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# Valorization of Corn Cobs for Xylitol and Bioethanol Production through Column Reactor Process

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**Abstract:** Corncobs are a plentiful lignocellulosic material that can be utilized for energy production as well as the generation of other high-value products. Within the modern concept of biorefineries, we present processes conducted in a column reactor for the valorization of corncobs as a substrate for ethanol and xylitol production. In the first step, corncobs were subjected to acid hydrolysis, resulting in a hemicellulosic hydrolysate rich in xylose sugars intended for xylitol production by *Candida tropicalis* UFMGBX12-a. The $Y_{P/S}$ (yield coefficient of product to substrate) and $Q_P$ (productivity) values were approximately 0.2 g/g and 0.15 g/L·h, respectively, for the assays conducted in the column reactor. Next, the remaining solid portion of cellulignin was used for ethanol production through semi-simultaneous saccharification and fermentation process by *Scheffersomyces parashehatae* UFMG-HM 52.2. This approach involved an intensified successive process consisting of alkaline pretreatment of cellulignin, followed by enzymatic hydrolysis and fermentative processes conducted in the same reactor without biomass transfer. After obtaining the enzymatic hydrolysate, a $Q_P$ value of 0.4 g/L·h for ethanol production was observed in the fermentation process conducted in the column reactor. The results demonstrate the potential of corncobs as a carbon source for biomolecules production, utilizing a process conducive to scale-up.

**Keywords:** corncobs; column reactor; xylitol; ethanol



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## 1. Introduction

Fossil fuels have been the primary source of energy for several decades, supporting various forms of transportation, industries, and domestic sectors. However, their extensive use has led to severe environmental consequences such as air and water pollution, greenhouse gas emissions and climate change [1]. Therefore, there is a current global need to transition to sustainable and renewable energy sources through environmentally friendly processes. According to the International Energy Agency (IEA), renewable energy sources are projected to become the largest source of electricity generation worldwide by 2025 [2]. This transition has been facilitated by the decreasing costs of renewable energy technologies, improved efficiency, and supportive policies and regulations.

Given this scenario, it is crucial to extract valuable resources and materials from agro-industrial waste. For example, lignocellulosic biomass waste is currently utilized to produce bioenergy materials such as bioethanol, biohydrogen, and biogas [3]. Lignocellulose is a complex combination of three major components: cellulose, hemicellulose, and lignin. These components constitute the cell walls of plants and are abundant in various biomass sources, including wood, grasses, and agricultural residues. The conversion of lignocellulosic materials into biofuels and high-value products contributes to a more sustainable and efficient utilization of biomass resources while reducing waste accumulation in the environment [4].

Within this context, corn is one of the primary grains globally and plays a significant role in bioenergy production. In 2021, global corn production exceeded 1.2 billion tons, with the United States being the largest producer (384 million tons), followed by China (272 million tons) and Brazil (88 million tons) [5,6]. In Brazil, corn is widely utilized in animal feed, the food industry, and bioenergy production. Corn ethanol, produced through grain fermentation, serves as a vital source of renewable energy in the country. Furthermore, biogas production from corn's residual biomass has proven to be a viable and clean energy alternative.

Corncobs, generated as a byproduct of corn cultivation, are an essential lignocellulosic biomass. They comprise a central core composed of both hard and soft woody material, along with lignin, cellulose, and hemicellulose [5]. Approximately 0.3 tons of corncobs are generated for every ton of processed corn, estimating the world's annual corncobs production to be around 363 million tons [7]. This substantial availability makes corncobs an excellent carbon source for fermentative processes [7]. Moreover, their utilization offers environmental benefits by substituting non-renewable resources such as fossil fuels, and corncobs have a lower carbon footprint compared to many other biomass sources. This makes them an environmentally friendly option for energy and chemical production.

However, the pretreatment of plant biomass, including corncobs, is necessary to obtain fermentable sugars from their carbohydrate components. The pretreatment process involves physical, chemical, or biological methods to break down or disrupt the biomass's primary components [8]. These processes aim to enhance cellulose accessibility to enzymes, thereby facilitating hydrolysis. During hydrolysis, cellulose and hemicellulose are converted into fermentable sugars, including C5 sugars (xylose and arabinose) and C6 sugar (glucose) [4]. Overall, pretreatment and enzymatic hydrolysis are critical steps in the production of biofuels from lignocellulosic biomass.

In addition to biofuels, the conversion of lignocellulosic biomass can be utilized to produce a wide range of other products, including bioplastics, biopigments, biopolymers, biochemicals, and other high-value-added products [3]. Two commonly used processes for this conversion are simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF) or semi-simultaneous saccharification and co-fermentation (SSSCF). SSSCF involves a prior saccharification stage followed by the simultaneous hydrolysis of lignocellulosic biomass and fermentation of the resulting C5 and C6 sugars to produce biofuels and other bioproducts. The advantage of SSF and SSSCF is that they reduce the cost of the process by combining two steps into one, increasing the overall yield of biofuels [9]. SHF, on the other hand, involves the hydrolysis of lignocellulosic biomass and fermentation in separate steps and reactors, usually increasing the overall cost of the process [10,11]. Indeed, saccharification followed by fermentation techniques can generate a wide range of bioproducts, including biofuel commodities and high-value chemicals [12]. Ethanol is one of the most promising alternative biofuels to replace gasoline in the transportation fuel market. Bioethanol offers advantages such as a low boiling point, higher heat of vaporization, and high-octane number. Currently, in many countries, bioethanol is blended with gasoline mixture [13–15]. The United States and Brazil are the major ethanol producers, accounting for approximately 80% of the world's production. In 2022, the US produced 15.4 billion gallons of biofuel, while Brazil produced 7.5 billion gallons [16]. Ethanol production can be classified into first and second generation. First-generation ethanol (1G) is produced on a large scale from feedstocks with high carbohydrate content intended for human and animal feed, such as sugarcane, corn, wheat, and soybean, among others [17]. Second-generation ethanol (2G) is one of the most promising technologies and is based on the production of biofuel from lignocellulosic materials such as sugarcane bagasse and byproducts from the processing of corn and rice [18]. Xylitol, a versatile platform chemical derived from lignocellulosic biomass, is classified as another important bioproduct. Xylitol is a key ingredient in formulations of products manufactured by the pharmaceutical, food, and dental industries due to its insulin-independent metabolism, low glycemic index, and anticariogenic nature [19]. The global xylitol market size is projected

to grow from $1.13 billion in 2022 to $1.2 billion in 2023, with a compound annual growth rate (CAGR) of 6.6%, and it is predicted to reach $1.56 billion in 2027 with a CAGR of 6.7% [20]. Industrial xylitol is currently produced through catalytic hydrogenation under high-pressure (up to 50 atm) and high-temperature (80–140 °C) conditions. This route has several disadvantages, including high operating and purification costs, high energy requirements, extensive purification and separation steps, and the generation of toxic by-products [21]. In recent years, the biotechnological route for xylitol production has gained attention due to it having several advantages compared to the chemical route. The biotechnological process for xylitol production is based on the conversion of biomass sources with xylitol-producing microorganisms under comparatively milder operating conditions. Furthermore, this bioprocess has a lower corrosive effect on reactors, releases fewer toxic by-products into the environment, and has a lower carbon footprint [21–23]. However, both second-generation ethanol and xylitol production still face several challenges related to efficient technologies for raw material pretreatment, hydrolysate fermentation, bioproduct recovery, and concerns regarding scale-up implementation [21,24]. Therefore, novel techniques need to be studied to enable more feasible and efficient bioprocesses. In this context, the use of column reactors represents a potential alternative for the intensification of chemical and fermentative processes due to their mild operating conditions, reduced pressures, improved oxygen mass transfer capability, homogeneity in the reaction fluid, and catalyst. Additionally, this type of reactor does not require the use of mechanical stirrers, providing high homogenization through air insertion and/or fluid circulation, and has a simple geometry and operability.

