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RESEARCH

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Production of bioplastics with chemical and enzymatic modificated xylan (lignin- and arabinose-free) from sugarcane bagasse

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Abstract

Hemicellulose is a renewable source to produce bio-based molecules with high added value. For this purpose, this study aimed to promote enzymatic and chemical modifications of xylan from sugarcane biomass to produce substrates as substitutes for scarce and expensive commercial xylan such as from beechwood and beachwood. Lignin- and arabinose-free xylans were produced through enzymatic and chemical treatments (using laccase (X3), α-L-arabinofuranosidase (X4) and H_2O_2 (X2)), compared to untreated/original xylan (X1). Xylan delignified with laccase (X3) showed interesting substrates as it induced a xylanase activity of 1350 IU/mL from *Aspergillus versicolor* growth, as a result of residual lignin removal, making xylan more accessible for enzymatic hydrolysis. These modified xylans were also used to produce bioplastics with potential technological applications that are sustainable, possibly biodegradable and obtained by a methodology that is easy to reproduce. The results indicate that the opacity (4.7%), moisture (7%) and solubility (35%) from bioplastic type 1 (B1: based on original xylan without modifications) showed that the presence of residual lignin contributed to a major opacity of the material, with moisture retention, increasing water solubility and decreasing tensile stress. It can be highlighted that the modifications of xylan resulted in improvements in the quality, integrity, and resistance of bioplastics, addressing the lack of information in the literature regarding the production and evaluation of xylan-based bioplastics described to date. Furthermore, this study addresses the scarcity and high cost of commercially available xylan by producing and evaluating an economically viable alternative, obtained through a simple and efficient protocol.

Keywords Biomass conversion, Hemicellulose, Biomolecules, Bioeconomy, Biorefinery

Introduction

Lignocellulosic materials represent a source of raw material in biotechnological processes as a large surplus of industrial residues of agricultural products is generated annually. Most of these residues are either used in animal feed or burned for alternative disposal and energy generation. However, these residues are a potential source for many compounds of industrial interest, such as antioxidant compounds and oligosaccharides with bioactive potential [1].

Hemicellulose is the second most abundant polysaccharide in lignocellulosic biomass. Its structure supports the cellulose microfibrils by hydrogen bonds and binds to the lignin by covalent bonds. Unlike cellulose, hemicellulose is classified as a heteropolysaccharide, comprising different carbohydrates, and presents an amorphous structure with a degree of polymerization of approximately 200 units. The composition of hemicellulose presents monosaccharides such as pentose, hexoses, acetylated sugars, and uronic acids. Its

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composition usually varies in different plant species [2]. The xylan main chain comprises a homopolymer of xylose or a heteropolymer of glucomannans, with the presence of arabinose, galactose, and 4-O-methylglucuronic acid that bind to each other through β-(1 → 4) glycosidic bonds, or also of the type β-(1 → 3), β-(1 → 6), α-(1 → 2), α-(1 → 3), and α-(1 → 6) [3].

Xylan is the predominant hemicellulose in sugarcane bagasse and accounts for one-third of the planet's renewable carbon sources [4]. In industry, the use of xylan to obtain bio-products has gained prominence, leading to the creation of packaging, hydrogels, ethanol, and lactic acid through the fermentation of monomeric sugars derived from xylan by microorganisms. Additionally, furfural can be produced from xylan hydrolysis and dehydration of xylose monomers, as well as the production of xylitol by hydrogenation routes of xylose [5].

Hemicellulose has been used to produce different value-added derivatives, such as xylooligosaccharides [6–8], films [9]. Furthermore, these derivatives have been widely used in medical applications [10] and bioplastic formulations [11]. In recent years, xylans have been frequently isolated and purified from various sources and used as substrates for endoxylanase characterization [12].

Considering that sugarcane bagasse is now one of the main raw materials, it is important that the hemicellulose of this biomass, xylan, is used as a substrate to determine xylanase activity and in microorganism cultivation for xylanase production [13, 14]. Normally, the hemicellulose extraction yield is high and shows the presence of residual lignin [14], however, hemicellulose with a low yield still presents residual lignin [15, 16]. The use of lignolytic enzymes, such as laccases could contribute to residual lignin removal and obtaining xylan with a higher degree of purity, as the laccase can oxidize electron-rich substrates of phenolic, reducing oxygen to water [17], depolymerizing the lignin structure.

Xylan can also be used as a substrate for characterizing xylanases and as a carbon source in microbial cultures. It is also the main component in the formulation of bioplastics. These xylan-based bioplastics have been produced and evaluated for opacity, moisture, solubility and tensile stress properties [11]. Other components, such as glycerol, serve as plasticizing agents that enhance the flexibility and elongation of bioplastics. However, glycerol may also reduce mechanical strength [18–20]. It has been used in the production of xylan-based bioplastic [11, 21], resulting in improved properties.

