HIGH-YIELD BIOREFINERY PRODUCTS FROM SUGARCANE BAGASSE: PREBIOTIC, ETHANOL, AND NANOPARTICLES

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ABSTRACT

An integrated biorefining strategy was applied to fractionate Sugarcane bagasse (SCB) into its major constituents, enabling high-yield conversion of the fractionated materials into high-value coproducts alongside cellulosic ethanol. A pilot-scale steam explosion produced a hydrolysate rich in low molecular weight xylooligosaccharides with a high in vitro efficacy as a prebiotic for different bifidobacteria. Lignin recovered after alkaline treatment of the steam-exploded SCB was converted into uniform spherical lignin nanoparticles (11.3 nm in diameter) colloids at approximately 100% by a green, innovative method. The spherical lignin nanoparticle colloid was used as a natural colorant for the eco-friendly dyeing of natural and synthetic textile fabrics. The resulting cellulose was hydrolyzed at 17.5% (w/v) consistency and low enzyme loading (17.5 mg/g) to yield a pure glucose hydrolysate at a high concentration (100 g/L) and a cellulosic solid residue that was defibrillated by disc ultra-refining into homogeneous cellulose nanofibrils (20.5 nm in diameter). Statistical optimization of the cellulosic hydrolysate fermentation led to ethanol production of 67.1 g/L, with a conversion yield of 0.48 g/g and productivity of 1.40 g/L.h.

Keywords: Lignin nanoparticles. Biorefinery. Nanocellulose. Prebiotic. Cellulosic biomass.

1 INTRODUCTION

To fractionate and recover hemicellulose from lignocellulose, hydrolysis under acid conditions is required, and hydrothermal treatments using only water (liquid hot water) or steam (steam explosion), also known as autohydrolysis pretreatments, have been considered as the most attractive options. First, they are environmentally friendly, relatively inexpensive (no need for chemicals or major corrosion issues due to the mild acidic medium), and have been successfully applied to various lignocellulosic biomass fields.[1] Another benefit is that mild conditions are widely employed to minimize the formation of sugar degradation products while still solubilizing a sufficient amount of hemicellulose, breaking down the hemicellulose chains, generating a hemicellulose-rich hydrolysate high in oligomers with varying degrees of polymerization.[2] This creates an opportunity to selectively fractionate hemicellulose directly into a marketable product in a single process step because oligomers can be used in areas with significant economic value (i.e., pharmaceutical products, food ingredients, fuels, chemicals, and bioplastics).

With significant hemicellulose dissolution, hydrothermal treatments result in a cellulosic material with low hemicellulose but high lignin content[3] because hydrothermal methods are inefficient for lignin solubilization, especially at low and mild severity conditions.[4] Among the several methods for isolating lignin, aqueous alkaline extraction with sodium hydroxide is a promising option when subsequent lignin applications are considered. This arises because this process uses sodium hydroxide to dissolve lignin, generating sulfur-free lignin. This characteristic directly influences the potential applications of lignin (the presence of sulfur seriously limits various applications). For example, sulfur is a poison for many metal-containing catalysts used for lignin depolymerization to aromatics and limits lignin utilization for biomedical applications. In addition, the soda method decreases the ash content, which is a major problem for agro-industrial residues,[5] and in terms of the biorefinery concept, it is also more attractive because hydrothermal treatment (autohydrolysis) increases lignin extractability with aqueous alkali.[6]

In this study, an integrated biorefining strategy was applied to sugarcane bagasse (SCB) to selectively fractionate it into its main constituents and allow the production of high value biorefinery products alongside ethanol production at a high yield. These products were xylooligosaccharides (XOS) with prebiotic properties from hemicellulose, lignin nanoparticles (LNP) from lignin, and cellulose nanofibrils (CNF) from the unhydrolyzed cellulosic solid residue.