In this work, we present a novel proposal to explore possibilities for valorizing all carbohydrate fractions of corncobs as a renewable and cost-effective source of fermentable sugars (5C and 6C) for the production of xylitol and ethanol. We presented a process intensification approach by synthesizing consecutive steps in the same column reactor operation. The hemicellulosic hydrolysate from the lignocellulosic biomass is directed to a fermentative process conducted in a column reactor operated under fluidized bed regime, while simultaneously, in pursuit of process intensification, cellulignin biomass undergoes alkaline pretreatment and the SSSCF process in another column reactor for ethanol production.

## 2. Materials and Methods

Figure 1 presents the flowchart related to the conducted experiments carried out in this work.

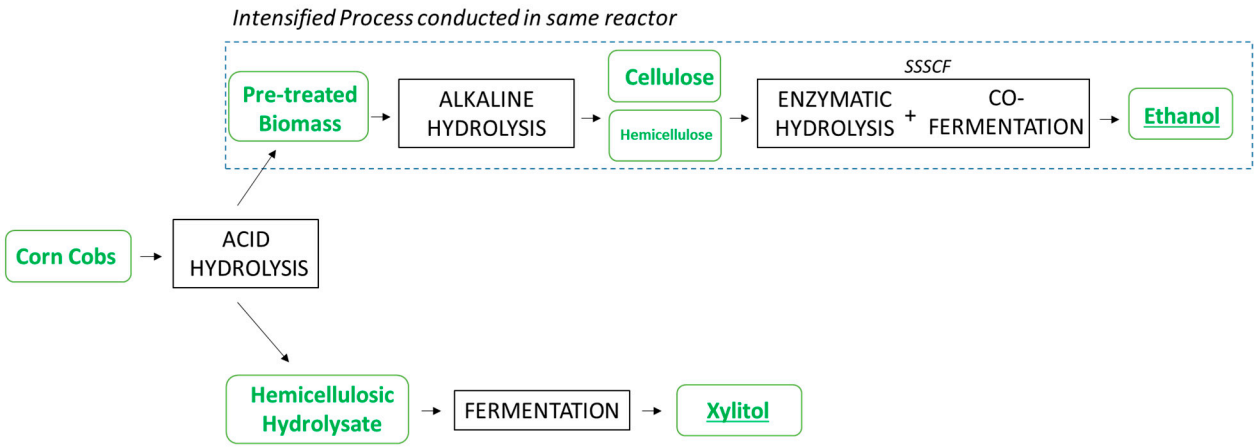


**Figure 1.** Flowchart outlining the experiments conducted for the valorization of corncobs in processes carried out in a column reactor for ethanol and xylitol production.

### 2.1. Raw Material and Column Reactor

Corncobs (Juiz de Fora, MG, Brazil) were used as the vegetal biomass in this study. The material was washed, dried, and milled using a Hammer mill Benedetti 270 (Mill Benedetti Ltd., Pinhal, SP, Brazil). After being ground, the material underwent classification using standardized Tyler series sieves. The remnant particles were found to have a size between 0.841 and 0.177 mm, corresponding to the 20 and 80 mesh sieves, respectively. This sieving process helped to determine the particle size distribution of the material.

The reactor consisted of a boron silicate glass column with a height (H) of 90 cm, an inner diameter (D) of 4 cm, and a surrounding jacket thickness of 1 cm, as shown in Figure 2A. The reactor was operated in a fluidized bed configuration for the alkaline hydrolysis, enzymatic saccharification, and fermentation experiments. Figure 2 illustrates the flowchart of the column reactor and a photograph of the actual apparatus.

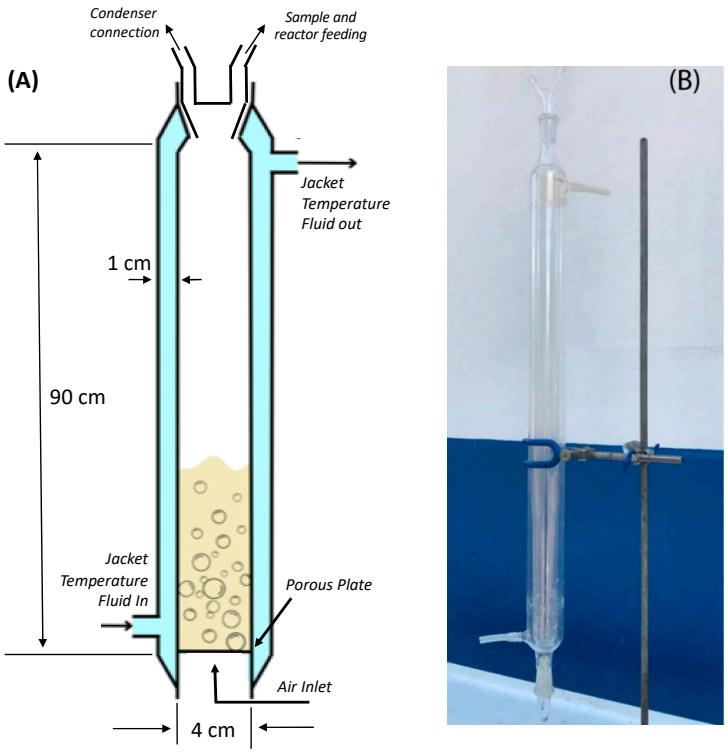


**Figure 2.** (**A**) Flowchart of the column reactor and (**B**) a photograph of the actual apparatus.

### 2.2. Microorganisms

The microorganisms *Candida tropicalis* UFMGBX12-a and *Scheffersomyces parashehatae* UFMG-HM 52.2 (wild Brazilian yeast) from the collection of the Federal University of Minas Gerais were used for the xylitol and ethanol production assays, respectively. The maintenance, inoculation, and cultivation of the microorganisms followed the methodology proposed by Chandel et al. [25].

### 2.3. Corncobs Hemicellulosic Hydrolysate

The acid hydrolysis of corncobs was conducted in a 30 L stirred tank under the conditions described by Chandel et al. [25] (100 mg $H_2SO_4$/g of dry corncobs, 20 min at 121 °C, 1:10 (mass of biomass/volume of solution)). Subsequently, the hemicellulosic hydrolysate of corncobs was filtered, and the remaining solid portion (cellulignin) was washed and dried for further processing. The hemicellulosic hydrolysate was then concentrated and detoxified using the overliming + active charcoal methodology as described by Antunes et al. [26].

### *2.4. Xylitol Production from Corn Cob Hemicellulosic Hydrolysate*

For the production of xylitol, 50 mL of corncob hemicellulosic hydrolysate was enriched with 5 g/L of potassium dihydrogen phosphate, 0.4 g/L of magnesium sulfate, and 10 g/L of yeast extract. Additionally, 0.5 g/L of *Candida tropicalis* UFMGBX12-a cells were added. The mixture was incubated in 125 mL Erlenmeyer flasks at 200 rpm in a rotary incubator at 30 °C for 96 h. In the experiments performed in the column reactor, 700 mL of supplemented corncobs hemicellulosic hydrolysate, along with 0.5 g/L of *Candida tropicalis* UFMGBX12-a, was introduced into the reactor bed with air microbubbles aeration at 0.3 $min^{-1}$ (air flow per volume of medium) using a porous plate-diffuser, as depicted in Figure 2. This provided homogenization, oxygen, and mass transfer to the bed column reactor. Fermentation was carried out for 48 h, and the temperature was controlled by water circulation in the surrounding jacket of the reactor (Figure 2). Samples were taken at different time points throughout the process for the analysis of fermentable sugars, ethanol, xylitol, and biomass concentration.

### *2.5. Intensified Process for Ethanol Production from Corncobs Enzymatic Hydrolysate*

The following three processes (Alkaline hydrolysis, enzymatic saccharification and fermentation (SSSCF)) were carried out by an intensification process method performed in the same device: Erlenmeyer flasks or a column reactor.

#### 2.5.1. Alkaline Pretreatment

The resting solid corncobs portion from the acid pretreatment (celulignin) was performed to the alkaline hydrolysis (alkaline pretreatment), aiming to raise enzymatic digestibility. In the assays carried out in Erlenmeyer flasks (125 mL), 70 mL of NaOH (0.5M) solution and 3 g of acid pretreated biomass were stirred at 200 rpm for 120 min at 90 °C. Thereafter, the obtained solid fraction after alkaline pretreatment was maintained in the flask, washed until a neutral pH was achieved and used in the following saccharification process.

