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Research Article

Optimization of hydrothermal-assisted alkali process for enhanced xylan recovery from banana fiber biomass

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Abstract
Banana fiber is a rich lignocellulosic biomass source that has not been widely explored. The hemicellulose components (15 - 20 %) of banana fiber can be a feedstock for producing high-value commodity chemicals. Hemicellulose is extracted by physical, chemical, and biological methods, in which combining hydrothermal treatment with alkaline mode of extraction provides an enhanced recovery percentage. Thus, the present study aimed to optimize the hydrothermal-assisted alkaline method of xylan extraction from the banana fiber biomass. Initially, xylan was extracted with a conventional-based alkali method. A maximum of about 43 and 35 % was recovered from pretreated and raw banana fiber at 12% NaOH concentration when incubated at 55 °C for 24 h. To improve the xylan yield, the hydrothermal assisted alkali method experimented in which 67.1% and 58.3 % of xylan were recovered when treated at 121 °C for 1 h at 12% NaOH. To further enhance the xylan recovery, a two-step alkali process by combining conventional and hydrothermal-assisted alkali methods resulted in the highest xylan (81%) recovery from pretreated banana fiber when incubated with 12 % alkali for 8 h followed by steam treatment. On the other hand, a maximum of 73 % of xylan was recovered when steam treated after incubation for 24 h from raw banana fiber. Thus, the alkali incubation followed by steam treatment significantly showed the highest xylan recovery from the banana fiber biomass. The extracted xylan might be utilized as a source for various xylan-based products, including furfural, xylooligosaccharides, xylose, and xylitol, all of which have significant roles in the pharmaceutical and food industries.

Keywords: Alkali extraction, Banana fiber, Hemicellulose, Two-stage process Xylan extraction

INTRODUCTION

Lignocellulosic biomass (LCB) is generated from waste agricultural residues and is considered as a rich source for biorefinery processes (Anwar *et al.*, 2014; Isikgor *et al.*, 2015 Machado *et al.*, 2016). Cellulose, hemicellulose, and lignin are the major components of lignocellulosic biomass. About 20–35% of lignocellulosic biomass is made up of hemicelluloses, the second most frequent polysaccharides in nature. According to Menon *et al.* (2010), xylan makes up to 30% of the cell wall material in annual plants, 15 –30% of the cell wall material in hardwoods, and 7 –10% of the cell wall material in softwoods. The biomass of lignocellulosic plants typically contains 20% to 30% hemicellulose (Limayem *et al.*, 2012; Barhoum *et al.*, 2020). Hemicellulose bioconversion has attracted a lot of attention recently due to its useful applications in several agro-industrial processes, including the effective conversion of hemicellulosic biomass to fuels and chemicals, improvement of animal feedstock digestibility, clarification of juices, and enhancement of beer consistency (Luo *et al.*, 2019). Hemicellulose is made up of D-xylose, L-arabinose, D-mannose, D-glucose, D-galactose, L-rhamnose, Lfructose, and D-glucuronic acid, which can be acetylated or methylated (Ebringerová *et al.*, 2005). The fundamentals of biorefinery, which integrate the utilization of every biomass component and produce zero- or almost zero-waste, are the most practical means of valorizing hemicellulose. A wide range of bioactive compounds, including xylitol, 2, 3-butanediol, polyhydroxy butyric acid, single-cell protein, pigments, and renewable ethanol, have been made from hemicellulosic sugars (Saha, 2003; Carvalheiro *et al.*, 2008; Chandel *et al.*, 2010; Qaseem *et al.*, 2021).

The three primary hemicellulose subgroups are mannans, xylans, and xyloglucans which have a backbone made up of β 1-4 linked -D-pyranose (hemiacetal sugars) residues. The most prevalent hemicellulose found in nature is called xylan, and it is made up of -d-xylopyranosyl (xylose) residues that are connected by β 1-4 glycosidic bonds. Glucuronic acid or arabinose may also be attached to the xylan backbone, resulting in the subcategories of xylan known as homoxylan, arabinoxylan, glucuronoxylan, and arabino-glucuronoxylan. (Limayem & Ricke, 2012; Van Dyk and Pletschke,, 2012). Xylan extracted from biomass has a wide range of applications like xylan based composite used in packaging industries (Nechita *et al.*, 2021), xylan-based hydrogels used in drug delivery systems (Chang *et al.*, 2021), value-added chemicals from xylan as a source like xylo-oligosaccharides as potential prebiotic (Capetti *et al.*, 2021), xylitol production by using xylose obtained from xylan which has application in pharmaceutical and food industries (Hilpmann *et al.*, 2019), the product furfural that can be obtained from hydrolysis of xylan and sequential dehydration of xylose formed (Morais *et al.*, 2020).

