

Alkaline pretreatment and enzymatic hydrolysis of corn stover for bioethanol production

Pré-tratamento alcalino e hidrólise enzimática de palha de milho para produção de bioetanol

Pretratamiento alcalino e hidrólisis enzimática de rastrojo de maíz para la producción de bioetanol

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Abstract

The demand for ethanol in Brazil is growing. However, although the country is one of the largest producers of this fuel, it is still necessary to diversify the production matrix. In that regard, studies with different raw materials are needed, mainly the use of low cost and high available wastes such as lignocellulosic residues from agriculture. Therefore, this study aimed to analyze the bioethanol production from corn stover. An alkaline pretreatment (CaO) was carried out, followed by enzymatic hydrolysis (Cellic Ctec2 and Cellic Htec2) to obtain fermentable sugars. The best experimental condition for the pretreatment and hydrolysis steps resulted in a solution with $0.31 \text{ g}_{\text{sugar}} \cdot \text{g}_{\text{biomass}}^{-1}$. Then, the fermentation was performed by the industrial strain of *Saccharomyces cerevisiae* (PE-2) and by the wild yeast strain *Wickerhamomyces* sp. (UFFS-CE-3.1.2). The yield obtained was $0.38 \text{ g}_{\text{ethanol}} \cdot \text{g}_{\text{dry biomass}}^{-1}$ was, demonstrating the potential of this process for bioethanol production.

Keywords: Lignocellulosic residue; Corn stover; Pretreatment; Hydrolysis; Fermentation; Biofuel.

Resumo

A demanda por etanol no Brasil está crescendo. Porém, embora o país seja um dos maiores produtores deste combustível, ainda é necessário diversificar a matriz produtiva. Nesse sentido, estudos com diferentes matérias-primas são necessários, principalmente a utilização de resíduos de baixo custo e alta disponibilidade, como os resíduos lignocelulósicos da agricultura. Portanto, este estudo teve como objetivo analisar a produção de bioetanol a partir da palha de milho. Foi realizado um pré-tratamento alcalino (CaO), seguido de hidrólise enzimática (Cellic Ctec2 e Cellic Htec2) para obtenção de açúcares fermentáveis. A melhor condição experimental para as etapas de pré-tratamento e hidrólise resultou em uma solução com $0,31 \text{ g}_{\text{açúcar}} \cdot \text{g}_{\text{biomassa}}^{-1}$. Em seguida, a fermentação foi realizada pela cepa industrial de *Saccharomyces cerevisiae* (PE-2) e pela cepa de levedura selvagem *Wickerhamomyces* sp. (UFFS-CE-3.1.2). O rendimento obtido foi de $0,38 \text{ g}_{\text{etanol}} \cdot \text{g}_{\text{biomassa seca}}^{-1}$, demonstrando o potencial deste processo para a produção de bioetanol.

Palavras-chave: Resíduo lignocelulósico; Palha de milho; Pré-tratamento; Hidrólise; Fermentação; Biocombustível.

Resumen

La demanda de etanol en Brasil está creciendo. Sin embargo, si bien el país es uno de los mayores productores de este combustible, aún es necesario diversificar la matriz productiva. En este sentido, se requieren estudios con diferentes materias primas, principalmente el uso de residuos de bajo costo y alta disponibilidad, como los residuos lignocelulósicos de la agricultura. Por tanto, este estudio tuvo como objetivo analizar la producción de bioetanol a partir del rastrojo de maíz. Se realizó un pretratamiento alcalino (CaO), seguido de hidrólisis enzimática (Cellic Ctec2 y Cellic Htec2) para obtener azúcares fermentables. La mejor condición experimental para los pasos de pretratamiento e hidrólisis resultó en una solución con $0,31 \text{ g}_{\text{azúcar}} \cdot \text{g}_{\text{biomasa}}^{-1}$. Luego, la fermentación fue realizada por la cepa industrial de *Saccharomyces cerevisiae* (PE-2) y por la cepa de levadura salvaje *Wickerhamomyces* sp. (UFFS-CE-3.1.2). El rendimiento obtenido fue de $0,38 \text{ g}_{\text{etanol}} \cdot \text{g}_{\text{biomasa seca}}^{-1}$, lo que demuestra el potencial de este proceso para la producción de bioetanol.

Palabras clave: Residuo lignocelulósico; Rastrojo de maíz; Pretratamiento; Hidrólisis; Fermentación; Biocombustible.

1. Introduction

Fossil fuels are the primary energy source utilized worldwide, and the demand is constantly growing due to population and industrialization increases; this scenario arouses not only social and economic issues but also environmental concerns (Cherubini & Strømman, 2011). With the increase in energy demand and the decrease in supplies of petroleum-derived fuels, there is a need to search for new renewable sources of low-cost energy (Akram et al., 2018). The use of biofuels such as bioethanol stands out as a suitable source to partially replace the use of fossil fuels (Zhao et al., 2018).