2 MATERIAL & METHODS

The sugarcane bagasse (SCB) underwent continuous steam explosion processing in a pilot unit. This process involved feeding SCB at 11.7 kg/h and saturated steam at 25−30 Kg/h under 15 bar pressure (approximately 190°C) for 15 min. The treated SCB was then added to a tumbling reactor (20% w/v) at 30°C for 30 minutes, followed by vacuum filtration. The filtrate, termed SCB-XOS hydrolysate, and a washing liquor obtained by further filtration were collected. The solid fraction, composed of cellulose and lignin, was used for lignin fractionation.To evaluate the prebiotic potential of SCB-XOS hydrolysate, an in vitro assay was conducted using four probiotic bacteria: *Bifidobacterium lactis*, *Bifidobacterium infantis*, *Bifidobacterium adolescentis*, and *Bifidobacterium brevis*. The fermentation was performed at 37°C for 72 h under anaerobic conditions in a specific Bifidobacterium medium. Comparative carbohydrates (xylose, glucose, commercial XOS, and fructooligosaccharides) were also tested at 5 g/L. Prebiotic activity was assessed by measuring cell growth (optical density at 650 nm), final medium pH, and the production of short-chain fatty acids.

Lignin was extracted from the steam-exploded SCB using aqueous alkaline extraction with NaOH, followed by acid precipitation with H_2SO_4. This process was carried out in a pressurized reactor vessel at 100°C for 1 h, with subsequent filtration and washing of the solid fraction. The liquid fraction was acidified to pH 2.0 to precipitate lignin, which was then centrifuged and washed multiple times. The recovered lignin was used to produce lignin nanoparticles, according to Marotti and Arantes.[7] The resulting nanoparticles applied in the aqueous dyeing of textile fabrics.

The cellulose fraction was bleached using H_2O_2 and NaOH at 80°C for 2 h to obtain a cellulose-rich pulp. Enzymatic hydrolysis of the cellulose-rich pulp was conducted using Cellic CTec 2 enzyme at 50°C for 72 h. The resulting mixture was centrifuged to separate solubilized sugars from unhydrolyzed cellulose. HPLC quantified the sugars, and the cellulose solid residue was used to produce cellulose nanofibrils (CNF) according to Berto and Arante.[8]

Saccharomyces cerevisiae MH36 yeast was used for fermentation. The yeast was cultured in a growth medium, centrifuged, and re-suspended in sterile water. Fermentation was conducted in flasks containing various glucose, yeast extract, and cells concentrations. Samples were taken periodically to measure glucose, glycerol, 2,3-butanediol, and ethanol concentrations by HPLC, with cell growth quantified by turbidimetry. A factorial design was used to determine the significance of variables on fermentative parameters and additional cultivation under optimal conditions was performed for verification.

3 RESULTS & DISCUSSION

The resulting SCB-XOS hydrolysate contained a 2:1 ratio of oligomers to xylose, with higher concentrations of xylobiose and xylotriose compared to commercial XOS. It included side chains like arabinose and acetyl groups. Non-saccharide compounds such as phenolics, furfural, and HMF were present in very low amounts due to the mild steam explosion conditions. *In vitro* prebiotic assays with four probiotic bacteria (*B. lactis*, *B. adolescentis*, *B. infantis*, and *B. brevis*) showed that SCB-XOS supported similar bacterial growth and pH reduction as commercial XOS (Figure 1A-D). SCB-XOS led to higher lactic acid production in three bacteria, while commercial XOS produced more acetic acid. The study confirmed that unpurified SCB-XOS has comparable prebiotic effects to commercial prebiotics despite containing additional compounds. The production of lactic and acetic acids suggests that SCB-XOS can positively impact gut health by enhancing microbiota function and inhibiting pathogen growth.

