allowing solvent and enzymes to access to the surface and intramolecular areas of biomass (Cantero et al. 2019). Alkaline pretreatment method has emerged as one of the most viable process options due to its selective action on ester linkage breakdow in lignin, fast reaction rates and process simplicity (Kim et al. 2016; Chandel et al. 2019). Alkaline pretreatment selectively removes lignin without degrading many carbohydrates and increases porosity and surface area, thereby enhancing enzymatic hydrolysis (Kim et al. 2016).
However, the major disadvantage of the alkaline process is the solubilization of considerable amount of lignin which needs to be recovered from the liquor for the energy generation in boilers or subsequent transformation into chemicals (Chandel et al. 2019). Pretreated biomass after enzymatic hydrolysis is then practically insoluble lignin cake accessible for further valorization into fuels and chemicals. Therefore, pretreatment causing specific molecular changes in the biomass rather than solubilization of lignin is an important advantage for reducing the processing cost for lignin recovery, saving of water and energy consumption (Sun et al. 2016; Kim et al. 2016). This concept may play a pivotal role in the lignin valorization within the goals of the sustainable matrix for the development of an advanced biomass-based industry (Beckham et al. 2016).

Materials and Methods

Lignocellulosic Material

Sugarcane bagasse (SCB) was obtained from Usina Ipiranga Agroindustrial (Descalvado, São Paulo, Brazil). The biomass was air-dried and milled in a Marconi knife mill, model MA 630 (Marconi, São Paulo, Brazil). The milled bagasse was screened using standard Tyler sieves (10 and 14 mesh), and powders were stored at room temperature in a sealed container and used for this study.

Commercial Detergents

Four commercial detergents (CDs) were purchased from local markets. They are sold in Brazil with the brand name as follows: Brilhante® (B), Omo® (O), Sabonito Flash® (F).

Commercial Detergents-Assisted Alkaline Pretreatment

During pretreatment, SCB was subjected to be pretreated by four commercial detergents. Detergents were used in concentrations of 5, 10 and 15% (w/v). The abovementioned concentrations for pretreatment were performed in combination with autoclaving at 121 °C under 15 psi for 60 min. All experiments were run with and without pH adjustment to 12. The pH of biomass and detergent solution was adjusted by 4 M NaOH. The ratio of the solid phase to liquid phase in each pretreatment was maintained at 1:10. The pretreated solid biomass was extensively washed with tap water to remove the solubilized lignin and neutralization of it. Biomass was dried at 60 °C to achieve a constant weight and was subsequently used for enzymatic saccharification.

Enzymatic Hydrolysis

Pretreated biomass was hydrolyzed using a commercial cellulase complex (CTec 2—0.5L) and was kindly provided by Novoymes Inc., Curitiba, Brazil. Enzymatic hydrolysis was carried out in 50-mL Erlenmeyer flask using enzyme loading of 20 FPU/g of pretreated SCB at 5% total solids, 50 °C for 72 h. The produced hydrolysate was recovered by filtration, and the total reducing sugars concentration was analyzed.

Analytical Methods

The chemical composition (cellulose, hemicellulose, and lignin) of raw and pretreated SCB was estimated using the protocol described by National Renewable Energy Laboratory—NREL, Colorado, USA (Sluiter et al. 2011). The reducing-sugar content in the enzymatic hydrolysates was determined using the dinitrosalicylic acid method of Miller (1959). Figure 1 presents the flow diagram showing experiments performed to carry out the study.

Results and Discussion

Effect of Commercial Detergent-Assisted Pretreatments on SCB Composition and Cellulose Availability