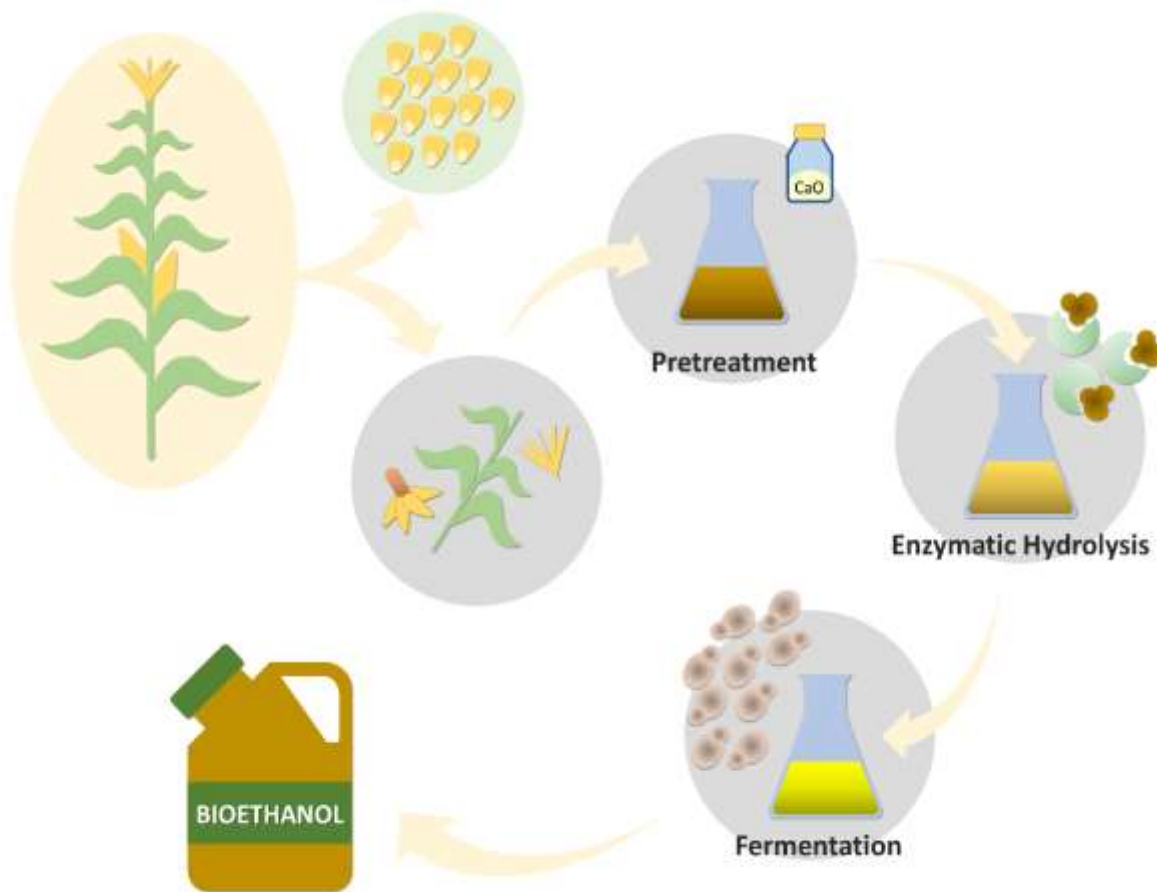
Bioethanol is a clean, sustainable and renewable energy source, produced from agricultural feedstock through fermentation processes (Chen et al., 2018; Zhao et al., 2018). The second-generation ethanol has advantages, as it does not compete with the food production, although its process still faces many operational and economic challenges compared to the production technologies of first-generation ethanol (Chen et al., 2018; Zhao et al., 2018). At present, the operational drawbacks are mainly in the pretreatment and enzymatic hydrolysis steps. The process of breaking down the lignin-polysaccharide matrix in an efficient way is still very difficult to achieve, even for the different types of pretreatment currently tested (Robak & Balcerek, 2020).

It is worth noting that Brazil is the world leader in the use of energy from renewable sources; the most widely used raw material is sugarcane juice, characterizing the country as the largest ethanol producer from that feedstock (Mączyńska et al., 2019). However, despite being one of the biggest producers, Brazil still needs to diversify the productive matrix in order to develop production routes from different raw materials (Cavali et al., 2020; Mibielli et al., 2020). Accordingly, since there is a growing demand for food and energy, lignocellulosic materials become a viable option for ethanol production (Talebnia et al., 2010).

