UTILIZATION OF COCONUT HUSK FOR BIOPRODUCTS OBTAINING: AN EXPERIMENT OPTIMIZATION

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ABSTRACT

The agro-industries processing of green coconuts results in a significant amount of byproduct known as coconut husk. This byproduct's large quantity necessitates the establishment of sustainable measures for its disposal. Coconut husk biomass comprises cellulose, hemicellulose, and lignin, among other constituents. It can be pre-treated to release fermentable sugars, such as xylose. These hydrolysates can be used to produce bioproducts through fermentative processes. This study aimed to optimize the acidic hydrolysis of coconut husk using a rotational central composite design (RCCD) for the release of fermentable sugars. The objective was to produce xylitol, a polyol widely used in the food industry, from the released xylose by a strain of yeast *Kluyveromyces marxianus*. The results demonstrated the feasibility of obtaining high xylose concentrations (11.45 g/L) under the optimized condition (0.94M and 21.36 minutes). Furthermore, obtaining xylitol through the fermentative process (with maximum production of 3.18 g/L) was also achieved. The yeast also produced ethanol, reaching concentrations close to 5.6 g/L. Thus, residual coconut husk can be utilized to obtain higher value-added products, such as xylitol, which finds applications as a sugar substitute in various food and pharmaceutical products.

Keywords: Xylose recovery. Coconut husk. Xylitol production. Ethanol. Bioprocess.

1. INTRODUCTION

The food processing industry produces significant agro-industrial waste, comprising organic materials with few practical uses. It is estimated that these components account for 1.3 billion tons generated globally. Unfortunately, most of this waste is discarded in the environment and deposited in landfills and dumps, generating pollution. Also, the lack of proper destinations for these organic materials leads to the proliferation of disease vectors, contamination of soil and water bodies, and the inevitable destruction of the urban landscape[1]. The originating lignocellulosic materials present in vegetable biomass discarded in the Agroindustry are potential sources of obtaining carbon and energy, constituting a valuable raw material in multiple biotechnological processes, enabling the synthesis of products with high added value. Brazil stands out on the world stage for its significant production of coconut, totaling more than 2 billion tons of annual fruit production, with its husk accounting for around 80% of its gross weight[2]. Green coconuts are primarily harvested for their water, sold locally, or processed for packaging. The remaining parts of the fruit, such as the bark material, are scarcely used for organic fertilizer, crafts, or making pots and are often discarded in cultivation areas, urban areas, or landfills.

The coconut husk primarily comprises fibers, accounting for 70% of its mesocarp. The remaining 30% of the husk comprises a powder filling the interfibrillar spaces, while the outermost layer is called the epicarp. The durability of the fibers in coconut husks is well-known, possessing a high concentration of lignin (40%) compared to other natural fibers. Additionally, the peel of the coconut contains 35.52% cellulose and 33.41% hemicellulose, both of which are crucial for producing xylose[3]. Xylose, a carbohydrate highly sought after in biotechnological processes, is a building block for producing xylitol[4]. Xylitol, a polyalcohol with the molecular formula $C_5H_{12}O_5$ (1,2,3,4,5-pentahydroxypentane), is preferred over traditional refined sugar (sucrose) for several reasons. It is safe for people with diabetes and is chemically and microbiologically stable, helping to extend the shelf life of food products by preventing the growth of microorganisms. Furthermore, xylitol does not react with amino acids, such as those involved in the Maillard reaction, which preserves the nutritional quality of food products. In summary, the coconut husk is a valuable source of fibers and carbohydrates, potentially utilized in various biotechnological and industrial applications.

The production of xylitol presents numerous advantages, making it an attractive compound. However, its current production method is costly and unsustainable, relying on traditional chemical hydrogenation processes in purified D-xylose solutions at high temperatures and pressure. To address this challenge, research has focused on alternative methods, such as using enzymes and fermentative microorganisms to convert xylose into xylitol. Considering this, the present study seeks to optimize the release of xylose from coconut husks through acid hydrolysis to produce xylitol via a fermentative process. By exploring this approach, this work aims to contribute to developing more efficient, sustainable, and cost-effective methods of xylitol production.