The physicochemical composition of untreated SCB was comprised of 35.4 ± 1.02% cellulose, 25.03 ± 0.82% of hemicellulose, and 33.9 ± 1.07% of lignin on dry weight basis (97.11 ± 1.98% mass balance). Table 1 compares the biomass composition of untreated and detergent pretreated SCB. The maximum increment for cellulose in pretreated samples (44.7 ± 2.38%) was obtained with the detergent B15pH. The pH adjustment was appeared to be as a major contributing factor in the removal of lignin from native bagasse showing 26.27% cellulose increase followed by O5pH, O10, O15 and O15pH, showing increments in available cellulose of 24.71, 24.71, 23.81 and 23.19%, respectively (Table 1). The results are in accordance with
the study of Hilares et al. (2016), showing 26.47% of available cellulose in the SCB when it was alkaline pretreated by hydrodynamic cavitation. In contrast, Maryana et al. (2014) reported an increment of 48% of cellulose in the bagasse pretreated with 1 M NaOH at 1:12 (total solid ratio). Sodium hydroxide is about 2.5-fold more expensive than commercial detergents (Americanas 2020; Didacticasp 2020; Phlab 2020). In this context, this study shows that the use of detergents, specially O, at 10 and 15% concentration showed similar cellulose and hemicellulose availability. Even though the lignin removal was not like the typical sodium hydroxide pretreatment (Chen et al. 2011, 2012; Yoon et al. 2011; Loow et al. 2016), it did not decrease the saccharification yield. These results suggest that detergents caused a structural modification in the bagasse cell wall that allowed the cellulases accessibility to the carbohydrates (cellulose and hemicellulose) available in the pretreated bagasse.

**Effect of Commercial Detergents on Lignin and Hemicellulose Composition**

Lignin and hemicellulose removal from pretreated biomass increases the amenability of cellulases toward cellulose available in the biomass. The lignin removal from the biomass is an important feature as the lignin devoid substrate yields the sugars without inhibitors after enzymatic hydrolysis (Shen et al. 2012). Typically, lignin percentage after acid or alkaline pretreatment of SCB remains in the range of 15–24% (Chen et al. 2011, 2012; Yoon et al. 2011; Loow et al. 2016). However, pretreatment conditions employed during the pretreatment play a key role in the extent of delignification of the lignocellulosic substrates. Here, we found that the maximum delignification (44.5%) when 5% of detergent O was used a catalytic agent with pH adjustment of 12 by 4 M NaOH. The least amount of lignin removal was observed with detergent F which showed the lignin removal from 4.55 to 11.05%. Detergents B and A when used with pH adjustment showed a direct relationship between delignification and detergent concentration.

### Table 1  Effect of commercial detergents on sugarcane bagasse (SCB) composition % d.wt (TS—total solids; HC—hydrodynamic cavitation; pH—pH adjustment to 12)

| Biomass pretreatment | Cellulose (%) | Hemicellulose (%) | Lignin (%) | Reference |
|----------------------|---------------|-------------------|------------|-----------|
| SCB UNTREATED        | 35.41 ± 1.02  | 25.03 ± 0.82      | 33.98 ± 1.07| Current study |
| SCB B15pH            | 44.70 ± 2.38  | 31.09 ± 2.06      | 22.26 ± 0.58| Maryana et al. (2014) |
| SCB O15              | 44.15 ± 2.72  | 29.71 ± 2.02      | 18.86 ± 0.41| Maryana et al. (2014) |
| SCB O10              | 44.15 ± 1.05  | 32.51 ± 1.39      | 25.34 ± 1.09| Maryana et al. (2014) |
| SCB O15              | 43.83 ± 0.51  | 30.26 ± 1.87      | 27.08 ± 1.00| Maryana et al. (2014) |
| SCB O15pH            | 43.61 ± 2.68  | 29.57 ± 1.86      | 20.00 ± 1.90| Maryana et al. (2014) |
| SCB UNTREATED        | 44.43 ± 0.86  | 22.9 ± 0.67       | 17.52 ± 0.65| Maryana et al. (2014) |
| SCB NaOH (1 M) -8.3% TS 100°C /15 min | 66.16 ± 0.13 | 17.06 ± 0.4 | 8.34 ± 0.35 | Maryana et al. (2014) |
| SCB UNTREATED Glucan  | 40.6           | 26.3              | 24.9       | Hilares et al. (2016) |
| SCB NaOH (0.5 M) -4.5% TSV 45 min- assisted by HC | 51.3 (Glucan) | 21.28 (Xylan) | 15.35 | Hilares et al. (2016) |

Reference:

Americanas 2020; Didacticasp 2020; Phlab 2020; Chen et al. 2011, 2012; Yoon et al. 2011; Loow et al. 2016; Hilares et al. (2016).
Similarly, Hilares et al. (2016) found that delignification effect was dependent of NaOH concentration. They observed that maximum lignin removal was reached with 0.5 M of NaOH, followed by 0.3 and 0.1 M (54.61%, 39.98% and 32.02%, respectively). In contrast, detergent O with pH adjustment showed an inverse effect, where the minimum concentration of detergent led more lignin removal (Fig. 2). Nevertheless, detergents without pH adjustments did not show any variation in lignin removal at any concentration of detergent.