The biofuel produced from lignocellulosic biomass is one of the most promising technologies under development, although its achievement depends mainly on the cellulose and hemicellulose content in the biomass (Chen et al., 2018; Zhao et al., 2018). Additionally, reducing the production cost is the main way to effectively market bioethanol. Hence, considering the

search for diversification of the energy matrix by developing processes using low-cost raw feedstocks, this study aimed to produce bioethanol from corn stover. As presented in Figure 1, three processes were assessed herein: alkaline pretreatment, enzymatic hydrolysis and fermentation.

Figure 1. Processes studied for bioethanol production from corn stover.



Source: Authors (2021).

2. Methodology

2.1 Materials

The corn stover (CS) used in experiments was collected from the field after the corn harvest in the municipality of Palmitos, state of Santa Catarina, Brazil. The commercial enzymatic preparations used in this work were Cellic CTec2 (cellulase complex) and NS22244 - Cellic HTec2 - (hemicellulase complex), both from Novozymes A/S (Bagsvaerd, Denmark) and supplied by Novozymes Latin America (Araucária, PR, Brazil). Two yeast strains were used in this study: a wild *Wickerhamomyces* sp. (UFFS-CE-3.1.2) isolated from rotten wood (Bazoti et al., 2017), and the industrial *Saccharomyces cerevisiae* (PE-2), supplied by the company Fermentec (Piracicaba/SP) (Basso et al., 2008). The reagents utilized in the experiments were all of analytical grade.

2.2 Biomass characterization

Initially, the CS was grinded in a knife mill to obtain smaller particles, which were sieved (Pavitest® - I-1016-A) to determine the granulometric distribution. Then, the physical-chemical characterization of the fraction with particles diameter less than 0.6 mm was performed through the following analyzes: granulometric distribution, moisture content, inorganic

materials, extracts content, structural carbohydrates and lignin, which were performed according to the standard procedures of National Renewable Energy Laboratory (NREL) (National Renewable Energy Laboratory, 2020).

2.3 Pretreatment and enzymatic hydrolysis

The pretreatment and enzymatic hydrolysis was based on other studies (Chang et al., 2001; Rabelo et al., 2009). Calcium oxide (CaO) was utilized for alkaline pretreatment, and, in order to select the best condition, a central composite design with two levels and two factors (CCD 2²), was carried out to evaluate the effects of incubation temperature (40, 55 and 70 °C) and the CaO concentration (0.2, 0.4 and 0.6 g·g_{dry biomass}⁻¹). Briefly, 20 g of CS were added into the 1.5 L lidded glass flasks together with 200 mL of CaO solution, resulting in a suspension with solid/liquid ratio of 100 g·L⁻¹. The pretreatment occurred in an orbital shaker (Shaker model SL - 223 from SOLAB) at 200 rpm during 24 hours, varying the temperature and CaO concentration according to the CCD 2².

Following the pretreatment step, the pH of the medium was corrected using a solution of citric acid (1 mol·L⁻¹), until reaching the ideal pH for the enzymes, around 5.0 – 5.5 (Novozymes, 2018). After pH correction, the enzymatic preparations Cellic Ctec2 (2 wt.% in relation to dry biomass) and Cellic Htec2 (0.5 wt.% in relation to dry biomass) were added, both diluted in sodium acetate buffer (1:10 v·v⁻¹). The bottles were conditioned again in the orbital shaker in rotation of 200 rpm, temperature of 50 °C for a period of 24 hours.

At the end of the enzymatic hydrolysis, the samples were centrifuged at 10,000 rpm for 5 minutes and filtered in vials, using non-sterile nylon filters with 0.45 µm pore size (Millipore) and 0.22 µm PVDF non-sterile, for quantifying sugars, acetate, furfural and hydroxymethylfurfural on high performance liquid chromatography (HPLC), according to the methodology expressed in the following items 2.5 and 2.6.

2.4 Batch fermentations of hydrolysate

From the best condition defined in pretreatment experimental design followed by enzymatic hydrolysis, a new hydrolysate was prepared, which was sterilized by vacuum filtration using nylon filters with 0.45 µm pores (Millipore). Subsequently, the micronutrients were adjusted by adding 3.0 g·L⁻¹ of monobasic potassium phosphate.

For the pregrowth of the yeasts, the cells were cultivated in yeast-extract-peptone-dextrose (YPD) medium (1% yeast extract, 2% peptone and 2% glucose) for 48 hours in a shaker at 145 rpm and 28 °C. The pre-grown cells were then transferred to fresh YPD medium (1 hundredth of the final volume); the inoculum preparations took place overnight until the cells reached the beginning of the exponential phase of growth, which was defined by optical density at 570 nm (OD₅₇₀ = 3.5). At this point, the cells were washed twice with distilled water at 4 °C and then suspended in the hydrolysates to reach a concentration of 10 mg of cells·mL⁻¹. Afterwards, a 200 µL aliquot was removed and centrifuged at 3,500 rpm for 05 minutes, with its supernatant stored at -20 °C. The rest of the culture was incubated at 28 °C and 145 rpm. Fermentation took place over 30 hours, and aliquots of 200 µL collected every two hours and centrifuged at 3,500 rpm for 05 minutes, with its supernatant stored at -20 °C. At the end of the fermentation period, the stored supernatants were thawed and filtered into vials, using nylon filters with a pore size of 0.45 µm (Millipore) to quantify sugars and ethanol in HPLC (Barrilli et al., 2020).