In this context, the innovation of the present study lies in the generation of xylan through a protocol that is easy to reproduce on a laboratory scale, making it an economically viable alternative to the currently scarce commercial xylan. In addition, xylan-based bioplastics were obtained through a sustainable and eco-friendly bioprocess using lignocellulosic raw materials, which can contribute to a disruptive technology aimed at reducing the reliance on non-renewable plastics.

Results and discussion

Production of lignin- and arabinose-free xylan

Xylan is recognized as an inducing substrate for xylanase production in microbial cultivation. However, the high cost and recent scarcity of commercial xylan render many processes unfeasible. Thus, there is a need to obtain a xylan that is easy to produce, reproducible, and low-cost. In this study, an alkali treatment with 6% hydrogen peroxide resulted in 200 g of xylan, achieving a yield of 50% (w/w).

This study sought to produce xylan with different characteristics produced by removing residual lignin and arabinose pendant groups as sugarcane xylan is classified as arabinoxylan, resulting in four different types of substrates: X1, corresponding to original xylan without modifications extracted with 6% H_2O_2 in an alkaline medium; X2, which was subjected to a second treatment with 6% H_2O_2 in an alkaline medium to remove residual lignin; X3, which corresponds to original xylan hydrolyzed with laccase to remove residual lignin; X4, which corresponds to original xylan subjected to enzymatic hydrolysis with α-L-arabinofuranosidase to remove pendant arabinose groups.

The first analysis of the study refers to the comparison by FTIR of the composition of the xylans with or without residual lignin (original, xylan modified with laccase and xylan modified with alkaline treatment) (Fig. 1).

Through the FTIR spectrum (Fig. 1), it can be observed that delignification of xylan occurred by comparing the presence of the band at 1510 cm^{-1}, which corresponds to the aromatic skeleton of the lignin molecule (highlighted with the symbol *) [22]. This can be seen when comparing the original xylan (red line), which still had a residual lignin content, with the modified xylans treated with laccase and hydrogen peroxide in an alkaline medium (black and green lines, respectively) (Fig. 1). X2 showed the lowest lignin content, with an absent band at 1510 cm^{-1}. X3 also showed some delignification when comparing its band at 1510 cm^{-1} with this same band of the original xylan, however, it can be observed that this band at 1510 cm^{-1} is still higher than that observed for X2, reinforcing that the alkaline treatment of xylan showed the best delignification effect. Moreover, the presence of bands can be observed between 1166 and 1000 cm^{-1} that are typical of xylan molecules [23].

After analyzing lignin removal from xylan through FTIR, the determination of the xylanase activity

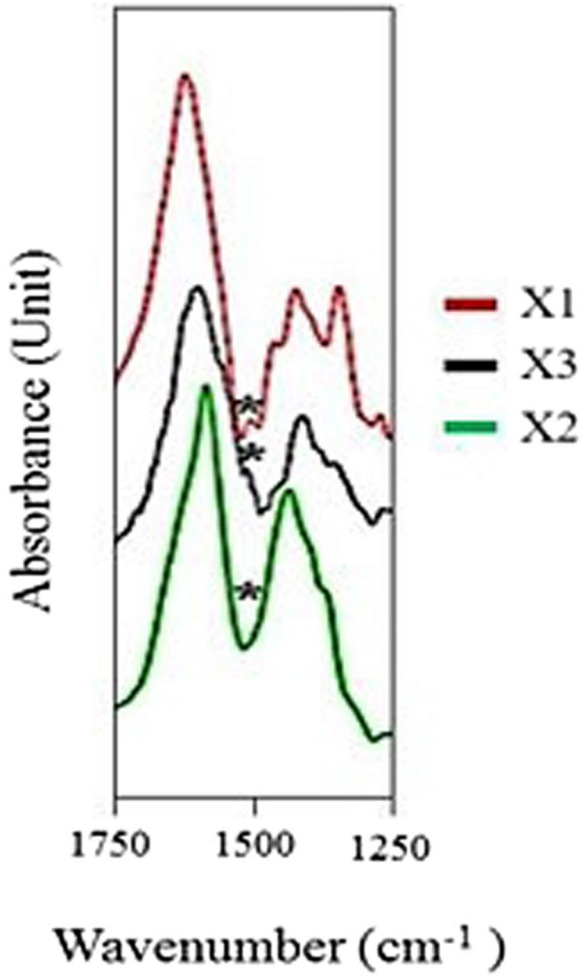

Fig. 1 FTIR analysis of xylan free-lignin and original xylan. Infrared spectrum ranging from 1250 cm^{-1} to 1750 cm^{-1} to identify the presence or absence of lignin according to each substrate. The green line corresponds to xylan subjected to a second alkaline treatment, where no lignin band can be seen, indicating that the treatment was effective. X1: untreated xylan; X2: xylan treated with 6% H_2O_2; X3: xylan hydrolyzed with laccase