To overcome the resistive character of associated cellulose and to preserve recovered xylan for further valorization, several physical, chemical, and biological pretreatment techniques can be used in a biorefinery to extract the hemicellulose component of the biomass (Teleman *et al*., 2003; Chaturvedi and Verma, 2013; Naidu *et al.*, 2018). The alkaline mode of hemicellulose extraction has more attention; chemicals like calcium hydroxide, sodium hydroxide, potassium hydroxide, or ammonium hydroxide are frequently employed for alkaline extraction. Hemicellulose is released from the lignocellulosic matrix and is then able to be extracted by the hydrolysis of the ester bonds between it and lignin. Alkaline extraction needs less demanding working conditions (lower temperatures and pressures) than other treatment techniques. The structure of the extracted hemicellulose differs from the native hemicellulose as a result of the removal of acetyl groups and uronic acids by alkaline pre-treatment (Badiei *et al.*, 2014; Naidu *et al.*, 2018). Hydrothermal-assisted alkaline extraction overcomes the duration and chemical cost of the conventional method. The primary benefit of steam explosion over other hydrothermal processes is the high temperatures required to solubilize hemicellulose and the short residence time needed to prevent structural changes in the hemicellulose (Naidu *et al.*, 2018). The enhanced extraction of hemicellulose (90–96%) from corn cobs by hydrothermal aided alkali pretreatment has been documented (Samanta *et al.*, 2012).

In developing countries, banana is a significant fruit crop, and each harvest yields vast quantities of byproducts. About 80 million metric tonnes of waste from banana pseudostems are produced annually in India. The banana pseudostem fiber contains 15-20% of hemicellulose, which can be extracted for conversion to value-added products (Gabhane et al., 2014). To the best of our knowledge use of a two-step process of xylan extraction by alkaline-based process is not reported. The present work aimed to explore the alkaline-based extraction of xylan from banana fiber. Hence, the present study focussed on the effect of different alkaline concentrations on xylan recovery and examined the two-step extraction process from banana fiber.

MATERIALS AND METHODS

Materials and chemicals used

Banana fiber was used as substrate in the present study, obtained from Eco Green Crafts, Coimbatore, Tamil Nadu, India. The collected biomass was dried in sunlight to remove moisture content to reach the constant weight. Further, the dried biomass was size reduced to 1- 3 cm using sharp scissors, followed by grinding using domestic mixie into powder form and passed through American Standard Test Sieve Series (ASTM) sieve, size no. 70 of size 212µm. Chemicals were purchased from Hi-Media Pvt. Ltd. (Mumbai). Double distilled water was used for the preparation of other reagents and solutions. The composition of banana fiber was analyzed as per the standard National Renewable Energy Laboratory (NREL) protocol (Sluiter *et al.*, 2008).

Extraction of hemicellulose from banana fiber

The experimental design for the extraction of xylan was performed. The design included the conventional method, hydrothermal (steam explosion) method, and two-step xylan extraction alkali treatment process. The following equation gives the actual recovery percentage of xylan.

Conventional alkaline method

Xylan from the banana fiber was extracted in raw and EnZolv pretreated banana fiber using sodium hydroxide (NaOH) as an alkaline source. The interaction of different parameters for maximum xylan recovery was analyzed using alkaline concentrations (2%, 4%, 8%, 12%, 16%); incubation temperatures (35 °C, 45 °C, 55 °C) and time (8 h, 16 h, 24 h). The experiment was carried out in an Erlenmeyer flask with a biomass concentration of 5% (w/v); the biomass alkali slurry was mixed thoroughly and incubated at the above said experimental runs with 100 rpm shaking speed. Following the incubation, the solid residue was filtered and washed with hot distilled water, dried at 45 °C, and saved for later examination in airtight containers. The filtrate was centrifuged at 5000 rpm for 15 min and the supernatant was neutralized using glacial acetic acid. From the neutralized supernatant, xylan was precipitated with the aid of 95% ice-cold ethanol and allowed to stand overnight. The precipitate was collected, washed with water, and dried at 50 °C to reach constant weight. The xylan recovery percentage was calculated using the equation above (Samanta *et al.*, 2012).