2.5 Determination of sugars, ethanol and acetic acid

A column for organic acids (Aminexce HPX-87H, Bio-Rad) was used to quantify glucose, xylose, cellobiose, acetic acid and ethanol with the RID-10A detector through HPLC (LC-MS 2020, SHIMADZU), with a refractive index detector (RID-10, SHIMADZU). The concentration of each component was determined with the aid of calibration curves, obtained initially with analytical standards, as described by Barrilli and co-workers (Barrilli et al., 2020). For the analysis, the following

conditions adapted from another work (Rabelo, 2010) were used: injection volume of 10 μL , using an aqueous solution of sulfuric acid ($5 \text{ mmol}\cdot\text{L}^{-1}$) as a mobile phase in a flow rate of $0.6 \text{ mL}\cdot\text{min}^{-1}$, and the oven and detector temperatures was 50 and 40 $^{\circ}\text{C}$, respectively, during 25 minutes.

2.6 Determination of Furfural and Hydroxymethylfurfural

The HPLC system (LC-MS 2020, SHIMADZU) was used, equipped with the NST-18 column, using the SPD-M20A detector. The concentrations of each component were determined with the aid of calibration curves, obtained initially with analytical standards. The analysis conditions were: injection volume of 20 μL , using as mobile phase a solution of ultrapure water with acetonitrile at a ratio of 85:15 ($\text{v}\cdot\text{v}^{-1}$) acidified with acetic acid ($1\% \text{ v}\cdot\text{v}^{-1}$), flow rate of $0.8 \text{ mL}\cdot\text{min}^{-1}$, and the furnace and detector was 40 $^{\circ}\text{C}$ during 15 minutes (Rabelo, 2010).

3. Results and Discussion

3.1 Characterization of the biomass

Corn stover (stem, leaf, cob and straw) have large and irregular sizes, making the transformation process into fermentable sugars difficult. Accordingly, the biomass was fractioned into smaller and more uniform particles to increase its accessible surface area in order to improve the action of enzymes (Madadi et al., 2017). The results are expressed in Table 1, and the particles in the size range of 0.3 – 0.6 mm were employed in the characterization experiments.

Table 1. Granulometric distribution of corn harvest residues.

| Size range (mm) | Retained Biomass (%) |
|-----------------|----------------------|
| 0.0 - 0.15 | 12.17 |
| 0.15 - 0.3 | 10.37 |
| 0.3 - 0.6 | 48.68 |
| 0.6 - 1.0 | 28.72 |
| 1.0 - 2.0 | 0.05 |
| > 2.0 | 0.01 |

Source: Authors (2021).

The biomass composition varies widely from one species to another; its major structural components (cellulose, hemicellulose and lignin) are present at different amounts according to the plant. It emphasizes the characterization analysis of the raw material before using in a process (Dayton & Foust, 2020). In this regard, the chemical composition of the lignocellulosic residue from corn harvest is shown in Table 2.

Table 2. Chemical composition of the corn stover.

| Components | Amount (wt.%) [*] |
|---------------|----------------------------|
| Ash | 7.27 ± 0.64 |
| Extractive | 22.53 ± 0.95 |
| Total Lignin | 13.3 ± 0.11 |
| Cellulose | 34.48 ± 1.18 |
| Hemicellulose | 22.67 ± 0.69 |
| Total | 100.08 ± 3.57 |

^{*}dry basis.

Source: Authors (2021).

Agricultural residues have lower content of cellulose and lignin than forest biomass, but the quantity of hemicellulose of the former is generally higher than in woody materials (Mussatto & Dragone, 2016). The amount of cellulose, hemicellulose and lignin present in the corn stover commonly range between 30.6–38.1, 19.1–25.3 and 16.7–21.3 wt.%, respectively (Mussatto & Dragone, 2016). The composition of corn stover described in another study showed values of 36 wt.% of cellulose, 29.2 wt.% of hemicellulose, 17.2 wt.% of lignin and 4.0 wt.% of ashes (Mao et al., 2010). Similarly, Amer and co-workers reported cellulose, hemicellulose, acid insoluble lignin and extractive contents of 32.1, 18.1, 11.1 and 29.4 wt.%, respectively (Amer et al., 2021). In second-generation ethanol production, the target is on cellulose and hemicellulose which must be available after pretreatment stage (Robak & Balcerek, 2020).