produced and purified from *Aspergillus versicolor* was determined using X1, X2 and X3 substrates in buffer sodium acetate pH 5.2. Moreover, all samples at the final reaction concentration of 1% were investigated, as well established in the literature [6]. This analysis was crucial to identify whether the total or partial removal of lignin in xylan led to an improvement in the activity of the enzyme on the substrate under optimal conditions such as the temperature at 50 °C and pH.

Xylan evaluation as a substrate for enzymatic activity

The results indicate that the highest activity of xylanase from *A. versicolor* was statistically similar when compared to X1 and X3 (624 IU/mL), as substrate for the enzymatic activity. X2 proved to be an uninteresting substrate for inducing xylanase activity (70 IU/mL) in *A. versicolor*. The hypothesis is that the hydrogen peroxide alkaline treatment (H_2O_2 6% (w/v), at pH 11.2), to which the unprocessed xylan was subjected, was probably severe and damaged the integrity of the xylan molecule in the new step of treatment. This most likely caused the xylanase to not perform as expected.

In the literature, there are reports that *Aspergillus niger* was genetically modified to obtain a higher thermostability xylanase. The different mutants generated were evaluated for xylanase activity with beechwood xylan (1%) as a substrate, and the highest specific activity observed in the study was close to 200 IU/mg [24]. A second one induced mutations in the fungus *Aspergillus niger* through UV radiation to enhance xylanase production. The xylanase activity in the presence of 0.5% beechwood xylan was determined, revealing that the highest xylanase activity in the *A. niger* mutants reached 9.36 IU/mL [25]. In the present study, the wild-type *A. versicolor* fungus showed a xylanase activity close to 624 IU/mL in the presence of 1% of X1 and X3 substrates.

Xylan evaluation as an inductor of xylanase production from A. versicolor

In addition to determining xylanase activities using the xylan with and without lignin residual, the growth of *A. versicolor* and the production of its endo-xylanase over 10 days were investigated by the enzymatic hydrolysis assay. In this growth, which was performed in a minimal medium, the only carbon source available was xylan (Fig. 2). The results for the X1 substrate were statistically similar to 3150 IU/mL compared with X3 at 192 h of cultivation (3120 IU/mL) (Fig. 2). For the X2 substrate, the highest activity of xylanase from *A. versicolor* was observed after 240 h of growth (540 IU/ml). The results of the growth of *A. versicolor* on different substrates indicate that X3 is a high-quality substrate compared to X1, which contains 5% residual lignin. Furthermore, total lignin removal through a second alkaline treatment did not yield the expected outcome in terms of maximum xylanase activity.

Therefore, X2 was the least effective substrate for microorganism growth among the three evaluated. It is hypothesized that the second alkaline treatment may have altered the polymerization degree of the xylan and potentially left NaOH residues on the xylan. Both of these factors likely hindered the action of xylanase, as substrate-related factors, including the presence of salt, can influence enzyme activity [26].

A study in the literature investigated the xylanase activity of *Aspergillus tamarii* over 120 h using 1% sugarcane bagasse xylan as the substrate, and the results