2. MATERIAL & METHODS

2.1 - Coconut husk processing

To optimize the action of sulfuric acid in hydrolysis, the coconut husk was subjected to processes that aimed to increase its surface area in contact with the acid solution. Initially, the green coconut was crushed using suitable equipment, such as a peeler, to obtain smaller husk fragments. These fragments were then cut with gardening scissors to reduce their size. Subsequently, the

resulting pieces were kept in an oven at 80 ºC for drying. With the peel dry, it was crushed in a grinder, followed by sieving for standardization. The remaining husk from the sieve was then subjected to a new crushing cycle until it reached the desired particle size.

2.2 - Experimental design to optimize xylose obtaining

A central composite experimental design was developed to investigate the influence of the solid fraction of green coconut (*Cocus nucifera* L.) biomass in different H_2SO_4 solutions, with varying concentrations (0.03 M, 0.02 M, 0.60 M, 1.00 M, 1.17 M), and different reaction times (5.9 min, 10 min, 20 min, 30 min, 34 min) according to a Central Composite Design (CCD) with three central points (see Table 1). The appropriate proportion of solid fraction was set at 10 % (w/v). After hydrolysis, 2 mL were removed from each sample to be centrifuged. Subsequently, the solid fraction was removed by filtration using a syringe, while the liquid fraction was analyzed to determine the content of released sugars.

2.3 - Production of xylitol from coconut husk hydrolysate

Producing xylitol from hydrolyzed coconut husks was produced in 125 mL Erlenmeyer flasks containing 50 mL of culture medium. This was done in a rotary shaker at a temperature of 30 °C and 200 rpm for 120 h. The initial concentration of cells was 1.0 g/L, and the temperature was maintained at 30 °C and 150 rpm. Samples were taken at predetermined intervals to evaluate cell growth, substrate concentration (xylose and glucose), and product concentration. The efficiency of xylitol production was evaluated by calculating the yield ($Y_{P/S}$) and productivity (Q_P).

2.4 - Analytical methods

2.4.1 - Carbohydrates and ethanol concentration and statistical analysis

The content of sugars such as glucose and xylose, and ethanol, was quantified using High-Performance Liquid Chromatography (HPLC), equipped with a Waters refractive index detector (Model 2414) and a 610-H column maintained at 30ºC. As eluent, phosphoric acid (H_3PO_4) was used with a concentration of 0.1% (w/v) and a flow rate of 0.5 mL/min with a sample volume of 20 µL. The experimental planning was analyzed and prepared using the Statistica v7.0 software (Statsoft), applying the response surface methodology (RSM) to analyze the results. Its statistical significance assessment was carried out using a one-way analysis of variance (ANOVA) with significance levels of 95%.

3. RESULTS & DISCUSSION

The results obtained from the experimental design to produce xylose and glucose are presented in Table 1. On average, the yields of both sugars at the central points were 9.64 g/L for xylose and 13.39 g/L for glucose. However, the highest yield for xylose extraction was achieved in run six (1.17 M for 20 min), with an average of 11.45 g/L, while run two (0.2 M for 30 min) was more effective in glucose extraction, resulting in 15.52 g/L of glucose. It is worth noting that the condition that led to the lowest release of both sugars was at an acid concentration of 0.03 M for 10 minutes. Therefore, it can be inferred that a higher acid concentration is beneficial for xylose extraction, while a longer time is preferable for glucose extraction.