3.2 Pretreatment and enzymatic hydrolysis

The structure of lignocellulosic materials comprises a stiff complex among cellulose, hemicellulose and lignin, which protects the material against enzymatic degradation (Cavali et al., 2020). Therefore, to obtain the carbohydrates, it is necessary the pretreatment of biomass to increase the exposure of cellulose and hemicellulose in order to facilitate the action of biocatalyst on those polysaccharides in the saccharification stage (Brandt et al., 2013; Silveira et al., 2015). Accordingly, the results achieved herein after pretreatment with CaO and the enzymatic hydrolysis of the corn stover are reported in Table 3.

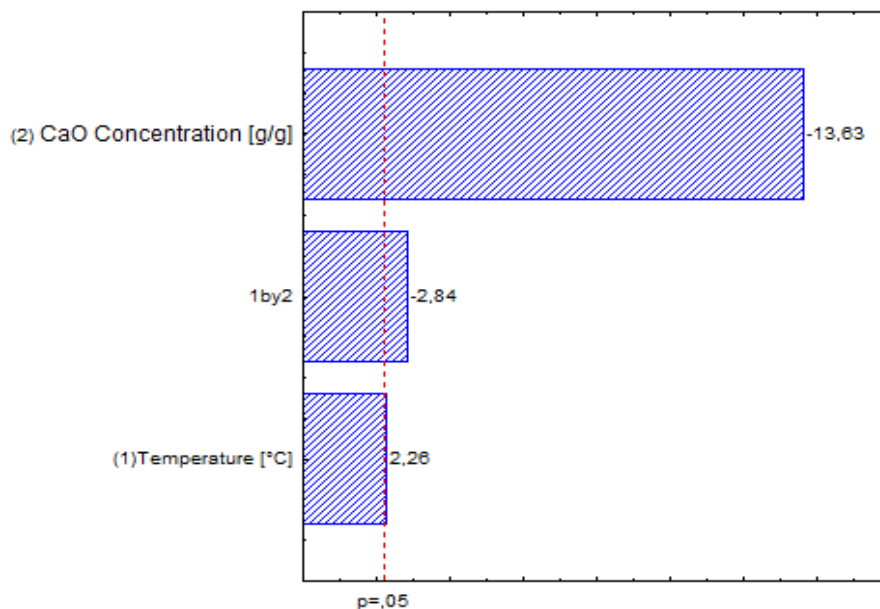
Table 3. Sugar yields of the corn stover after pretreatment and enzymatic hydrolysis.

| Test ^a | Temp. (°C) | CaO (g·g ⁻¹ dry biomass ⁻¹) | Glucose (g·L ⁻¹) | Xylose (g·L ⁻¹) | Cellobiose (g·L ⁻¹) | TS ^b (g·L ⁻¹) | TS ^c (g·g ⁻¹ biomass ⁻¹) | Yield ^d (%) | AA ^e (g·L ⁻¹) |
|-------------------|---------------|---|---------------------------------|--------------------------------|------------------------------------|---|---|---------------------------|---|
| 01 | (-) 40 | (-) 0.2 | 14.71 ± 0.08 | 6.73 ± 0.02 | 0.77 ± 0.28 | 22.21 ± 0.29 | 0.22 | 37.27 | n.d. |
| 02 | (+) 70 | (-) 0.2 | 20.41 ± 1.59 | 10.05 ± 0.83 | 0.59 ± 0.29 | 31.04 ± 1.82 | 0.31 | 52.08 | 3.06 ± 0.06 |
| 03 | (-) 40 | (+) 0.6 | 1.71 ± 1.55 | 1.17 ± 0.52 | 0.48 ± 0.20 | 3.36 ± 1.65 | 0.03 | 5.64 | n.d. |
| 04 | (+) 70 | (+) 0.6 | 0.78 ± 0.01 | n.d. | 0.53 ± 0.17 | 1.31 ± 0.17 | 0.01 | 2.19 | 2.34 ± 0.10 |
| 05 | (0) 55 | (0) 0.4 | 5.57 ± 1.53 | 3.63 ± 1.70 | 1.10 ± 0.10 | 10.31 ± 2.29 | 0.10 | 17.30 | 2,68 ± 0.11 |

^aTests performed in triplicate; ^bTotal sugars; ^cCorresponding value for 20.0 g of biomass and 0.2 L of suspension; ^dPercentage of cellulose and hemicellulose converted into fermentable sugars; ^eAcetic acid; n.d.: not detected. Source: Authors (2021).

The test 02 presented a higher yield regarding total sugars followed by test 01, both performed with the lowest concentration of CaO. When the highest amount of CaO was employed a reduction in total sugar concentration was noticed, which emphasizes the greater influence of this factor on reaction than temperature, as depicted in Figure 2. The CaO concentration presented a negative effect, whereas temperature effect was almost insignificant and positive on total sugar yield.