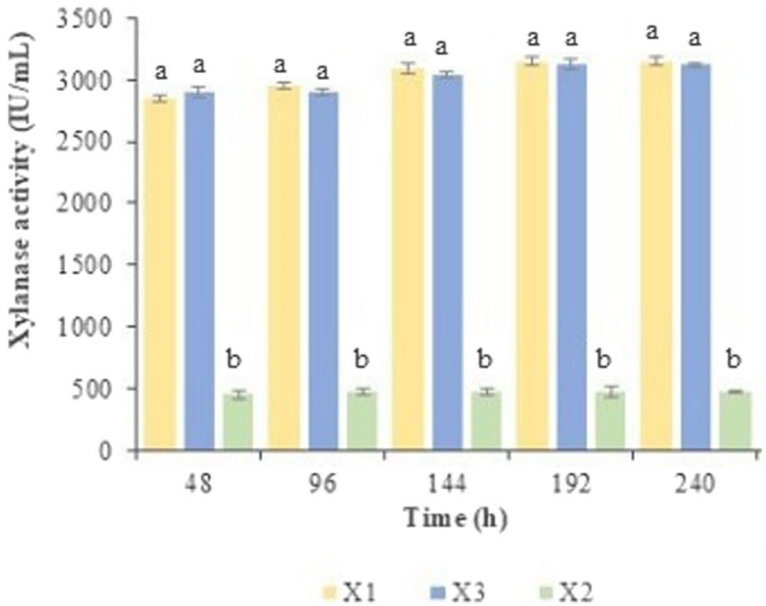

Fig. 2 Xylanase activity from *A. versicolor* during 10 days on three different substrates (xylans). Items "a" and "b": comparison of variance means using ANOVA with the Tukey test. X1: untreated xylan; X2: xylan treated with 6% H_2O_2; X3: xylan hydrolyzed with lacase

indicated that the highest xylanase activity (1.91 U/mL) occurred after 72 h of growth [27]. Another study investigated the xylanase activity of *Aspergillus heteromorphus* in the presence of rice straw (1–5%) as a substrate over 5 days. At the end of this fermentation period, the highest xylanase activity identified was 11.6 IU/mL [28]. The results of the present study for the three xylans evaluated as the substrate were higher than those observed in the literature, even when comparing the X2 that presented the lowest values of xylanase activity (540 IU/mL after 10 days of cultivation).

Production and evaluation of the properties from xylan-based bioplastics

Four types of bioplastics were developed based on xylan and complexed with starch, glycerol, and bis-acrylamide (Supplementary Fig. 1). Then, the properties of generated bioplastics were evaluated (opacity, moisture, solubility and tensile stress) (Fig. 3). The production and property evaluation of the bioplastics produced in this study were compared to the only two reports of xylan-based bioplastics described in the literature, as described below.

The production and property evaluation of the bioplastics produced in this study were compared to the few reports of xylan-based bioplastics described in the literature, as described below. The results for the opacity of the xylan-based bioplastic original (B1, 3.2%) (Fig. 3) were close to the data described in the literature, as close to 3% for the bioplastic made from xylan in a proportion of 5% (w/v) of polysaccharides [11], as in this study this proportion was also followed. Compared to the study of Abe et al. (2022) [21], where the opacity described was 2.54% for this same concentration of xylan in the bioplastic formulation, the data of the present study indicate that the bioplastic B1 presents higher opacity (3.2%).

For the bioplastics prepared with lignin-free xylan (B2 and B3), which presented opacity of 2 and 2.3% (Fig. 3), it is noteworthy that the opacity of both was lower than the values described in Macedo et al. (2022) [11] and ABE et al. (2022) [21], with values close to 3% and exactly 2.54%, respectively. The data corroborate that the delignification of xylan can contribute to the reduction of the brown coloration and the transparency of the bioplastic, because as previously described, lignin contributes to the coloration of the film [29].

Regarding the moisture content of the bioplastic, bioplastics B1 to B4 showed values equivalent to 7, 4, 4 and 4%, respectively (Fig. 3). The bioplastic based on original xylan (B1) presented a higher percentage of moisture in relation to the other bioplastics formulated with delignified xylan (B2 and B3) and without arabinose pendent groups (B4). Concerning the literature, all bioplastics obtained in this work presented results for moisture below that observed in the study of Macedo et al. (2022) [11], where the moisture was equivalent to 13.34% for the bioplastic made from xylan under the same concentration conditions as those used in the present study.

The bioplastics B1 to B4 showed solubility equal to 15, 12.3, 12.9 and 14%, respectively (Fig. 3). In the literature, the results for solubility of xylan and starch-based bioplastics formulated using 5% polysaccharides were