According to Unilever, the active detergent components in OMO powders are surfactants, such as sodium dodecylbenzenesulfonate. Some OMO detergent powders also contain lipase, amylase and other enzymes that help break down protein-based stains. Sodium compounds, such as sodium carbonate and sodium sulfate, are common building and bulking agents in these formulations. These agents comprise most of the actual mass of the powder. Other binding agents, and stabilizers, such as cellulose, sodium polyacrylate and tetrasodium etidronate, ensure that the components of the detergent remain well-mixed. This information could have several modifications depending on the region, and regulations for each country, other detergents used in the current work could contain similar compositions and the variation of the quality of each component could depend on the brand and cost of the product.

In regard to the hemicellulose removal, all pretreatment conditions showed an increment in its content in the pretreated bagasse. When pH was adjusted, the range of hemicellulose increment had a variation between 7.83 and 24.21%, but when pH was not adjusted, the increment of this fraction was between 9.34 and 29.88%. Some reports in literature indicated that the use of NaOH in pretreatment tends to remove lignin and hemicellulose (de Carvalho et al. 2016; Ascencio et al. 2019). However, we found in the in the current results, the use of some commercial detergents allows to enhance cellulose and hemicellulose in the pretreated SCB as shown in pretreatments in Table 1, even without high levels of delignification. During the pretreatment, the retainment of maximum carbohydrates in the biomass is an advantageous strategy to get high sugars concentration after enzymatic hydrolysis (Ascencio et al. 2019; Adsul et al. 2020).

Table 2  Relation between total sugar conversion and total lignin of natural pretreated samples

| Sample   | Total reducing sugars yield (%) | Available lignin in the pretreated bagasse (% wt) |
|----------|---------------------------------|-----------------------------------------------|
| Natural bagasse | 14.13                           | 33.98 ± 1.07                                  |
| NaOH 0.1 M    | 32.49                           | 28.63 ± 0.61                                  |
| O5          | 27.78                           | 27.05 ± 0.46                                  |
| O5pH        | 55.32                           | 18.86 ± 0.41                                  |
| O10         | 43.46                           | 25.34 ± 1.09                                  |
| O10pH       | 66.5                            | 19.22 ± 0.62                                  |
| O15         | 37.20                           | 27.08 ± 1.00                                  |
| O15pH       | 51.50                           | 20.00 ± 1.90                                  |
| B5          | 30.80                           | 26.75 ± 2.08                                  |
| B5pH        | 59.20                           | 30.92 ± 2.34                                  |
| B10         | 25.6                            | 26.92 ± 0.78                                  |
| B10pH       | 54.70                           | 27.19 ± 1.48                                  |
| B15         | 44.70                           | 26.81 ± 0.18                                  |
| B15pH       | 57.30                           | 22.26 ± 0.58                                  |
| F5          | 13.4*                           | 30.54 ± 0.15                                  |
| F5pH        | 38.6                            | 32.45 ± 0.74                                  |
| F10         | 15.05*                          | 31.23 ± 0.52                                  |
| F10pH       | 34.4                            | 30.24 ± 4.33                                  |
| F15         | 17.2*                           | 30.22 ± 1.58                                  |
| F15pH       | 33.78                           | 31.70 ± 1.21                                  |

*It is inferred that some detergent components interfered in the saccharification process and analysis protocols for sugars