Hydrothermal-assisted alkaline extraction

The raw and pretreated banana fiber was subjected to hydrothermal assisted extraction of xylan-involved treatment at 121 °C for 1 h (without any previous incubation) with different concentrations of alkali (2%, 4%, 8%, 12%, 16%) maintaining the same biomass concentration as mentioned above. After the reaction time of 1 h, the autoclave was depressurized and the solution was filtered. The filtered residue was washed, dried, and stored in an airtight container. After neutralizing with glacial acetic acid, the xylan from the filtrate was precipitated using 95% ice-cold ethanol and left overnight. The precipitate was collected after centrifugation and the recovery percentage was calculated as mentioned in the equation (Banerjee *et al.*, 2019).

Two-stage process of xylan extraction

For enhanced recovery of xylan from the biomass the combination of the above-mentioned method was carried out by following the conditions; the raw and pretreated banana fiber was first subjected to the alkaline treatment of 12% with different time intervals (0,1,8,16, 24 h) at 45 °C with 100 rpm shaking speed. Following the conventional treatment, the slurry was subjected to hydrothermal treatment at 121 °C for 1 h. After the reaction time, the filtrate was then precipitated and the xylan % was calculated as mentioned in the above sections (Singh *et al.*, 2018).

Statistical analysis

Each experiment was run in triplicate, presenting the results as mean ± standard error. The experimental data were subjected to analysis of variance (ANOVA), and significant differences between means were examined with Duncan's Multiple Range Test (DMRT), which was estimated at $P<0.05$ using SPSS software. GraphPad Prism version 8.0.1 was used to create the graph displayed for data representation.

RESULTS AND DISCUSSION

Compositional analysis of pretreated banana fiber

The data on the composition of the banana fiber showed that the raw banana fiber contains 60.64% cellulose, 16.25% of hemicellulose, 16.02% of lignin, 4.87% of moisture content, and 8% of ash content. A similar composition was obtained in the banana (*Musa paradisiaca*) pseudostem fiber (Cecci *et al.*, 2020). The EnZolv pretreatment was optimized in our previous study and the composition of pretreated banana fiber includes 73.2% Cellulose, 15.85% of hemicellulose, 6.57% of lignin, 1.07% of moisture content, 2.50% extractives, and 11.6% of ash content (Table 1).

Xylan recovery from conventional alkaline method

The alkaline mode of xylan extraction is widely used and the mechanism involves disruption of the biomass cell wall and hydrolysis of ester linkage between the hemicellulose and lignin portions (Chaturvedi and Verma, 2013; Puitel *et al*., 2022). In the conventional alkaline method, xylan was extracted using 5 different alkali concentrations followed by incubating with three different incubation times (8 h,16 h,24 h) and temperatures (35 °C, 45 °C, 55 °C) for higher recovery. In both raw and pretreated banana fiber, maximum recovery of xylan was obtained at 12% NaOH concentration with a yield of 37.6% and 46.2% followed by 8% NaOH treatment with a yield of 20.2% and 24.4%, when incubated at 35 °C respectively (Fig 1). Like the present study, Samanta *et al.* (2012) extracted xylan from corn cob and the same alkaline concentration of 12% showed maximum xylan recovery. The xylan extraction from different raw materials like pineapple peel waste, wheat straw, and corn stalk was studied and reported recov-

Table 1. Composition of raw and pretreated banana fiber

Substrate	Extractives %	Moisture %	Ash content %	Cellulose %	Hemicellulose %	Lignin %
Raw Banana fiber	2.2 ± 0.34	4.87± 1.24	8.00 ± 0.63	60.6 ± 1.04	16.25 ± 1.87	16.02 ± 1.58
Pretreated banana fiber	2.5 ± 0.17	1.07 ± 0.02	11.6 ± 0.59	73.2 ± 1.24	15.85 ± 1.17	6.57 ± 1.60

Values in each column represent the mean of three replicates ± standard error (SE) ery from different alkali concentration ranges (Banerjee *et al.* 2019, Puitel *et al*., 2022)

The impact of incubation time and temperature on the yield of xylan recovery was estimated. Maintaining the 12% NaOH concentration as a constant factor, the other two parameters (incubation time and incubation temperature) were analyzed at different ranges. The results showed that an increase in temperature causes an increase in xylan recovery from the banana fiber. At 55 °C, significant xylan recovery of 33.1 and 35% was obtained from raw and pretreated banana fiber followed by 45 °C with a yield of 25 and 29% from the raw and pretreated biomass. Concerning incubation time, the highest xylan yield was obtained at 24 h with 35 and 43 % followed by incubation at 16 h with 31.4 and 27.2% from the raw and pretreated banana fiber when incubated at 55°C (Table 2). Similar to the present results, Ruzenne *et al.* (2008) have reported an increase in xylan recovery from wheat straw biomass and an increase in temperature. Banerjee *et al.* (2019) have suggested that increasing temperature beyond the optimum level may cause xylan disintegration, leading to other product formation. Similarly, Singh *et al.* (2018) reported that maximum xylan recovery was at 24 h of alkali treatment of biomass. The present study also suggested that pretreated, i.e., the delignified banana fiber has shown increased xylan recovery than xylan extracted from raw banana fiber. Similar results were reported by Nacos *et al.* (2006) that delignified kenaf wood increases the xylan recovery from 5 to 30%.