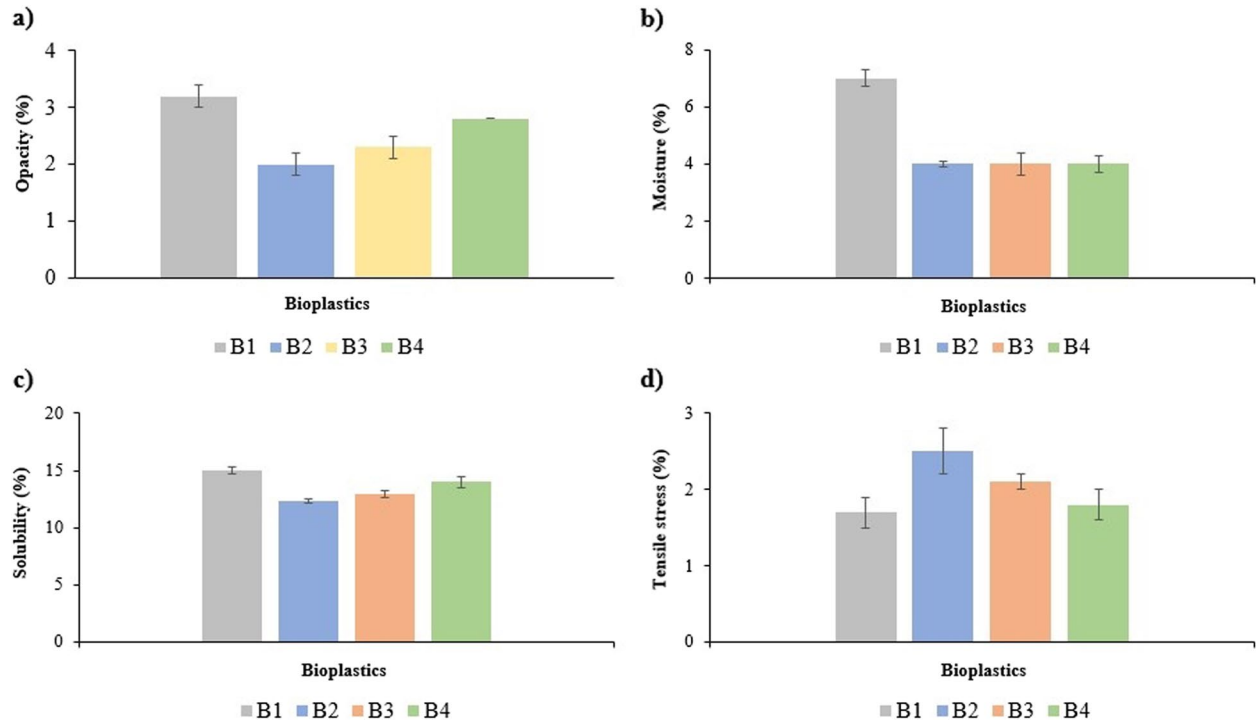

Fig. 3 Evaluation of the xylan-based bioplastics properties such as opacity, moisture, solubility and tensile stress. B1: bioplastic comprising original xylan, starch, bis-acrylamide and glycerol. B2: bioplastic composed of xylan/laccase, starch, bis-acrylamide and glycerol. B3: bioplastic composed of xylan/hydrogen peroxide in an alkaline medium, starch, bis-acrylamide and glycerol. B4: bioplastic composed of xylan/arabinofuranosidase, starch, bis-acrylamide and glycerol. Data label (a and b): comparison of variance means using ANOVA with the Tukey test

equivalent to 16.40% (Abe et al., 20) and 20.25% [11]. The hypothesis of the higher values found for solubility in the present study is that bis-acrylamide may have contributed to decreasing water absorption as cross-linking agents reduce the solubility of hemicellulose in liquid and increase water resistance [29].

Regarding mechanical strength, the bioplastics B1 to B4 showed a tensile stress of 1.7, 2.5, 2.3 and 1.8 MPa, respectively (Fig. 3). The original xylan-based bioplastic (B1) presented higher tensile stress as the residual lignin makes the bioplastic more mechanically vulnerable due to the tendency of lignin to form aggregates [11, 30]. Starch is also known to be a reinforcing agent and helps improve the mechanical properties of films [29]. In comparison with literature data, bioplastic X2 showed tensile stress higher than 2.35 MPa, as described by Macedo et al. (2022) [11], where the same concentrations of polysaccharides and sugarcane bagasse xylan (12.87% residual lignin) were used. On the other hand, all other bioplastics in this present study showed tensile stress lower than 2.35 MPa.

The influence of glycerol, starch and bys-acrilamide into the properties from xylan-based bioplastics

Regarding the composition of bioplastics, the modifications in xylan extracted from sugar cane bagasse addressed in this study, such as delignification and arabinose removal, led the bioplastics to present better quality in their properties, such as reduction in moisture (%), solubility (%) and opacity (%), and contributed to a higher mechanical strength compared to the bioplastic B1.

The presence of starch contributed to the mechanical strength of all bioplastics as it is a reinforcing agent due to its chemical composition rich in straight-chain amylose. Due to this property, starch has been used in the formulation of bioplastics [31]. Moreover, bis-acrylamide likely contributed to water resistance, leading to reduced solubility of the bioplastic. As a cross-linker agent, bis-acrylamide decreases the solubility of hemicellulose in liquid, which consequently enhances water resistance [29], as can be observed.

Glycerol is a plasticizing agent widely described in the literature for the formation of bioplastics. Its presence is known to reduce internal hydrogen bonds in intermolecular bonds in biodegradable starch-based plastics in their formulations, helping to improve water resistance [32], which was also observed in this study.