Effect of Recalcitrant Lignin on Enzymatic Saccharification

The effect of the detergents-assisted pretreatment showed different responses on sugars release after enzymatic hydrolysis. Sugars recovery predominantly depends on the amount of lignin in pretreated substrate. Lignin is considered the most important factor in the accessibility of cellulases to the cellulose in the substrate (Kim et al. 2016). Detergents did not remove a very high amount of lignin...
from the substrate but might have caused changes in the cellulose–lignin network at molecular level. Table 2 shows the amount of lignin in all detergents pretreated bagasse samples. This is evident with the table that approximately 18–25% of lignin was removed from the native bagasse after pretreatment with all three detergents used in the study. Detergent O after pH adjustment of 12 caused maximum lignin removal (Table 2), which in turn showed the higher sugar recovery (50–67%) after enzymatic hydrolysis. Ladeira et al. (2020) found 22% lignin in the liquid hot water-pretreated SCB showing 52% of glucose yield after enzymatic hydrolysis. Seo et al. (2017) observed 67% lignin removal from lignocellulose after a mixed pretreatment with acids and surfactants. The pretreated biomass when enzymatically hydrolyzed showed 6 g/l sugars (21% yield), while Maheswari et al. (2020) found saccharification yields of 28% on SCB with alkali pretreatment and 33% for assisted alkali pretreatment with humic acid and CaCl₂ (5% of improvement). In the current study, detergents F5- and F10-pretreated SCB showed similar results like NaOH-pretreated SCB. Maximum sugars were obtained from the SCB after pretreatment with detergents B and F.

Detergent F presented similar results in every concentration tested, yielding high sugar recovery with the available lignin, 13–17 g/L and 30–33%, respectively (Fig. 3). The best results are considered those that released the highest amounts of reducing sugar while maintaining the highest amount of lignin. B5pH-based pretreatment had the best performance releasing the highest amount of sugar (59.20%, 22.13 g/L) and retaining 30.92% of lignin. This is important feature in recovering the high amount of sugars (59.2%, 22.13 g/L) while retaining considerably high amount of lignin in the substrate indicating the potential of residual solids for new applications in biorefinery after the sugars extraction. The high amount of lignin in the hydrolyzed substrate could be valorized for the adhesives, resins and important phenolic compounds in biorefinery (Garlapati et al. 2020).

Pretreatments of SCB at B10pH, B15 and B15pH released 44–57% reducing sugars with the retainment of 22–29% of residual lignin. Pretreatment conditions (F5pH, F10pH and F15pH) showed 33–39% of reducing sugars release, with 30–33% residual lignin in the pretreated SCB. Similar glucose yields were as reported by Seo et al. (2017) after surfactant-based pretreatment of SCB. The role of catalytic agents in biomass pretreatment is pivotal for the lignocellulosic sugars recovery after enzymatic hydrolysis. For instance, Rabeya et al. (2020) observed 61.75% and 71.49% degradation of hemicellulose and lignin, respectively, after 2% NaOH pretreatment of corn stalk at 100 °C for 1 h. Sodium hydroxide acts on lignin removal from the lignocellulosic substrate leaving cellulose and lignin together improving the amenability of cellulolytic enzymes.
for renewable sugars production. On the other hand, dilute sulfuric acid pretreatment mechanistically acts on hemi-cellulose degradation from the lignocellulosic substrate. For example, sugarcane straw pretreatment by sulfuric acid pretreatment (130 °C, 2.9% w/v H₂SO₄ solution, solid–liquid ratio (1:4) and 30 min of residence time) showed a xylose concentration of 56.5 g/l (Moutta et al. 2012).

The choice of treatment will depend on the process to be coupled or the subsequent use of biomass, depending on which of the two fractions (sugars or lignin) will be used in a greater proportion.

Conclusion

Alkaline pretreatment has been considered one of the most effective methods for the cellulosic sugars production. Our results clearly indicate that pretreatment of lignocellulosic biomass with commercial detergents followed by enzymatic hydrolysis greatly enhances the enzymatic conversion of carbohydrate polymers into fermentable sugars. These results also support the possibility of retaining residual lignin in the pretreated biomass while yielding the high amount of sugars after enzymatic hydrolysis. Commercial detergent-assisted pretreatment provides an alternative strategy for pretreatment of vegetal biomass and presents a new sustainable application of detergents in economic 2G sugars production which can be used as an excellent feedstock in biorefineries for the biofuels, biochemicals and biomaterials production.

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Availability of data and material The data have been originated from the experiments. Sugarcane bagasse was collected locally, cellulase was gifted from Novozymes Inc, Curitiba, Brazil, and commercial detergents were procured from local market.

Declarations

Conflicts of interest All authors declare no conflicts of interest.

Consent to participate All authors have consented their participation.

Consent for publication All authors have consented the publication of this work.

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