Xylan recovery from hydrothermal-assisted alkaline extraction

Hydrothermal-assisted alkaline extraction was experimented on to enhance the maximum recovery of xylan from the banana fiber biomass. One of the key benefits of a conventional alkali-based technique is that the hydrothermal pretreatment requires no incubation period. In the present study, hydrothermal treatment was processed at 121 °C for 1 h for the same above-mentioned alkali concentration and the results showed that at 12% level of alkali concentration, a significant yield of xylan was obtained in both raw (58.3%) and pretreated (67.1%) followed by 8% alkaline concentration with the yield of 50.56% from raw biomass and 59.1% from pretreated banana fiber respectively (Fig 2). One of the most important factors is the alkali concentration, which greatly impacts the process economics because high alkali concentrations require more expensive downstream processing. Similarly, Samanta *et al.* (2012) have reported increased xylan extraction from corn cob when treated with 12% NaOH with steam application for 1 h . Jnawali *et al.* (2018) reported that treatment of coconut husk with 20% NaOH with 60 min steam treatment resulted in 93% relative xylan recovery; similar findings were obtained by Jayapal *et al.* (2013) in sugarcane bagasse with 85% xylan; Mou *et al.* (2023) reported that hydrothermal combined alkali treatment in cotton stalk recorded 67% of xylan yield.

Xylan yield from two-step process

Since the yield of xylan obtained was not up to the margin level, the experiment was moved to the following process involving the combination of conventional alkali incubation and hydrothermal treatment at 121 °C for 1 h for significant xylan recovery from banana fiber biomass. In the present study, incubation of biomass with 12% NaOH for different time incubation followed by steam treatment for 1 h was performed and the result obtained showed that in pretreated banana fiber, incubating the biomass with 12% alkali for 8 h at 55 °C followed by hydrothermal treatment at 121 °C for 1 h re-

Table 2. Effect of various incubation temperatures and time on the xylan recovery

Source	Raw Banana fiber (BF)	Xylan from PTBF
Temperature (°C)		
35°C	20 ± 1.4^{c}	23 ± 1.5^{bc}
45°C	25 ± 1.3^{b}	27 ± 1.8^{b}
55°C	33 ± 1.2^{a}	35 ± 1.7^{a}
R^2 value	0.987	0.993
Incubation time (h)		
8 h	21 ± 1.2^{c}	23 ± 1.2^{c}
16 h	29 ± 1.3^{b}	31 ± 1.1^{b}
24 h	41 ± 1.2^{a}	43 ± 1.8^{a}
R^2 value	0.995	0.998

Alkali concentration was maintained at 12 % NaOH for the biomass. Each value in the column represents the mean of three replicates ± standard error (SE) and the same letters within the treatment are not significantly different from each other as determined by DMRT ($p<0.05$); BF- Banana fiber; PTBF- Pretreated banana fiber

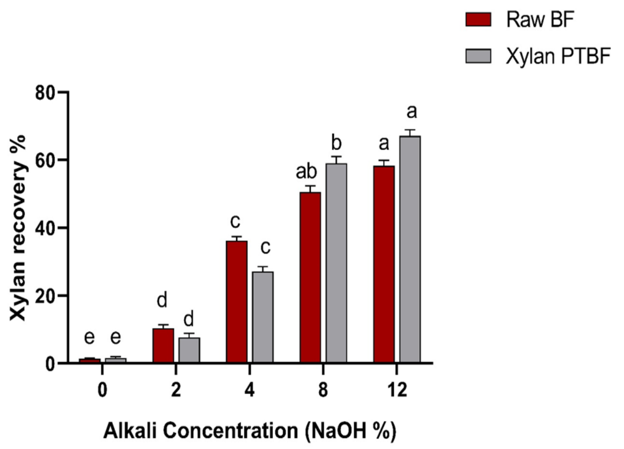

Fig. 1. *Effect of different alkali concentrations on xylan recovery pretreated banana fiber (Data represent the mean of three replicates ± standard error (SE) and the same letters within the treatment are not significantly different from each other as determined by DMRT (p<0.05). (R^2 value Raw Banana fiber- 0.993, pretreated banana fiber- 0.992).*