In the assays conducted in the column reactor, 30 g of biomass and 700 mL of NaOH (0.5M) solution were introduced at reactor bed homogenized by air insertion at 0.3 $min^{-1}$ (air flow per volume of medium), for 120 min at 90 °C. From then on, the obtained solid fraction after alkaline pretreatment was maintained in the reactor, washed until a neutral pH was achieved (by inserting water and sucking the liquid by a peristaltic pump in successive times) and used in the following saccharification process.

#### 2.5.2. Semi-Simultaneous Saccharification and Fermentation

The remaining acid and alkaline pre-treated corn cob cellulignin was performed to the enzymatic hydrolysis, aiming to obtain fermentable sugars. In the process performed in Erlenmeyer flasks (125 mL), 23.4 mL of citrate buffer (pH 4.8, 50 mM)/g of acid-alkaline pretreated corncobs and 20 FPU (filter paper unit) of cellulase enzyme complex (Cellulase CP CONC/Dyadic)/g of biomass were added within 0.3 mL of tween at a temperature of 50 °C, stirred at 200 rpm, for 48 h. After 48h, temperature was reduced to 30 °C for the assays starting and sodium hydroxide was used in medium to accurate the pH to 5.5. Thus, medium supplementation (3 g/L of yeast extract, 5 g/L of ammonium sulfate, and 3 g/L of malt extract) and 0.5 g/L cells of *Scheffersomyces parashehatae* UMFG-HM 52.2 were added in the flasks. The fermentation assays were conducted for 72 h at 200 rpm (in rotatory movement incubator) and 30 °C.

Correspondingly, 23.4 mL of citrate buffer (pH 4.8,50 mM)/g of acid and alkaline pretreated corncobs and 20 FPU (filter paper unit) of cellulase enzyme complex (Cellulase CP CONC/Dyadic)/g of biomass, added within 0.3 mL of tween solution were introduced at the reactor bed homogenized by air insertion at 0.3 $min^{-1}$ (air flow per volume of medium) at a temperature of 50 °C for 48 h. After 48 h, temperature was reduced to 30 °C for the assays starting and sodium hydroxide was used in medium to accurate the pH to 5.5. Thus, medium supplementation (3 g/L of yeast extract, 5 g/L of ammonium sulfate,

and 3 g/L of malt extract) and 0.5 g/L cells of *Scheffersomyces parashehatae* UMFG-HM 52.2 were added in column reactor. The fermentation assays were performed at the reactor bed homogenized by air insertion at 0.3 $min^{-1}$ conducted for 48 h at 30 °C. Samples were taken in different times along process for fermentable sugars, ethanol, xylitol, and biomass concentration analysis.

### *2.6. Analytical Methods*

The corncobs chemical composition (raw material, after acid pretreatment and after acid and alkaline pretreatment) was quantified according to the Laboratory Analytical Procedure (LAP) (titled "Determination of Structural Carbohydrates and Lignin in Biomass", Technical Report NREL/TP-510-42618, Version 07-08-2011), as methodology used by Antunes et al. [26], expressing percentage of cellulose, hemicellulose and lignin for each dry sample. The analysis of glucose, xylose and alcohol concentration was carried out by High Performance Liquid Chromatography (HPLC) [26].

The biomass concentration was verified by turbidimetry by using spectrophotometer (Beckman DU 640 B Fullerton, CA) at 600 nm and associated with the dry weight of cells (g/L) by related calibration curve [26]. The fermentation parameters were (i) yield of fermentable sugars in bioproduct ($Y_{P/S}$), fermentable sugar yield in biomass ($Y_{X/S}$), volumetric productivity ($Q_P$) and sugar consumption (%) [25]. Glucose and xylose concentration after enzymatic saccharification were used to calculate glucan and xylan yield conversion, according to the equations used by Antunes et al. [26], as follows: G = ([(Glucose * V* 0.9)/(glucan content * m)] * 100), where G is cellulose conversion yield in glucose, glucose is glucose concentration in the enzymatic hydrolysate (g/L); V is volume of enzymatic hydrolysate (L); m is mass oven-dried WIS (g) and X = ([(Xylose * V * 0.88)/(xylose content * m)] * 100), where X is hemicellulose conversion yield in xylose, Xylose is xylose concentration in the enzymatic hydrolysate (g/L); V is volume of enzymatic hydrolysate (L) and m is mass oven-dried WIS (g).

## 3. Results

### *3.1. Biomass Characterization and Pre-Treatment*

Lignocellulosic materials consist primarily of cellulose (35 to 50%), hemicellulose (20 to 35%), and lignin (5 to 30%) in terms of composition. However, there can be variations in their compositions depending on the species, variety, origin, and cultivation [27]. Figure 3 illustrates the chemical characterization of corncob in its natural state, after acid pretreatment (performed in a stirred tank reactor), and after acid and alkaline pretreatment (performed in a column reactor).

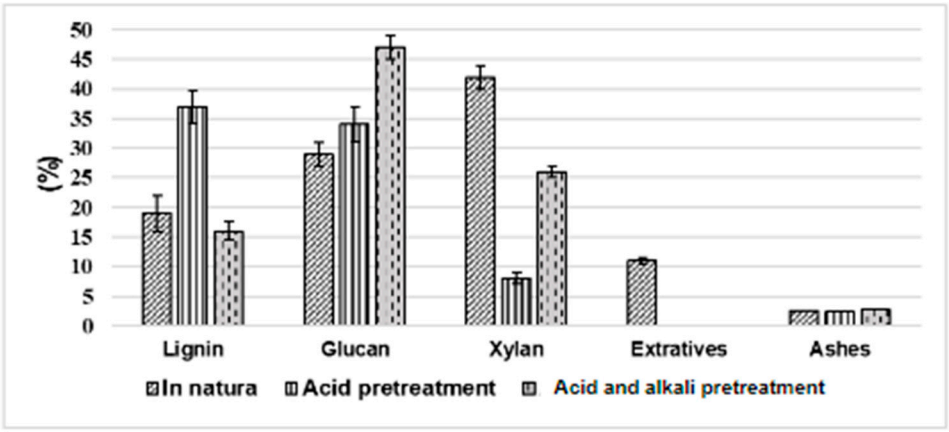


**Figure 3.** Chemical characterization of corncobs in nature, after acid pretreatment and after acid and alkaline pretreatment.

Corncobs in their natural state contained approximately 28% cellulose, 42% hemicellulose, and 19% lignin. In a study by Biswas et al. [28] on corncob biomass from India, it was found to contain 32% glucan, 25.9% hemicellulosic, 22% lignin, and 2.6% ash. Acid pretreatment of the corncob resulted in hemicellulosic hydrolysate with around 19.00 g/L of xylose. Biswas et al. [28] conducted acid hydrolysis of corncob using sulfuric acid as a catalyst under various temperature, time, and acid concentration conditions. They tested sulfuric acid concentrations ranging from 0.5% to 3% (*w*/*w*), temperatures from 120 °C to 160 °C, and reaction times from 30 to 120 min. The results indicated that higher temperature and sulfuric acid concentration led to more efficient acid hydrolysis of corncobs. The highest conversion of cellulose to glucose in their study was achieved using 3% (*w*/*w*) sulfuric acid at 160 °C for 120 min.

In the present work, the solid portion of the remaining biomass after acid pretreatment underwent intensified processes, starting with alkaline pretreatment to reduce lignin content and enhance biomass enzymatic digestibility. This was carried out in shake flasks and column reactor. After alkaline pretreatment in the column reactor, samples showed 16% lignin, 47% cellulose, and 26% hemicellulose. Although the acid and alkaline pretreated solid portion already contained some lignin, its reduction was effective in improving biomass digestibility and the remaining portion did not pose any negative effects or hinder the subsequent semi-simultaneous saccharification and co-fermentation process. In a recent study by Ayeni et al. [29], alkaline pretreatment of corncobs with 0.5% NaOH and 1% *v*/*v* peroxide at 105 °C for 3 h resulted in 89% glucan hydrolysis yield at 48 h. In this work, after alkaline pretreatment, the alkaline black liquor containing solubilized lignin was removed, and the acid and alkaline pretreated biomass was retained in the same vessel (shake flasks or column reactor). It was washed and subjected to enzymatic saccharification. After 72 h of enzymatic hydrolysis, the intensified acid and alkaline-enzymatic hydrolysis process in the column reactor yielded around 19 g/L of glucose and 9 g/L of xylose, with approximately 84% glucan and 86% xylan yield. Similarly, the intensified alkaline-enzymatic hydrolysis process in shake flasks yielded around 21 g/L of glucose and 10 g/L of xylose, with approximately 93% glucan and 92% xylan yield.