The enzymatic hydrolysis of cellulose-rich pulp at high solids (17.5% w/w) and low enzyme loading (15 mg/g) for 72 hours achieved a cellulose conversion rate of 55.6% and produced a high glucose concentration of 100 ± 0.9 g/L. This effective hydrolysis was due to the prior removal of most hemicellulose and lignin. The conversion and glucose concentration were slightly lower than those Pereira and Arantes (2020) reported under similar conditions, likely because this study avoided using acetate buffer at pH 4.8 to prevent inhibition of alcoholic fermentation by acetate. Despite this, the glucose concentration achieved (100 g/L) was significantly higher than most reported concentrations (40–80 g/L). The unhydrolyzed solid residue from this process was used to produce CNF.

CNF and LNP are high-value nanomaterials with enhanced physical and chemical properties. Produced by disc ultra-refining, both materials showed significant particle size reductions and surface area increases. Initial lignin particle sizes were reduced from PS_{90} 7.96 μm to PS_{90} 27.7 nm after one cycle, with LNPs displaying a zeta potential of -35.8 mV, indicating good colloidal stability. The wet specific surface area (SSA) of lignin increased from 631.6 to 118,800 m²/kg. The spherical LNPs were used as eco-friendly natural colorants for dyeing natural and synthetic fabrics. The LNP colloids effectively dyed fabrics in shades of brown, with higher color uptake observed in wool, polyamide, acetate, and cotton compared to acrylic and polyester (Figure 1). Additionally, thermal degradation analyses showed increased char-forming capacity in dyed fabrics, indicating fire-retardant properties. This study demonstrates the potential of stable LNP colloids as sustainable, efficient natural colorants with added fire-retardant benefits. CNF production from cellulose solid residue (CSR) showed particle sizes reduced from PS_{90} 8.83 μm to PS_{90} 1.99 μm after 13 cycles, forming long fibrils with 2-60 nm diameters. The SSA of CNF increased from 234.8 to 652.3 m²/kg, and the zeta potential was -27.3 mV, suggesting good suspension stability. The dynamic viscosity of the CNF suspension was 10.95 cP, which is advantageous for reducing operational costs in applications. Energy consumption for CNF production was 19 kWh/kg, lower than reported for other methods, partly due to residual lignin facilitating defibrillation.