Table 1. Experimental design and response for dilute acid hydrolysis of coconut husk using H_2SO_4

Run	H_2SO_4 (M)	Time (min)	Xylose (g/L)	Glucose (g/L)
1	0.20	10.00	1.87±0.02	2.15±0.00
2	0.20	30.00	10.19±0.22	15.52±0.32
3	1.00	10.00	7.26±0.00	8.93±0.06
4	1.00	30.00	9.22±0.02	10.45±0.00
5	0.03	20.00	2.92±0.17	3.35±0.13
6	1.17	20.00	11.45±0.06	14.58±0.12
7	0.60	5.90	4.70±0.09	9.75±0.12
8	0.60	34.10	7.52±0.06	14.14±0.01
9	0.60	20.00	10.56±0.27	14.29±0.31
10	0.60	20.00	9.53±0.01	13.11±0.01
11	0.60	20.00	8.84±0.07	12.77±0.30

Figure 1 displays the response surface, which outlines the ideal area for obtaining xylose and glucose. The F-test and ANOVA analysis were employed as criteria to determine the significance of the fitted models. The fitted models produced satisfactory correlation coefficients (R^2= 0.86 for Equation 1 and R^2= 0.81 for Equation 2). Therefore, the main focus of this work is the extraction of xylose, which is used by *Kluyveromyces marxianus* strains for xylitol production.

Xylose (g/L) = -10.87126128461+21.299390061008x-6.8408172460124x2+1.0796772919901y-0.016561076441074y2-0.3975xy (R^2=0.86) (1) Glucose (g/L) = -13.792864385244+38.867228549151x-15.467959426975x2+1.1228172348277y-.010353930923977y2-0.740625xy (R2 = 0.81) (2)

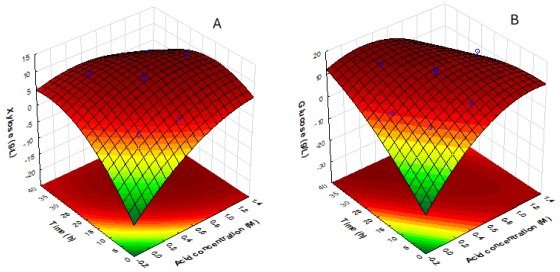

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Figure 1. Response surface for xylose (A) and glucose (B) released from the coconut husk hydrolysate.

According to the conducted study, it has been determined that the most effective concentration of H_2SO_4 for releasing xylose from coconut husk is 0.94M and 21.36 minutes. At these conditions, concentrations higher than 10 g/L of xylose were obtained. The results indicate the efficiency of the treatment in decomposing hemicellulose into xylose. Thus, the produced xylose may be applicable for synthesizing high-value products.

Figure 2 depicts the xylitol production profile in the hydrolyzed coconut husk medium. It is noticeable that xylitol production was achievable, reaching a maximum concentration of 3.18 g/L. A xylitol yield and productivity of 0.198 g. g^{-1} and 0.027 g/L/h were observed within 24 h. Furthermore, rapid glucose consumption was noted, leading to ethanol production in the medium (reaching 5.63 g/L).

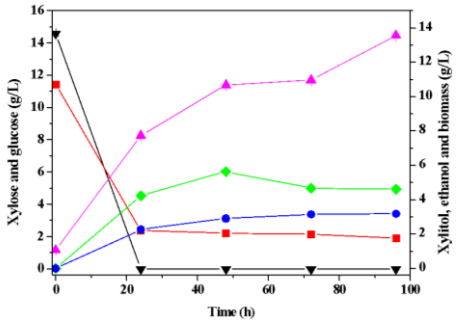

Figure 2. Xylitol production kinetics by *Kluyveromuces marxianus* ATCC 36907 at 30 ºC and 200 rpm, for 120h:(▼) Glucose, (■) xylose, (●) xylitol, (◆) ethanol, and (▲) cell concentration.

4. CONCLUSION

The hydrolysis of coconut husk has proven to be effective in releasing high concentrations of xylose (>10 g/L) under optimal conditions (0.94 M for 21.36 minutes), presenting its potential applicability in subsequent biorefinery processes to produce high-value products. Additionally, the observed xylitol production, reached a maximum concentration of 3.18 g/L, along with yields and productivities of 0.198 g. g^{-1} and 0.027 g/L/h within 24 h, respectively, highlight the potential of this biosynthetic process. These findings underscore the efficiency of the treatment in decomposing hemicellulose from coconut husk into xylose, thus supporting the application of the produced xylose to produce high-value products.

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