Pereira et al. [30], e.g., performed steam explosion pretreatment of corncobs prior to enzymatic hydrolysis. The cobs were subjected to temperatures of 200 °C and 220 °C for 10 min and then rapidly cooled. The pretreatment resulted in a reduction in cob mass and partial breakdown of the lignocellulosic structure, facilitating enzymatic hydrolysis. Enzymatic hydrolysis was carried out using a mixture of cellulolytic enzymes and xylanases, with variations in hydrolysis time and enzyme concentration. The results showed that enzymatic hydrolysis was more efficient for cobs pretreated at 220 °C, resulting in a glucan yield of 44.2% and xylan yield of 56.9% compared to cobs pretreated at 200 °C.

The results of the intensified chemical-enzymatic processes demonstrated the potential for obtaining enzymatic hydrolysate, with profitable values achieved in both shake flasks and column reactors. This approach is suitable for use in sequential fermentation steps.

### *3.2. Xylitol Production Form Corncobs Hemicellulosic Hydrolysate by Using Candida tropicalis UFMGBX12-a*

In the fermentation process using *Candida tropicalis* UFMGBX12-a yeast, the hemicellulosic hydrolysate obtained from the acid hydrolysis pre-treatment of corncob, which is rich in fermentable sugars such as xylose and glucose, was used as the substrate.

The profile of fermentable sugars consumption, xylitol production, and biomass production in Figure 4 provide a visual representation of these changes over time, allowing for a better understanding of the fermentation process and the performance of *Candida tropicalis* UFMGBX12-a yeast in producing xylitol from corncob hemicellulosic hydrolysate.

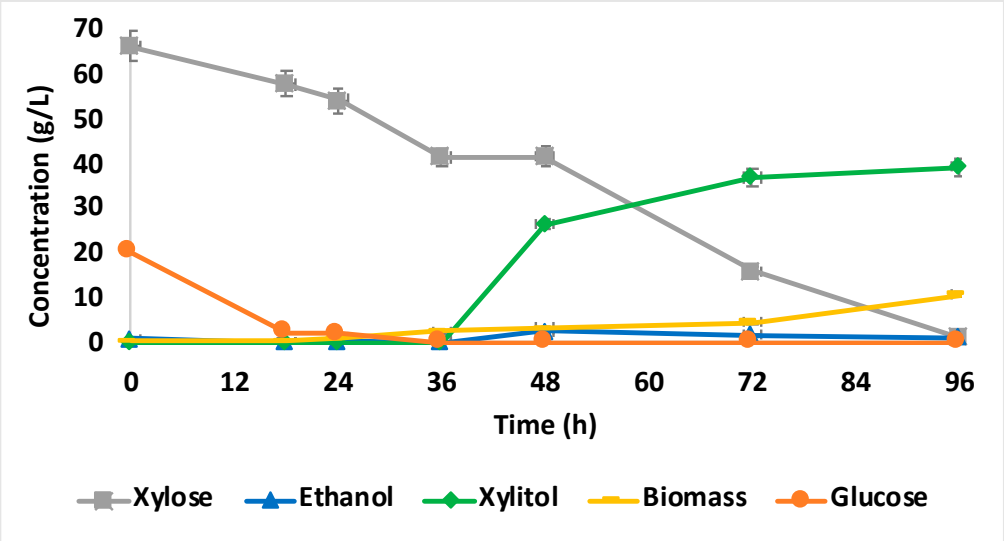


**Figure 4.** Xylitol production from corncob hemicellulosic hydrolysate using *Candida tropicalis* UFMGBX12-a in shake flasks.

In this fermentative process, the initial concentration of hydrolysate was 66.39 g/L and 20.21 g/L of xylose and glucose, respectively. It was observed that all fermentable sugars were completely consumed within 96 h. Glucose was initially consumed within 12 h, mainly for energy and biomass production through the Embden-Meyerhof pathway, while xylose was consumed over the course of 96 h for xylitol production via the xylose reductase enzyme, which is commonly found in yeast [31]. Xylose was also concurrently used for cell maintenance. The yield of biomass to substrate ($Y_{X/S}$) was 0.12 g/g. The maximum concentration of xylitol reached 39.43 g/L in 96 h, with a yield of product to substrate ($Y_{P/S}$) of 0.60 g/g and a volumetric productivity of 0.41 g/L·h for xylitol production. The concentration of ethanol was relatively low, reaching 2.72 g/L at 72 h, which is a common occurrence in C5 converter yeast. Ethanol production could be partially attributed to glucose metabolism through the Embden-Meyerhof pathway and the conversion of xylitol to xylulose via the xylitol dehydrogenase enzyme, followed by metabolization through the pentose phosphate pathway. The higher $Y_{P/S}$ compared to $Y_{X/S}$ indicated that the fermentation conditions favored xylitol production.

To the best of our knowledge, this is the first report of xylitol production from corncobs hydrolysate using the wild yeast strain *Candida tropicalis* UFMGBX12-a from the Brazilian ecosystem.

In fact, research on the production of xylitol from corncobs primarily relies on the use of genetically modified microorganisms (GMOs) engineered to utilize xylose as a carbon source [32–34]. Specifically, the genetically modified *Saccharomyces cerevisiae* PE-2-GRE3 demonstrated a high productivity of 0.83 g/L·h [33]. Similarly, engineered *Escherichia coli* achieved an impressive productivity of up to 1.04 g/L·h, setting a record for microbial fermentation [34].

Nevertheless, the use of GMOs for industrial applications is limited due to regulatory challenges, potential environmental risks, and other disadvantages when compared to native microorganisms. Native microorganisms are more scalable, cost-efficient, sustainable, and therefore better suited for industrial production.

Native microorganisms such as *Debaryomyces nepalensis* NCYC 3413 (21.5 g xylitol/L) [35], *Meyerozyma caribbica* InaCC Y67 (6.49 ± 0.12 g xylitol/L) [36], and various species of *Candida* spp. including *C. tropicalis* CCTCC M2012462, *C. tropicalis* PNL3, *C. tropicalis* MTCC 6192, *C. tropicalis* KUEN 1022, *C. guilliermondii* ATCC 201935, among others, have been utilized for the production of xylitol from corncobs [37–39]. However, there have been no reports found regarding *Candida tropicalis* UFMGBX12-a [37–39].

Additionally, we studied xylitol production from corncobs hydrolysate in a column reactor, using a simple homogenization system achieved by introducing air microbubbles. Experimental verification determined that a minimal aeration rate of 0.3 $min^{-1}$ (air flow per volume of medium) provided feasible homogenization in the bioreactor. Figure 5 illustrates the profile of fermentable sugar consumption, xylitol production, and biomass production in a fermentation process conducted in column reactor using the microorganism *Candida tropicalis* UFMGBX12-a.