In general, the characteristics of the components used in this study to form xylan-based bioplastics contribute to the development of a cohesive, non-brittle,

malleable material with potential properties for industrial applications. It should be noted that there are few reports in the literature on xylan-based bioplastics [11, 21, 33], especially when a previously modified xylan is used as a base [34]. This is the first report of the addition of a cross-linker, such as bis-acrylamide, in the formulation of such bioplastics.

Challenges and prospects for xylan-based bioplastics

Although there are few studies in the literature on xylan-based bioplastics [5, 11, 21, 33], their properties and applicability are interesting and promising. In addition to the properties evaluated in this study, such as opacity, solubility, humidity and tensile stress, xylan-based bioplastics can be biodegradable [21], since they can be composed of a polysaccharide matrix, as demonstrated in this study.

One of the interesting industrial applications of bioplastics is their use as packaging in the food industry, especially as primary packaging that comes into direct contact with food [35]. To this end, new studies involving the evaluation of essential properties for primary packaging such as UV-blocking [36], antioxidant and antimicrobial actions [37] are needed. Xylan, which is a heteropolysaccharide that can exhibit antioxidant [38] and antimicrobial [39] action, could be the ideal raw material for the production of new sustainable and economically viable bioplastics for industrial applications.

However, the major challenges in the production and application of xylan-based bioplastics are probably its large-scale production and the cost-effectiveness and competitiveness with conventional petroleum-based plastics [40], as the xylan fraction needs to be extracted from lignocellulosic biomass with a high yield and low residual lignin content, avoiding the promotion of breaks in its structure. Among the alternatives for solving these challenges include the large availability of Brazilian lignocellulosic biomass feedstock resulting from the country's agro-industrial production, and the 3D-filament printing method, since this technology has already been used to print second-life waste plastics [41] and reinforced plastics [42] in recent years.

Conclusion

This study demonstrated that both original xylan (X1) and partial lignin-free xylan (X3) enabled the endo-xylanase from *A. versicolor* to show an activity of 624 IU/mL under optimal temperature and pH conditions. Moreover, these substrates served as optimal inducers for *A. versicolor* growth, as seen by the high endo-xylanase activity detected over 10 days of microbial growth, exceeding 3000 IU/mL. Commercial xylan is economically unviable due to its high cost and scarcity in the market, creating a need for a low-cost and easy laboratory protocol to obtain xylan, such as the one developed in this study, to address this gap. While the literature includes reports on the use of glycerol and starch in xylan-based bioplastic formulations, the use of a cross-linker such as bis-acrylamide, and the removal of arabinose and lignin from xylan, has not been previously published.

It can be concluded that the bioplastics formulated with lignin-free xylan showed lower opacity, moisture, and solubility, as well as greater tensile strength compared to those containing original xylan. These findings also align favorably with the limited reports on xylan-based bioplastics available in the literature. There is a clear need to reduce petroleum-based plastics through more sustainable and eco-friendly processes, such as the ones presented in this study. The bioplastics produced in this study have the potential for future technological applications such as packaging, making bags, cutlery, dishes, and medical devices, among others. It is recommended that further studies on the technical and economic feasibility of xylan-based bioplastics, both those already described in the literature and those presented in this study, should be conducted to establish their low-cost viability for biotechnological applications in the industry.

Material and methods

The illustration below summarizes the methodological stages of this study and highlights the objectives of the work, such as obtaining xylan (substrates) free of lignin and arabinose, as well as the production of xylan-based bioplastics (Fig. 4).

Raw material

The biomass of sugarcane bagasse was chosen as the raw material because it is well-characterized and widely used to obtain xylan fractions and produce derivatives. The sugarcane bagasse for this study was provided by a local sugarcane mill (São João, Araras/SP, Brazil). The material was washed using distilled water to remove impurities and residual sugar, and was submerged in distilled water for 3 days. After this, the raw material was dried at 55 °C for 48 h and milled in a 20-mesh knife mill (0.825 mm) [43].

Hemicellulose extraction and measurement of lignin residual content

The hemicellulose fraction was obtained through sugarcane bagasse treatment with 0.2% (w/v) ethylenediamine tetraacetic acid (EDTA) (Sigma, 99.5% purity) solution