Figure 2. Pareto chart of the experimental design of the corn stover pretreatment.



Source: Authors (2021).

Regarding the interaction between the two factors analyzed, it was noticed a significant negative effect. Accordingly, when the CaO concentration decreases, the temperature must be increased to maximize the yield of fermentable sugars. However, it has been reported that the CaO pretreatment improves the degradation of both lignin and hemicellulose, increasing the availability of the cellulose fraction (Zhang et al., 2020). Thus, delignification is important to improve the enzymatic hydrolysis to achieve higher sugar yields (Silveira et al., 2015). In addition, another study also reported the utilization of low concentrations of CaOH₂ for pretreating corn stover (Kaar & Holtzapple, 2000).

The sugar concentration obtained after enzymatic hydrolysis with assay 02 (0.31 g·g_{biomass}⁻¹) can be considered a satisfactory conversion since it was similar to other work in which elephant grass was used (Siqueira et al., 2016). Considering the conversion of hemicellulose and cellulose to sugars, the best condition yielded 52.08%. It is in agreement with other studies which also used lignocellulosic biomass aiming ethanol production, such as rice straw (Bak et al., 2009) and sugarcane bagasse (Krishnan et al., 2010).

Another factor taken into account was the yeasts inhibitors. Lignocellulosic hydrolysates might produce weak organic acids such as acetic acid, lignin degradation products as well as furfural and hydroxymethylfurfural (HMF), which came from sugar degradation (Bellissimi et al., 2009). The acetic acid content was also presented in Table 3. Hemicellulose presents acetyl groups that are released as acetic acid in a process named de-acetylation (Parawira & Tekere, 2011). Furfural and HMF were not detected in any condition tested and, therefore, they were not reported. These compounds are not present under alkaline condition (Steinbach et al., 2017).

Although test 02 had shown the highest glucose yield, it also produced higher concentration of acetic acid than test 04 and 05. This value of acetic acid might be related to the pretreatment efficiency, as it is generated by the breakdown hemicellulose structure (Mussatto & Dragone, 2016). However, acetic acid is considered an inhibitor during fermentation due

to its toxic effects on yeasts, which comes from the ability to penetrate the yeast cytoplasm causing intracellular acidification and then disturbing the proton transport system at concentrations higher than $3.0 \text{ g}\cdot\text{L}^{-1}$ (Bellissimi et al., 2009).