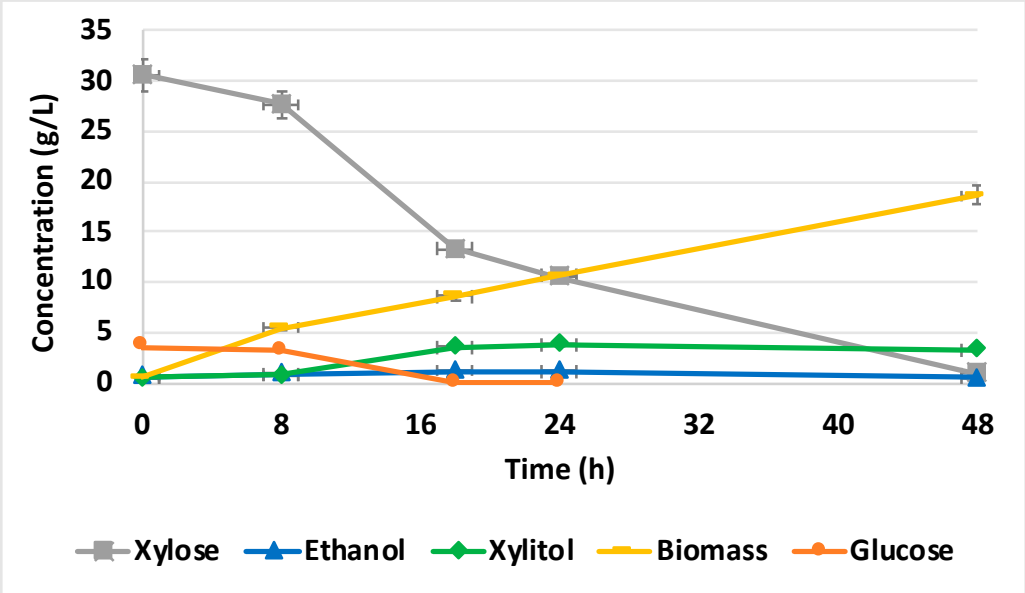


**Figure 5.** Xylitol production from corncob hemicellulosic hydrolysate using *Candida tropicalis* UFMGBX12-a in column reactor.

In this fermentative assay, the initial concentration of hydrolysate was 30.59 g/L and 3.61g/L of xylose and glucose, respectively. It was observed that all fermentable sugars were completely consumed within 48 h. Glucose was consumed first, followed by xylose. The maximum concentration of xylitol reached 3.82 g/L in 24 h, with a yield of product to substrate ($Y_{P/S}$) of 0.19 g/g and a volumetric productivity of 0.15 g/L·h for xylitol production. The maximum yield of biomass to substrate ($Y_{X/S}$) was 0.54 g/g. It was noticed that the highest xylitol production occurred within the first 24 h when xylose was readily available in the medium, and no further increase in xylitol concentration was observed after that time. From 24 to 48 h, xylitol production remained constant while biomass concentration increased. Only small amounts of ethanol were detected, reaching 1.17 g/L at 18 h. In the column reactor experiments, the higher $Y_{X/S}$ compared to $Y_{P/S}$ indicated that the fermentation conditions favored biomass production. This could be attributed to the high level of aeration provided to the system. Oxygen concentration is a crucial parameter for xylitol production by yeast. Under low oxygen availability conditions, there is a redox imbalance that limits the rate of xylitol dehydrogenase activity, leading to more xylitol accumulation and excretion by the cells. However, under higher oxygen concentration conditions, the reaction rate catalyzed by xylitol dehydrogenase is not limited, allowing for the conversion of xylitol to xylulose and its further metabolization through the pentose phosphate pathway [40]. In this experiment, it is suggested that the aeration rate in the column reactor should be decreased to promote less oxygen availability and enhance xylitol production instead of biomass. However, experimental verification showed that an aeration rate lower than 0.3 $min^{-1}$ (air flow per volume of medium) did not provide feasible homogenization in the reactor. Therefore, an alternative approach could be the addition of an inert gas to the system to improve fluid homogenization while reducing oxygen availability. Stirred tank reactors are commonly used for xylitol production from

yeast at the bench and pilot scale, but research on xylitol production in column reactors is scarce. Some studies have reported the use of column reactors for other applications, such as ethanol production from wheat straw and the growth of lipid-rich bacterial biomass. However, to the best of our knowledge, there is a lack of research on the use of column reactors for fermenting corncobs hydrolysate.

Indeed, stirred tank reactors are commonly reported for xylitol production from yeast at bench and pilot scale [41]. However, research on the production of xylitol in column reactors is limited. For instance, there is a study highlighting the use of the yeast *Candida guilliermondii* immobilized in calcium-alginate in a column reactor at an aeration rate of 0.66 to 1.33 $min^{-1}$ (air flow per volume of medium), which achieved a maximum volumetric productivity of 0.21 g/L·h from sugarcane bagasse [42]. Furthermore, bubble column reactors have been employed in various applications, such as fermenting wheat straw rich in cellulose to produce ethanol (9.31 g/L) [43], integrating enzymatic pretreatment and hydrolysis of apple pomace with *Rhodococcus opacus* [44], and growing lipid-rich bacterial biomass utilizing refinery wastewater [45].

### *3.3. Ethanol Production from Acid and Alkali Pretreated Corncobs through Integrated Semi-Simultaneous Saccharification and Co-Fermentation Processes*

After acid hydrolysis, the celulignin corncobs underwent subsequent alkali enzymatic pretreatment, followed by co-fermentation. These steps were conducted in shake flasks and a column reactor. Semi-simultaneous saccharification and co-fermentation were initiated after 72 h of enzymatic hydrolysis of the acid-alkaline pretreated biomass at 50 °C. *S. parashehatae* UFMG-HM 52.2 cells and nutritional supplementation were added to the system, gradually reducing the temperature to 30 °C, to initiate the simultaneous saccharification and co-fermentation process. Figure 6 illustrates the fermentation profile of ethanol production from corncobs enzymatic hydrolysate using *Scheffersomyces parashehatae* UMFG-HM 52.2 in shake flasks.

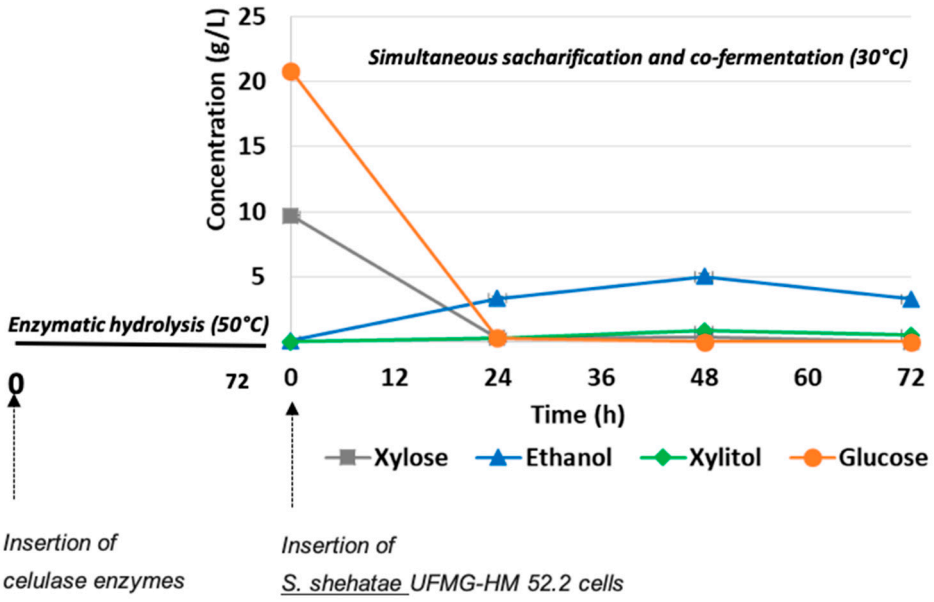


**Figure 6.** Ethanol production from corn cob enzymatic hydrolysate using *Scheffersomyces parashehatae* UMFG-HM 52.2 in shake flasks.

Figure 6 shows the fermentation profile of ethanol production from corn cob enzymatic hydrolysate by using *Scheffersomyces parashehatae* UMFG-HM 52.2 in shake flasks.

Ethanol fermentation was initiated with an initial concentration of 20.86 g/L of glucose and 9.68 g/L of xylose. It was observed that both sugars were completely consumed by the yeast. The maximum ethanol production reached 5.0 g/L at 48 h of simultaneous saccharification and co-fermentation. The specific ethanol production rate ($Q_P$) was 0.1 g/L·h, and

the yield of ethanol to substrate ($Y_{P/S}$) was approximately 0.16 g/g. The initial glucose and xylose yields were 93% and 92%, respectively. Although the conversion rate was low, even at 30 °C and pH 5.5, hydrolysis of remaining cellulose continued concurrently with sugar consumption by the yeast. Due to this ongoing hydrolysis, it was not possible to precisely calculate the conversion yield, but it slightly affected the $Y_{P/S}$ value due to the high initial values observed at the beginning of fermentation.