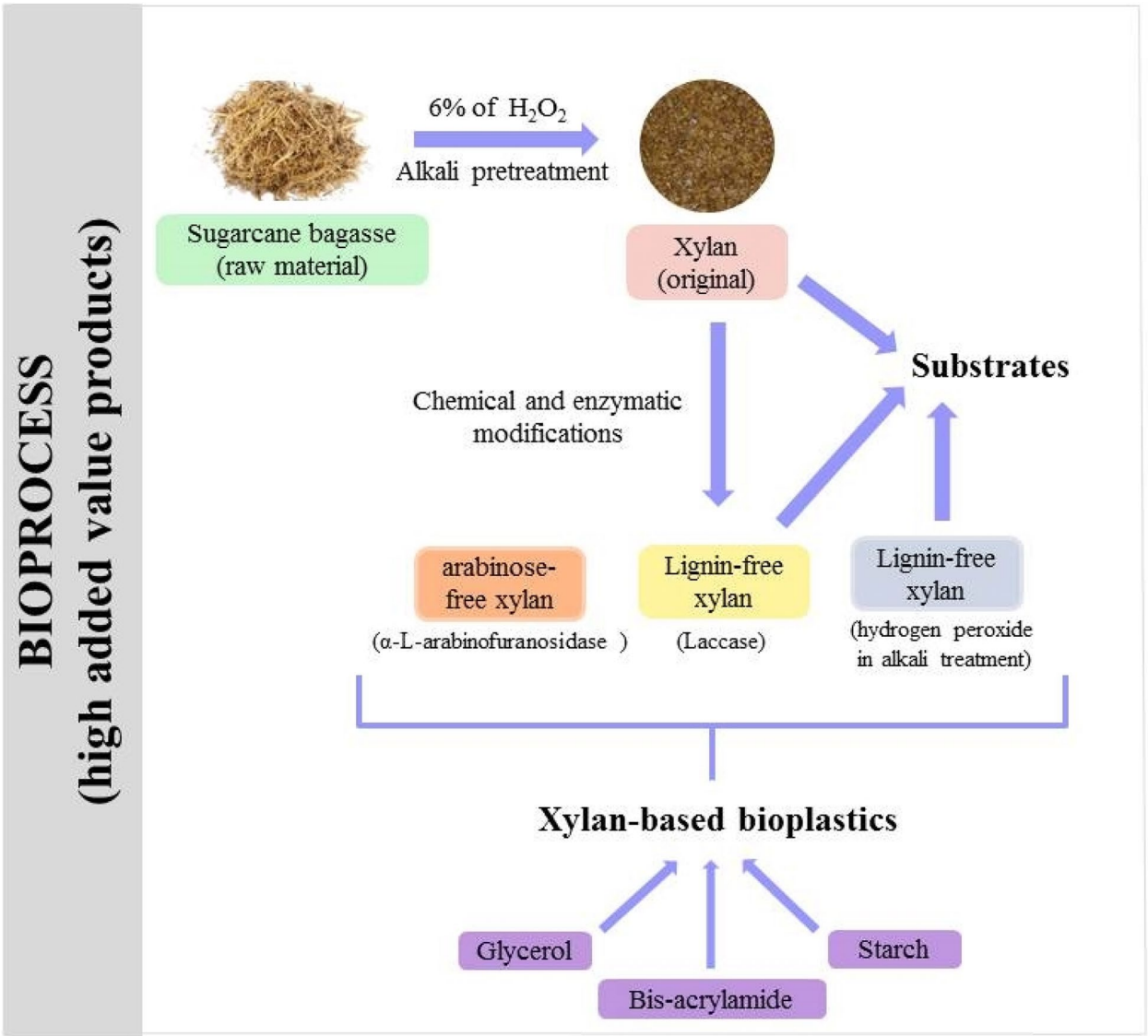

Fig. 4 Summary of the bioprocess stages and obtaining high added value products

by 1 h at 90 °C for metal removal. The extraction process followed the method with optimized conditions for bagasse, which include using 6% H_2O_2 (w/v) (Merck, quality level 400) at 25 °C for 4 h [14, 44]. After this step, 10 g of bagasse was placed in 1 L flasks and prepared reagents were added at a volume of 200 mL (5% w/v). Then, the pH was adjusted to 11.6 with 5 mol/L NaOH (Synth, impurity-free) and placed under agitation at 80 rpm. The material was filtered using filter paper. The pH was immediately readjusted to 6 with 6 mol/L of HCl (Synth, impurity-free). Finally, 3 vol ethanol was added to xylan precipitation. The liquid fraction was changed 3 times to wash it. Then, the xylan fraction was isolated from the liquid and oven-dried at 45 °C.