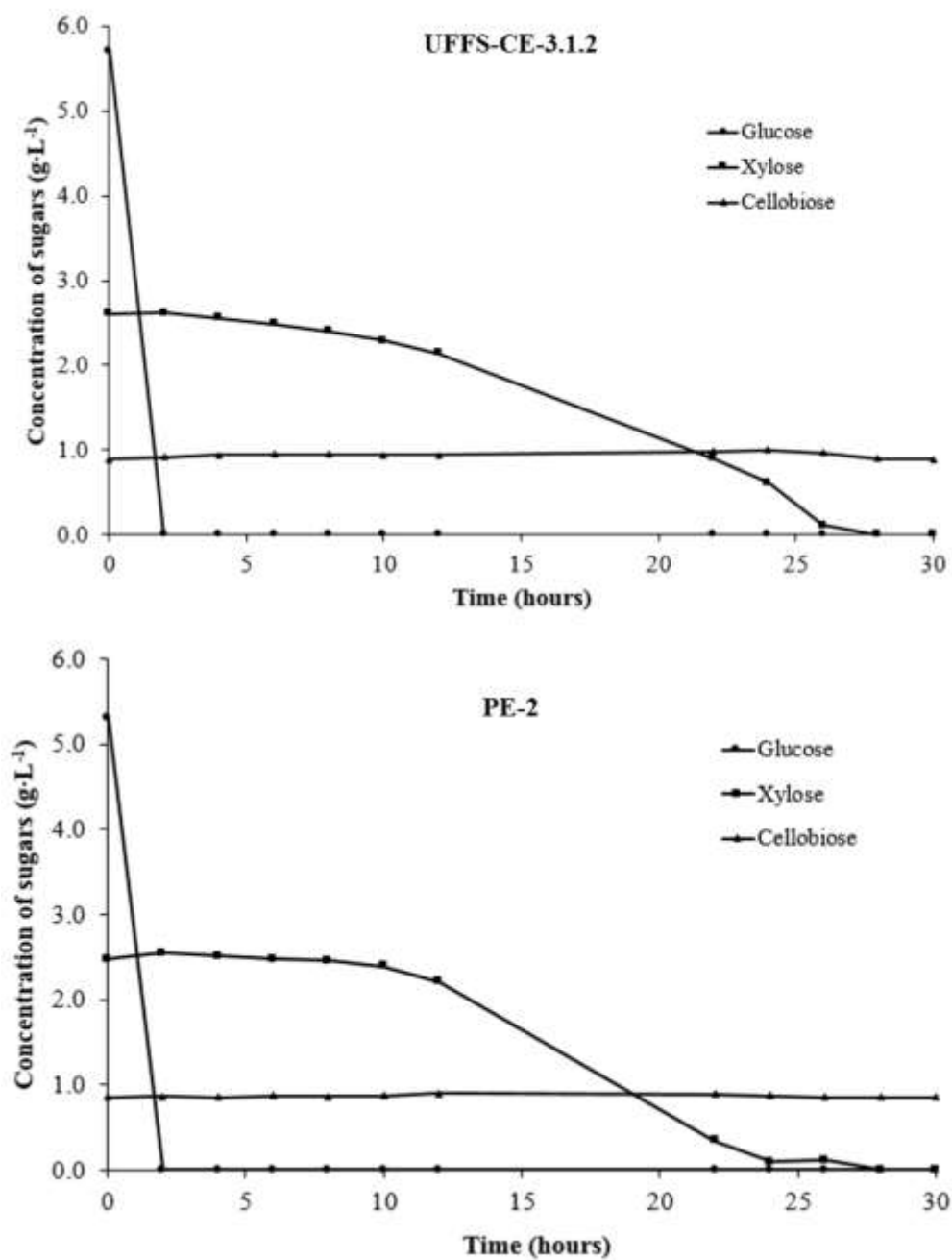
3.3 Fermentation

The enzymatic hydrolysate obtained from pretreated corn stover at conditions reported in the assay 02 was used in fermentation using two yeast strains. Figure 3 shows the sugar consumption during the fermentation step by the UFFS-CE-3.1.2 and PE-2 strains. The initial concentrations of glucose, xylose, cellobiose and acetic acid were 8.23, 3.07, 1.04 and $2.55 \text{ g}\cdot\text{L}^{-1}$, respectively.

It can be seen in Figure 3 that both strains showed total glucose consumption at the beginning of the fermentation, since it is the most preferable sugar for those microorganisms (Kim et al., 2010; Lucaroni et al., 2019). In contrast, the xylose metabolization was only completed after 25 hours for UFFS-CE-3.1.2 and PE-2 strains. However, the consumed xylose was not fermented to ethanol as showed in Figure 4 because there was not an increase in ethanol concentration corresponding to xylose consumption. Indeed, the commercial yeast (PE-2) do not use xylose as energy source as already demonstrated in another work even after 336 hours of incubation; actually, this yeast produces xylitol, which justifies the consumption of xylose reported herein (Lopes et al., 2017).

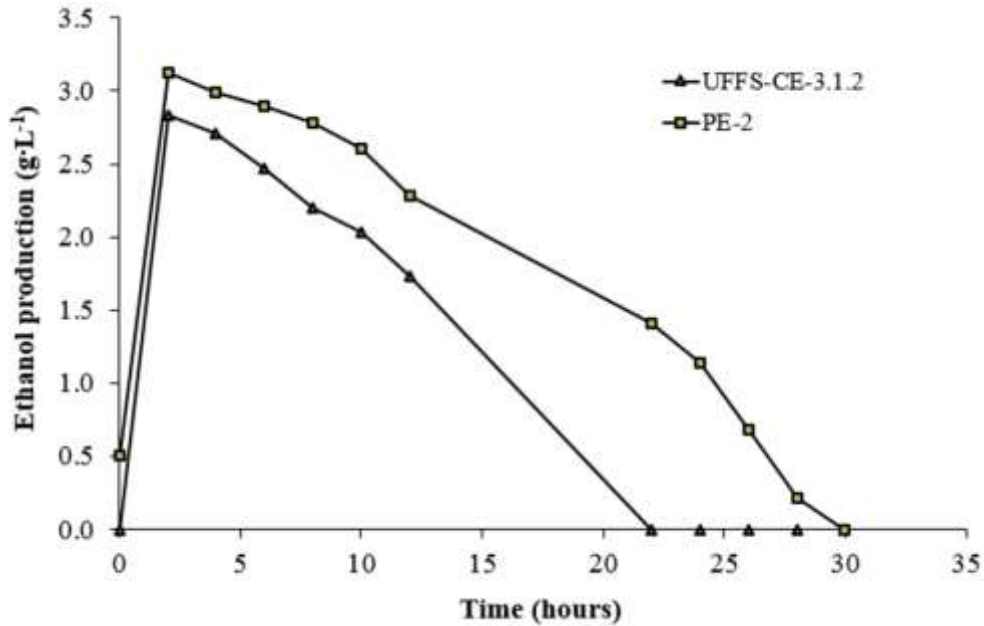
Nevertheless, the consumption of xylose by the UFFS-CE-3.1.2 is noteworthy. The wild yeast strain UFFS-CE-3.1.2 belongs to a species not yet described in the genus *Wickerhamomyces* (Bazoti et al., 2017). As shown in Figure 4, this yeast performed very similarly to the industrial yeast used in this study (PE-2). Industrial strains undergo a long selection process to present the best performance in converting glucose into ethanol, productivity and tolerance to inhibitory factors, whereas this process did not occur with wild yeasts (Lopes et al., 2016).