A depletion of ethanol was observed because the yeast utilized the produced ethanol as a carbon source during periods of carbohydrate scarcity [26], even though it resulted in self-damage. A slight concentration of xylitol in the medium was also detected, as xylitol is an intermediate compound in the metabolism of C5 sugars, leading to ethanol production.

There are reports available on the production of ethanol from corncobs with different productivities compared to the reported in the present study. However, these studies employed longer fermentation periods or different fermentation approaches. For instance, *Kluyveromyces marxianus* CICC 1727-5 achieved an ethanol productivity of 0.41 g/L·h. However, the fermentation period was longer, lasting 120 h, in comparison to the 24 h fermentation period of *S. parashehatae* UFMG-HM [46]. Similarly, *S. cerevisiae* TC-5 demonstrated a productivity of 0.291 g/L·h in three different fermentation approaches: hydrolysis and fermentation (SHF), prehydrolysis-SSF (pre-SSF), and simultaneous saccharification and fermentation (SSF) [47].

Following the same experimental approach conducted in shake flasks, Figure 7 presents the fermentation profile of ethanol production from corn cob enzymatic hydrolysate using *Scheffersomyces parashehatae* UFMG-HM 52.2 in a column reactor.

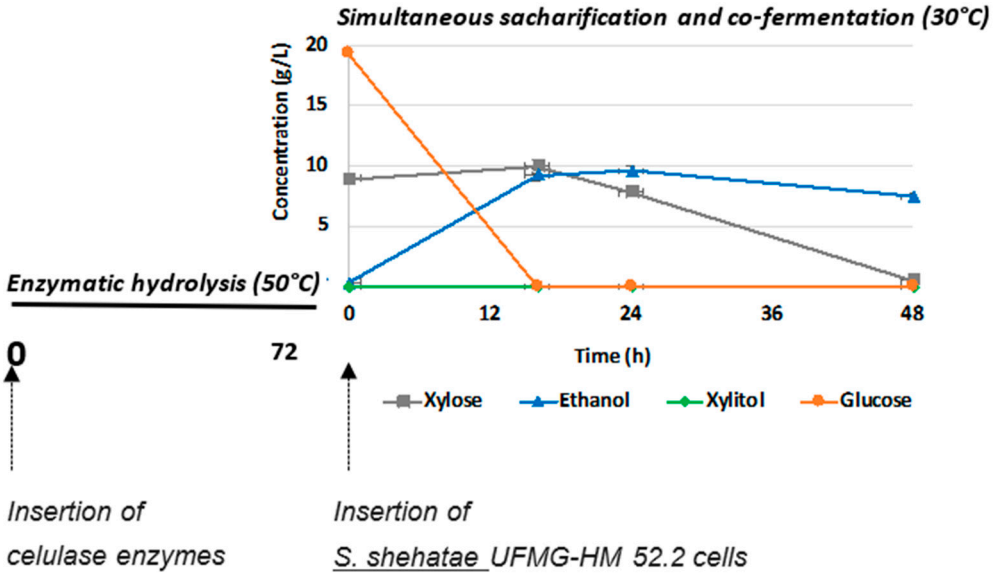


**Figure 7.** Ethanol production from corn cob enzymatic hydrolysate using *Scheffersomyces parashehatae* UMFG-HM 52.2 in column reactor operated as fluidized bed reactor.

Similar to the experiments conducted in shake flasks, total assimilation of sugars from enzymatic hydrolysate was observed in the column reactor. Glucose sugars were consumed first, followed by xylose. Maximum ethanol production was observed at 24 h of the process, with a specific ethanol production rate ($Q_P$) of 0.4 g/L·h and a yield of ethanol to substrate ($Y_{P/S}$) of 0.43 g/g. The initial glucose and xylose yields were 84% and 86%, respectively, at the beginning of the fermentation time. It is worth noting that the $Y_{P/S}$ value could be slightly changed due to the high conversion yield of carbohydrates at the start of fermentation, considering possible hydrolysis of the remaining fraction and sugar consumption during the process. For this experiment, xylitol concentration was less than 0.5 g/L.

After 24 h, the remaining xylose sugars were suggested to be utilized for cell maintenance rather than ethanol production, as no increase in ethanol concentration was observed.

The productivity achieved in the column reactor was similar to what was found for *Kluyveromyces marxianus* CICC 1727-5, but over a longer period (120 h). Furthermore, the productivity of *S. parashehatae* UFMG-HM in the column reactor was higher that of the thermotolerant yeast *Saccharomyces cerevisiae* TC-5 in an SSF process (0.291 g/L·h) [47].

The column reactor demonstrated high feasibility for process intensification, encompassing chemical, enzymatic, and fermentative processes. The reactor was initially loaded with biomass, and subsequent steps including alkaline hydrolysis, enzymatic hydrolysis, and fermentation were carried out without the need for transferring the solid portion. This approach avoids mass transfer issues and eliminates the requirement for separate reactors and configurations for different processes.

In addition to the simple system of homogenization by air insertion, the reactor temperature can be easily adjusted for each process by recirculating the fluid through the reactor jacket. Most studies on ethanol production from corncobs utilize the simultaneous saccharification and fermentation (SSF) approach. For instance, when employing unwashed corncob residues with *Saccharomyces cerevisiae* in SSF, a yield of approximately 0.4 g/L·h of ethanol was achieved [48]. On the other hand, by using genetically engineered *Pichia kudriavzevii* N-X, which performs well under high temperatures and low pH conditions, SSF resulted in 20.8 g/L of ethanol at 84.3% of the theoretical value [49]. In line with these findings, both simultaneous hydrolysis and co-fermentation (SHCF) and simultaneous saccharification and co-fermentation (SSCF) techniques were applied to corn cob with *S. cerevisiae* CRD5HS at solid loadings of 25–35% (*w*/*w*) in 250 mL flasks. The comparison between SHCF and SSCF revealed that SSCF offered the advantages of shorter process time and lower risk of contamination [50]. These advantages are also observed in the fermentation process conducted in the column reactor employed in our current research. Consequently, this study contributes by demonstrating ethanol production from corncobs with productivities equal to or even higher than those achieved in SSF processes, while reducing the likelihood of contamination. A similar intensification strategy was applied by Antunes et al. [26] for second-generation ethanol production using sugarcane bagasse in a column reactor with a central tube in a fluidized bed configuration. This highlights the potential of the current approach for intensification using a column reactor for corncobs processing and the production of various bioproducts from its fermentable sugars.

## 4. Conclusions

Corncobs have significant potential as a raw material for energy and biomolecule production. Through acid pretreatment, the hemicellulosic hydrolysate obtained from corncobs can be used for xylitol production in a fermentation process conducted in column reactors, yielding profitable results in terms of xylose conversion.

Furthermore, the remaining solid portion of the corncobs after acid pretreatment contains a substantial amount of cellulose. In this study, an intensified approach combining chemical, enzymatic, and fermentative processes was employed in a single column reactor to utilize the cellulignin fraction for bioethanol production. Alkaline hydrolysis was conducted to reduce the lignin content and enhance the enzymatic digestibility of the biomass, while keeping the biomass within the bed reactor.

Subsequently, after pH correction, a semi-simultaneous saccharification and co-fermentation process was carried out. This involved an initial enzymatic hydrolysis period, followed by the introduction of cells for fermentation. This approach demonstrated remarkable potential for ethanol production by enabling the sequential execution of three different processes in the same reactor, without the need for mass transfers between separate vessels.

Indeed, we highlight the use of a reduced number of reactors as an intensification strategy, along with the ability to obtain different bioproducts through our sugar extraction method. These factors are key to achieving economic profitability in future sugarcane biorefineries. In addition to sugar extraction, the selection of promising microorganisms capable

of metabolizing C5 and C6 sugars derived from cellulose and hemicellulose is crucial for maximizing the utilization of available fermentable fractions in biomass. Therefore, we used two wild Brazilian yeasts, *Scheffersomyces parashehatae* UFMG-HM 52.2 and *Candida tropicalis* UFMGBX12-a, isolated from the Brazilian ecosystem, for the production of ethanol and xylitol, respectively.