Glucose consumption, cell growth, and production of glycerol, butanediol, and ethanol were monitored in 11 cultivation experiments. Glucose was fully consumed within 1-3 days, depending on initial concentration. Maximum cell concentrations of 2.31-2.92 g/L were reached with an initial cell concentration of 0.38 g/L, and 5.47-5.97 g/L with 4.56 g/L initial cell concentration. Higher initial glucose led to greater ethanol production, averaging 65.2 g/L in concentrated hydrolysate versus 18.4 g/L in diluted hydrolysate over 2-3 days. Glycerol and butanediol production peaked at 3.3 g/L and 3.4 g/L, respectively. After 2 days, glucose consumption ranged from 70.3% to 99.9%, with higher initial yeast extract and cell concentrations improving consumption. Cell growth varied from 0.6 to 2.1 g/L, influenced significantly by initial cell concentration. Ethanol production ranged from 15.8 to 67.7 g/L, with the highest yields and productivity in conditions with high glucose and yeast extract but low initial cell concentrations. Statistical analysis showed that initial glucose concentration significantly impacted glucose consumption, ethanol production, and productivity. Optimal conditions (experiment 4) produced 67.1 ± 5.1 g/L ethanol with a yield of 0.48 ± 0.05 g/g and productivity of 1.40 ± 0.11 g/L h, achieving 96% of the theoretical maximum yield. These results demonstrate efficient ethanol production, essential for the economic viability of biofuel processes.
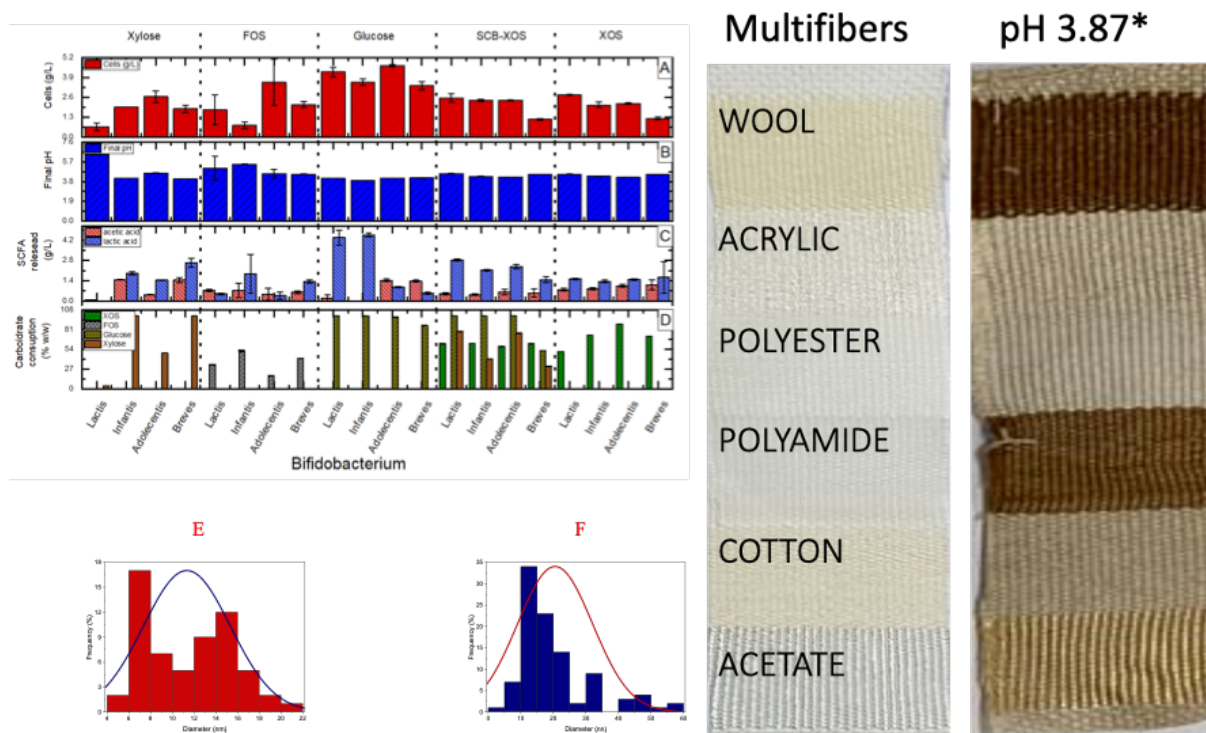
Figure 1 Figure title Co-products from sugarcane bagasse: A) Metabolic parameters and carbohydrate consumption by bifidobacteria over 72 h. A – final cell concentration (g/L) calculated by optical density at 600 nm; B – pH after prebiotic fermentation; C – Short chain fatty acid released during prebiotic fermentation; D – consumption of carbohydrates after prebiotic fermentation. E) Diameter class distribution, determined by AFM, of the lignin nanoparticles and of the F) cellulose nanofibrils obtained after one and thirteen cycles in the disc ultra-refiner, respectively.

4 CONCLUSION

Pilot-scale steam explosion of SCB under mild conditions effectively fractionated hemicellulose, resulting in a (XOS)-rich hydrolysate with strong prebiotic efficacy. Lignin recovered through alkaline treatment and acid precipitation was mechanically converted into uniform, spherical LNP at a high yield. Cellulose was hydrolyzed at high dry matter and low enzyme loading, producing a hydrolysate fermented into ethanol at a concentration close to 7% w/w and a yield near the theoretical maximum. The remaining unhydrolyzed cellulose was defibrillated into homogeneous CNF. This study successfully demonstrates the biorefinery concept for producing high-value products and ethanol with high yields.

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