Figure 3. Sugar consumption profiles by UFFS-CE-3.1.2 and PE-2 strains during the hydrolysate fermentation.



Source: Authors (2021).

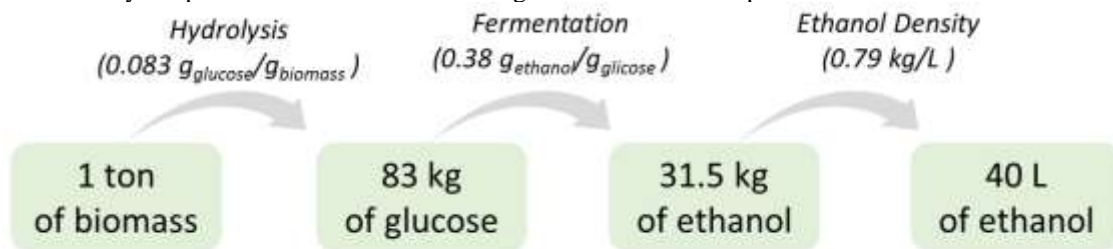
Figure 4. Ethanol production by strains UFFS-CE-3.1.2 and PE-2 during the hydrolysate fermentation.



Source: Authors (2021).

The two experiments for ethanol production started with a concentration of 8.23 g/L glucose, obtaining 2.80 g/L and 3.10 g/L of ethanol for strains UFFS-CE-3.1.2 and PE-2, respectively, as presented in Figure 4. Thus, a yield of 0.34 $\text{g}_{\text{ethanol}}/\text{g}_{\text{glucose}}$ for UFFS-CE-3.1.2 and 0.38 $\text{g}_{\text{ethanol}}/\text{g}_{\text{glucose}}$ for PE-2 was achieved. Regarding the yield obtained with yeast strain UFFS-CE-3.1.2, the result attained is in agreement with another study (Lucaroni et al., 2019). The study performed by Bonatto and co-workers (Bonatto et al., 2020) with sugarcane bagasse also showed the UFFS-CE-3.1.2 ability to consume xylose, revealing a superior yield after 120 hours compared to that of this study, in which the fermentation was conducted during 30 hours. Taking into account the pretreatment and fermentation steps, the global yield of the process is shown in Figure 5.

Figure 5. Ethanol yield per ton of corn stover according to the conditions of pretreatment and fermentation determined.



Source: Authors (2021).

The 2019/2020 Brazilian corn crop was about 102.31 million tons as reported by National Supply Company (Companhia Nacional de Abastecimento (CONAB), 2020). Considering the rate of 1 dry kg of corn stover per dry kg of corn grain (Ruan et al., 2019) and 14% of moisture, which is the ideal percentage for corn harvesting according to Brazilian Agricultural Research Corporation (Brazilian Agricultural Research Corporation - EMBRAPA, 2021), about 88 million tons of corn stover were produced. Commonly, 45% of the corn stover can be sustainably removed following each corn crop from high high-yielding fields with minimum till practices (Iowa Corn Promotion Board, 2013). It suggests then that almost 40 million tons of corn stover were available to be used as raw material for ethanol production. According to the conversions

presented in in this study, it would generate about 1,600 million liters of ethanol. However, it is important to point out that the process described in this study might be improved in order to obtain higher yields and boost the bioeconomy development.

4. Conclusion

The growing biofuel demand can be supplied by introducing alternative raw materials, such as residual lignocellulosic biomass. In this study, corn stover was proposed as feedstock for bioethanol production. The processes assessed were the alkaline pretreatment with CaO, enzymatic hydrolysis and fermentation performed by the industrial strain of *Saccharomyces cerevisiae* (PE-2) and by the wild yeast strain *Wickerhamomyces* sp. (UFFS-CE-3.1.2). With the pretreatment used (0.2 g_{CaO}·g_{biomass}⁻¹ and 70 °C) followed by enzymatic hydrolysis, a concentration of 0.31 g_{sugar}·g_{biomass}⁻¹ was obtained. The fermentation yielded around 0.38 g_{ethanol}·g_{dry biomass}⁻¹, using commercial and wild yeasts. Accordingly, corn stover has a high potential for bioethanol production, although further studies to improve the process and evaluate the production at larger scales are required.

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