Moreover, the use of a column reactor offers advantages due to its simple geometry and ease of operation, making it conducive for scaling up the process. While this work focused on xylitol and ethanol production, the same approach can be applied to other biomolecules production in possible future developments of the research field. This would involve utilizing C5 sugars derived from hemicellulosic hydrolysate for one product, while simultaneously employing semi-simultaneous saccharification and simultaneous co-fermentation for the remaining biomass to produce a second product.

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**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author, on request.

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## References

1. Zhao, L.; Sun, Z.F.; Zhang, C.C.; Nan, J.; Ren, N.Q.; Lee, D.J.; Chen, C. Advances in pretreatment of lignocellulosic biomass for bioenergy production: Challenges and perspectives. *Bioresour. Technol.* **2022**, *343*, 126123. [CrossRef]
2. Kharisova, A.Z.; Shvayko, I.V.; Shalina, O.I.; Ostryakova, A.F.; Singizov, I.Y. Ensuring Economic Security by Modifying Renewable Energy Systems. In *Geo-Economy of the Future: Sustainable Agriculture and Alternative Energy*; Springer: Cham, Switzerland, 2022; pp. 851–858.
3. Wang, D.; Tian, J.; Guan, J.; Ding, Y.; Wang, M.L.; Tonnis, B.; Liu, J.; Huang, Q. Valorization of sugarcane bagasse for sugar extraction and residue as an adsorbent for pollutant removal. *Front. Bioeng. Biotechnol.* **2022**, *10*, 893941. [CrossRef]
4. Philippini, R.R.; Martiniano, S.E.; Chandel, A.K.; De Carvalho, W.; Da Silva, S.S. Pretreatment of sugarcane bagasse from cane hybrids: Effects on chemical composition and 2G sugars recovery. *Waste Biomass Valorization* **2019**, *10*, 1561–1570. [CrossRef]
5. Zou, H.; Jiang, Q.; Zhu, R.; Chen, Y.; Sun, T.; Li, M.; Zhai, J.; Shi, D.; Ai, H.; Gu, L.; et al. Enhanced hydrolysis of lignocellulose in corn cob by using food waste pretreatment to improve anaerobic digestion performance. *J. Environ. Manag.* **2020**, *254*, 109830. [CrossRef]
6. FAO—Food and Agriculture Organization of the United Nations. FAOSTAT. 2021. Available online: http://www.fao.org/faostat/en/#data/QCL (accessed on 20 April 2023).
7. Gandam, P.K.; Chinta, M.L.; Pabbathi, N.P.P.; Baadhe, R.R.; Sharma, M.; Thakur, V.K.; Sharma, G.D.; Ranjitha, J.; Gupta, V.K. Second-generation bioethanol production from corncob–A comprehensive review on pretreatment and bioconversion strategies, including techno-economic and lifecycle perspective. *Ind. Crop. Prod.* **2022**, *186*, 115245. [CrossRef]
8. Konde, K.S.; Nagarajan, S.; Kumar, V.; Patil, S.V.; Ranade, V.V. Sugarcane bagasse based biorefineries in India: Potential and challenges. *Sustain. Energy Fuels* **2021**, *5*, 52–78. [CrossRef]
9. Ishizaki, H.; Hasumi, K. Ethanol production from biomass. *Res. Approaches Sustain. Biomass Syst.* **2014**, 243–258. [CrossRef]
10. Cotana, F.; Cavalaglio, G.; Gelosia, M.; Coccia, V.; Petrozzi, A.; Ingles, D.; Pompili, E. A comparison between SHF and SSSF processes from cardoon for ethanol production. *Ind. Crop. Prod.* **2015**, *69*, 424–432. [CrossRef]
11. Dahnum, D.; Tasum, S.O.; Triwahyuni, E.; Nurdin, M.; Abimanyu, H. Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. *Energy Procedia* **2015**, *68*, 107–116. [CrossRef]

12. Takkellapati, S.; Li, T.; Gonzalez, M.A. An overview of biorefinery-derived platform chemicals from a cellulose and hemicellulose biorefinery. *Clean Technol Environ. Policy* **2018**, *20*, 1615–1630. [CrossRef]
13. 13] Mood, S.H.; Golfeshan, A.H.; Tabatabaei, M.; Jouzani, G.S.; Najafi, G.H.; Gholami, M.; Ardjmand, M. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renew. Sustain. Energy Rev.* **2013**, *27*, 77–93. [CrossRef]
14. Sindhu, R.; Binod, P.; Pandey, A.; Ankaram, S.; Duan, Y.; Awasthi, M.K. Biofuel production from biomass: Toward sustainable development. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 79–92.
15. Dhamodaran, G.; Esakkimuthu, G.S.; Palani, T.; Sundaraganesan, A. Reducing gasoline engine emissions using novel bio-based oxygenates: A review. *Emergent Mater.* **2023**, 1–21. [CrossRef]
16. Aizarani, J.; Statista. Ethanol Fuel Production in Top Countries 2022. Available online: https://www.statista.com/statistics/281606/ethanol-production-in-selected-countries/ (accessed on 1 March 2023).
17. Ganguly, P.; Sarkhel, R.; Das, P. The second- and third-generation biofuel technologies: Comparative perspectives. In *Sustainable Fuel Technologies Handbook*; Academic Press: New York, NY, USA, 2021; pp. 29–50.
18. Soares, L.B.; da Silveira, J.M.; Biazi, L.E.; Longo, L.; de Oliveira, D.; Furigo Júnior, A.; Ienczak, J.L. An overview on fermentation strategies to overcome lignocellulosic inhibitors in second-generation ethanol production using cell immobilization. *Crit. Rev. Biotechnol.* **2022**, 1–22. [CrossRef]
19. Kaur, S.; Guleria, P.; Yadav, S.K. Evaluation of Fermentative Xylitol Production Potential of Adapted Strains of Meyerozyma caribbica and *Candida tropicalis* from Rice Straw Hemicellulosic Hydrolysate. *Fermentation* **2023**, *9*, 181. [CrossRef]
20. Xylitol Global Market Report 2023—By Type (Wood Fibrous Sweetener, Birch Xylitol, Corn Xylitol, Other Types), by Form (Solid, Liquid), by Application (Food and Beverages, Oral Hygiene Products, Cosmetics and Toiletries, Pharmaceuticals, Other Applications)—Market Size, Trends, and Global Forecast 2023–2032. Available online: https://www.thebusinessresearchcompany.com/report/xylitol-global-market-report#:~:text=The%20global%20xylitol%20market%20size,(CAGR)%20of%206.6%25 (accessed on 14 March 2023).
21. Jain, V.; Awasthi, A.; Ghosh, S. Biosynthesis of xylitol by cell immobilization: An insight. *Biomass Convers. Biorefin.* **2023**, 1–13. [CrossRef]
22. De Albuquerque, T.L.; Da Silva, I.J., Jr.; De Macedo, G.R.; Rocha, M.V.P. Biotechnological production of xylitol from lignocellulosic wastes: A review. *Process Biochem.* **2014**, *49*, 1779–1789. [CrossRef]
23. Arcaño, Y.D.; García, O.D.V.; Mandelli, D.; Carvalho, W.A.; Pontes, L.A.M. Xylitol: A review on the progress and challenges of its production by chemical route. *Catal. Today* **2020**, *344*, 2–14. [CrossRef]
24. Felipe Hernández-Pérez, A.; De Arruda, P.V.; Sene, L.; Da Silva, S.S.; Kumar Chandel, A.; De Almeida Felipe, M.D.G. Xylitol bioproduction: State-of-the-art, industrial paradigm shift, and opportunities for integrated biorefineries. *Crit. Rev. Biotechnol.* **2019**, *39*, 924–943. [CrossRef]
25. Chandel, A.K.; Antunes, F.A.; Anjos, V.; Bell, M.J.; Rodrigues, L.N.; Polikarpov, I.; de Azevedo, E.R.; Bernardinelli, O.D.; Rosa, C.A.; Pagnocca, F.C.; et al. Multi-scale structural and chemical analysis of sugarcane bagasse in the process of sequential acid–base pretreatment and ethanol production by *Scheffersomyces shehatae* and *Saccharomyces cerevisiae*. *Biotechnol. Biofuels* **2014**, *7*, 63. [CrossRef]
26. Antunes, F.A.F.; Chandel, A.K.; Brumano, L.P.; Terán-Hilares, R.; Peres, G.F.D.; Ayabe, L.E.S.; Sorato, V.S.; Santos, J.C.; da Silva. A novel process intensification strategy for second-generation ethanol production from sugarcane bagasse in fluidized bed reactor. *Renew. Energy* **2017**, *124*, 189–196. [CrossRef]
27. Islam, M.K.; Wang, H.; Rehman, S.; Dong, C.; Hsu, H.Y.; Lin, C.S.K.; Leu, S.Y. Sustainability metrics of pretreatment processes in a waste derived lignocellulosic biomass biorefinery. *Biores. Tech.* **2020**, *298*, 122558. [CrossRef]
28. Biswas, B.; Pandey, N.; Bisht, Y.; Singh, R.; Kumar, J.; Bhaskar, T. Pyrolysis of agricultural biomass residues: Comparative study of corn cob, wheat straw, rice straw and rice husk. *Bioresour. Technol.* **2017**, *237*, 57–63. [CrossRef]
29. Ayeni, A.O.; Agboola, O.; Daramola, M.O.; Grabner, B.; Oni, B.A.; Babatunde, D.E.; Evwodere, J. Kinetic study of activation and deactivation of adsorbed cellulase during enzymatic conversion of alkaline peroxide oxidation-pretreated corn cob to sugar. *Korean J. Chem. Engin.* **2021**, *38*, 81–89. [CrossRef]
30. Pereira, L.; Sarmento, M.H.R.S.S.; Faria, J.L.C.; Aires-Barros, R.M.F. Enzymatic hydrolysis of corncobs pretreated by steam explosion. *J. Biotechnol.* **2020**, *135*, 275–283.
31. Antunes, F.A.F.; Santos, J.C.; Cunha, M.A.A.; Brumano, L.P.; Milessi, T.S.S.; Teran-Hilares, R.; Peres, G.F.D.; Dussan, K.J.; Silva, D.D.V.; Dalli, S.S.; et al. Biotechnological Production of Xylitol from Biomass. In *Production of Platform Chemicals from Sustainable Resources, Biofuels and Biorefineries*, 1st ed.; Fang, Z., Ed.; Springer: Singapore, 2017; Volume 311, p. 342.
32. Ariyan, M.; Thankappan, S.; Ramachandran, P.; Uthandi, S. Xylitol Production from Corncob Hydrolysate by an Engineered Escherichia coli M15 as Whole-Cell Biocatalysts. *Waste Biomass Valorization* **2022**, 1–16. [CrossRef]
33. Baptista, S.L.; Carvalho, L.C.; Romaní, A.; Domingues, L. Development of a sustainable bioprocess based on green technologies for xylitol production from corn cob. *Ind. Crop. Product.* **2020**, *156*, 112867. [CrossRef]
34. Yuan, X.; Cao, J.; Wang, R.; Han, Y.; Zhu, J.; Lin, J.; Yang, L.; Wu, M. Genetically Engineering *Escherichia coli* to Produce Xylitol from Corncob Hydrolysate without Lime Detoxification. *Molecules* **2023**, *28*, 1550. [CrossRef]
35. Mohanasundaram, Y.; Nambissan, V.D.; Gummadi, S.N. Optimization of sequential alkali/acid pretreatment of corn cob for xylitol production by *Debaryomyces nepalensis*. *Biomass Conv. Bioref.* **2023**, 1–18. [CrossRef]