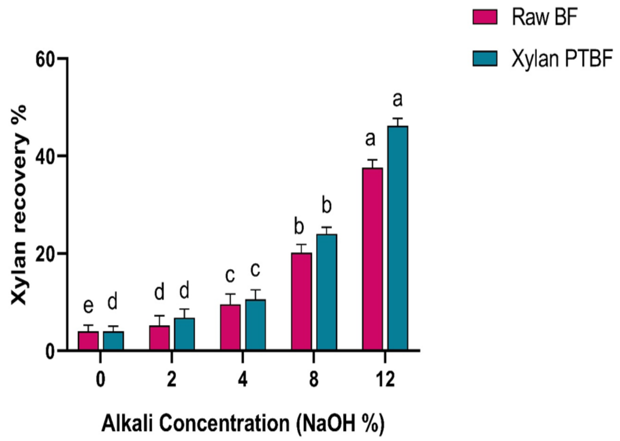

Fig. 2. *Effect of different alkali concentrations with hydrothermal treatment on xylan recovery from the pretreated banana fiber (Data represent the mean of three replicates ± standard error (SE) and the same letters within the treatment are not significantly different from each other as determined by DMRT (p<0.05). (R^2 value Raw Banana fiber- 0.980, pretreated banana fiber- 0.991)*

sulted in 80.2% xylan recovery; incubation with alkali for 16h followed by hydrothermal treatment resulted in 71% recovery. Compared to pretreated banana fiber the xylan recovery from raw biomass took longer incubation time of about 24 h with a maximum xylan yield of about 73%, followed by incubation with 16 h with a yield of 62.5% (Fig 3). This might be due to the disruption of lignin content present in the untreated biomass. Similar to the present study, Singh *et al.* (2018) reported 94% xylan recovery when areca nut husk was treated with 10% NaOH for 8 h followed by steam treatment at 121 °C for 1 h; Banerjee *et al*., (2019) has reported

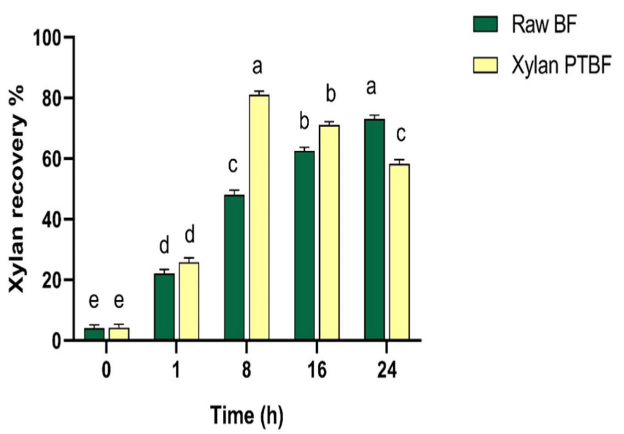

Fig. 3. *Effect of two-step alkali hydrothermal treatment on xylan recovery from the pretreated banana fiber at different incubation times (Data represent the mean of three replicates ± standard error (SE) and the same letters within the treatment are not significantly different from each other as determined by DMRT (p<0.05). (R^2 value Raw Banana fiber- 0.990, pretreated banana fiber- 0.994)*

87% relative recovery of xylan using the alkali assisted hydrothermal treatment from pineapple waste biomass. Compared to the reports, the present study showed 80.2% yield, which may be attributed to the loss of xylan chain during extraction.

Conclusion

Banana fiber was found to be a rich source of lignocellulosic content; its abundance, easy availability, and less explored information make banana fiber a suitable substrate for hemicellulose extraction. The present study demonstrated that using the conventional mode of alkali extraction resulted in less recovery of xylan, while hydrothermal-assisted alkaline extraction also showed little increase in xylan recovery. In contrast, the two-step process combining both methods showed significant enhancement in xylan recovery of about 80.2% from the pretreated banana fiber when incubated with 12% NaOH for 8 h followed by steam treatment at 121° C for 1 h. Concerning raw banana fiber, incubation for 24 h following steam treatment has resulted in 73% xylan recovery. Thus, the two-stage process of alkaline incubation followed by steam treatment has shown a two-fold increase in xylan recovery compared to conventional alkaline treatment within a short period of time. In future aspects, the extracted xylan may be converted to xylo-oligosaccharides, highly recognized as potential prebiotic and other high-value commodity chemicals.

Conflict of interest

The authors declare that they have no conflict of interest.

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