Chemical and enzymatic modifications of xylan for residual lignin removal

Firstly, xylan extract by hydrogen peroxide in an alkali medium was subjected again to a novel treatment such as that described in Section "Production of lignin- and arabinose-free xylan". After this step, the original xylan (2% w/v) was submitted to enzymatic hydrolysis using 1.67 IU/g of laccase (Sigma) solubilized in 0.05 mol/L sodium tartrate buffer pH 6.5 at 30 °C for 4 h following the method proposed by Rabia et al. (2018) [45] for depolymerizing residual lignin present on solubilized xylan, and also using 10 IU/g α-L-arabinofuranosidase (Megazymes) in a reaction at 50 °C for 24 h for the hydrolysis of pendent groups of arabinoses on xylan. The enzymatic

treatments aimed to remove residual lignin content and arabinose pendent groups on xylan, successively. To facilitate the understanding, the different xylans obtained through chemical and enzymatic modifications were described as X1: untreated xylan; X2: xylan treated with 6% H_2O_2; X3: xylan hydrolyzed with lacase; X4: xylan hydrolyzed with α-L-arabinofuranosidase.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

All the samples were directly placed onto ZnSe crystal. Then, pellets were produced containing 0.001 g of the sample and 0.25 g of KBr (Synth, impurity-free). For this analysis, lignin bands were observed at 1510 and 1700 cm^{-1}, and arabinose bands were detected between 980 and 1100 cm^{-1} using a Fourier transform infrared spectrometer (Shimadzu IRAfnity-1S). This analysis aimed to determine the total or partial removal of residual lignin and the pendant arabinose groups as a result of the enzymatic action of laccase and α-L-arabinofuranosidase.

Cultivation of Aspergillus versicolor

The modified xylans were used at 2% concentration as a substrate for the growth of *A. versicolor* in a minimal medium (MM) [46] for 10 days at 37 °C without agitation in 250 mL Erlenmeyers. Then, some collections were performed at 48, 96, 144, 192, and 240 h. The samples were centrifuged at 2000 x*g* for 5 min to remove cells and to obtain the supernatant, which was then submitted to a determination of xylanase activity (IU/mL) according to the method [47].

Purification step and determination of enzymatic activity of xylanase

The xylanase from *A. versicolor* was purified following the method [48]. The enzymatic activity was performed applying the methodology [47]. The enzymatic reaction was prepared using 1% of each modified xylan (w/v) and 100 µl of xylanase in a final volume of 1 ml for 5 min at 50 °C. All reactions were performed in triplicate.

Xylan-based bioplastics

The bioplastic formulations followed the methodology of [47], with modifications such as the addition of 0.5% (w/v) bis-acrylamide (Sigma), as bis-acrylamide is a conventional organic crosslinker that improves the mechanical strength of hydrogels as described by [49]. All bioplastics comprised 5% of total polysaccharides (m/m total mass of the filmogenic solution), including xylan (X1, X2, X3, and X4), starch (Sigma, impurity-free) and 20% glycerol (Sigma, 99.5% purity) (w/w of polysaccharides), as well as using bis-acrylamide as described previously. The bioplastic gelatinization process was performed at 80 °C for 2 min. The content was distributed in Petri plates and submitted at 30 °C during 24 h for drying. Triplicates were made of each of the four types of bioplastics produced.

Bioplastic properties

i) Opacity.

To assess opacity, all bioplastic samples measuring 3.0 × 0.9 cm were analyzed in a spectrophotometer at 450 nm wavelength (Biospectro, SP-22). The opacity was determined by the division between the absorbance and the thickness of bioplastics [50].

ii) Moisture and solubility.
Samples (X1 to X4) in triplicate in sizes equivalent to 0.9 × 1.5 cm were weighed and oven-dried at 105 °C by 24 h. The procedure was repeated until a constant mass had been obtained, and its moisture percentage was then determined from the difference in masses before and after drying, which represents the presence of water in the bioplastic [51]. The samples were inserted in a different beaker containing 50 mL of distilled water and then they were stirred at 110 rpm at room temperature for 24 h. After this step, they were oven-dried again at 105 °C until the the final dry mass was obtained [51]. After being removed from the oven and before being weighed, the samples were kept in a desiccator to cool.

iii) Mechanical test.
Tensile stress and elongation at the break of samples (X1 to X4 xylan-based bioplastics) were measured by an Engco Texturometry machine. The thickness of specimens of 2 cm in length and 0.6 cm in width were previously measured in different and random regions by a digital 0–25 mm micrometer of 0.001 mm resolution (Mitutoyo 293–230). The test was conducted at a 1 $mm.min^{-1}$ displacement speed. It should be noted that the tests to evaluate the properties of the bioplastics (opacity, humidity, solubility and mechanical test) were carried out in triplicate.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44316-024-00024-9.

Supplementary Material 1.

Authors' contributions

Michel Brienzo and Danilo Bueno: Conceptualization, Methodology; Danilo Bueno.: Investigation, Data curation, Software, Writing- Original draft preparation, Editing. Michel Brienzo: Supervision, Reviewing, Editing.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

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