36. Pramasari, D.A.; Oktaviani, M.; Thontowi, A.; Purnawan, A.; Ermawar, R.A.; Sondari, D.; Ningrum, R.S.; Laksana, R.P.B.; Lianawati, A.; Fahrezi, M.Z.M.; et al. The use of hemicellulose acid hydrolysate for hydrolysis of sugarcane trash and its fermentation for producing xylitol. *Ind. Crop. Prod.* **2023**, *193*, 116163. [CrossRef]
37. Hidayah, N.; Adiandri, R.S.; Rahayu, E. Microbial xylitol production from corn cob using *Candida guilliermondii*. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *1024*, 012077.
38. Rahayu, E.; Hidayah, N.; Adiandri, R.S. Production of Xylitol from Corn Biomass using *Candida* sp. As Microbial Agent. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *1024*, 012075.
39. West, T.P. Xylitol Production by Candida Species from Hydrolysates of Agricultural Residues and Grasses. *Fermentation* **2021**, *7*, 243. [CrossRef]
40. Winkelhausen, E.; Kusmanova, S. Microbial Conversion of D-Xylose to Xylitol. *J. Ferment. Bioengin.* **1998**, *86*, 1–14. [CrossRef]
41. Prado, C.A.; Antunes, F.A.F.; Rocha, T.M.; Sánchez-Muñoz, S.; Barbosa, F.G.; Terán-Hilares, R.; Cruz-Santos, M.M.; Arruda, G.L.; Da Silva, S.S.; Santos, J.C. A review on recent developments in hydrodynamic cavitation and advanced oxidative processes for pretreatment of lignocellulosic materials. *Bioresour. Technol.* **2022**, *345*, 126458. [CrossRef] [PubMed]
42. Branco, R.F.; Santos, J.C.; Murakami, L.Y.; Mussatto, S.I.; Dragone, G.; Silva, S.S. Xylitol production in a bubble column bioreactor: Influence of the aeration rate and immobilized system concentration. *Process Biochem.* **2007**, *42*, 258–262. [CrossRef]
43. González-Gloria, K.D.; Rodríguez-Jasso, R.M.; Saxena, R.; Sindhu, R.; Ali, S.S.; Singhania, R.R.; Patel, A.K.; Binod, P.; Ruiz, H.A. Bubble column bioreactor design and evaluation for bioethanol production using simultaneous saccharification and fermentation strategy from hydrothermally pretreated lignocellulosic biomass. *Biochem. Eng. J.* **2022**, *187*, 108645. [CrossRef]
44. Niglio, S.; Procentese, A.; Russo, M.E.; Piscitelli, A.; Marzocchella, A. Integrated enzymatic pretreatment and hydrolysis of apple pomace in a bubble column bioreactor. *Biochem. Eng. J.* **2019**, *150*, 107306. [CrossRef]
45. Paul, T.; Sinharoy, A.; Pakshirajan, K.; Pugazhenthi, G. Lipid-rich bacterial biomass production using refinery wastewater in a bubble column bioreactor for bio-oil conversion by hydrothermal liquefaction. *J. Water Process Eng.* **2020**, *37*, 101462. [CrossRef]
46. Du, C.; Li, Y.; Zong, H.; Yuan, T.; Yuan, W.; Jiang, Y. Production of bioethanol and xylitol from non-detoxified corn cob via a two-stage fermentation strategy. *Bioresour. Technol.* **2020**, *310*, 123427. [CrossRef]
47. Boonchuay, P.; Techapun, C.; Leksawasdi, N.; Seesuriyachan, P.; Hanmoungjai, P.; Watanabe, M.; Srisupa, S.; Chaiyaso, T. Bioethanol Production from Cellulose-Rich Corncob Residue by the Thermotolerant *Saccharomyces cerevisiae* TC-5. *J. Fungi* **2021**, *7*, 547. [CrossRef]
48. Zheng, T.; Lei, F.; Li, P.; Liu, S.; Jiang, J. Stimulatory effects of rhamnolipid on corncob residues ethanol production via high-solids simultaneous saccharification and fermentation. *Fuel* **2019**, *257*, 116091. [CrossRef]
49. Ji, H.; Xu, K.; Dong, X.; Sun, D.; Jin, L. Sequential Production of D-xylonate and Ethanol from Non-Detoxified Corncob at Low-pH by *Pichia kudriavzevii* via a Two-Stage Fermentation Strategy. *J. Fungi* **2021**, *7*, 1038. [CrossRef]
50. Liu, S.; Yu, Y.; Xu, Z.; Chen, S.; Shen, G.; Yuan, X.; Deng, Q.; Shen, W.; Yang, S.; Zhan, C.; et al. Efficient corncob biorefinery for ethanol initiated by a novel pretreatment of densifying lignocellulosic biomass with sulfuric acid. *Fermentation* **2022**, *8*, 661. [CrossRef]