

Agricultural Waste to Value-Added Xylooligosaccharides: The Potential of Banana *Pseudostem*

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Abstract

Lignocellulosic biomass is a sustainable and renewable resource to be used as feedstock for high value biochemicals especially xylooligosaccharides (XOS) which are attracting great interest because of its well-established prebiotic functionality. Banana pseudostem is an agro industrial residue available in high quantities and presents a moderate xylan content which makes it potential substrate for XOS production within integrated biorefinery systems. The following review evaluates the current advancement on strategies to valorize banana pseudostem generating XOS specifically addressing the effects of physicochemical pretreatment approaches on biomasses deconstruction and xylan accessibility. In this connection pretreatment procedures exemplified by alkaline dilute acid and steam explosion leading to lingo cellulosic structural

changes as well as hemicellulose solubilisation with effect on downstream enzymatic digestibility are reviewed. Furthermore, the evaluation of endo- β -1,4-xylanases as an important biocatalytic step ruling the XOS yield and product specificity for the enzymatic depolymerization of xylan is discussed. It also synthesizes the effects of process parameter's reaction temperature pH enzyme loading on hydrolysis efficiency and degree of polymerization of resultant oligosaccharides as reported in individual studies. Although new findings have been published on XOS biosynthesis from lignocellulose confounding factors such as feedstock heterogeneity low substrate selectivity and lack of techno economic validation impede optimization efforts for banana pseudostem derived systems. Integrated & synergistic pretreatment enzymatic conversion strategies along with scale up studies and life cycle assessment must become the focus of future studies to develop economically feasible and environmentally friendly XOS production routes. The valorization of banana pseudostem may promote the implementation of circular bioeconomy models by collaborating with agricultural residues being transformed into functional food ingredients

1. Introduction

The Xylooligosaccharides (XOS) are a novel class of prebiotic oligosaccharides that have generated considerable attention due to their physiological function as gut microbiota modulators and their versatility in food pharmaceutical and nutraceutical formulations. In its structure XOS are made of D-xylose units linked through β -(1 $\rightarrow$ 4) bonds with a typically degree of polymerization (DP) from 2 to 10. XOS has other substituents including arabinose acetyl and glucuronic acid groups depending on the biomass source and processing conditions which affect solubility, stability and bioactivity. One of the functional advantages of XOS is that they are resistant to hydrolysis by human digestive enzymes making them selectively fermentable by probiotics such as Bifidobacterium and Lactobacillus species in the gut. This specific fermentation is linked to better gut microbiotic balance and elevation of short chain fatty acids (SCFAs) production which provide good support for intestinal health and metabolic regulation (Aachary & Prapulla, 2011; Samanta et al. In addition XOS have high thermal stability and pH tolerance enabling the incorporation of XOS into many processed food systems.

The rising prevalence of gastrointestinal disorders coupled with an increase in consumer demand for functional and health promoting foods are driving the interest in prebiotic ingredients. Consequently, there is an increasing research interest in developing sustainable and economically feasible synthetic pathways for XOS.

Lignocellulosic biomass has become one of the most desirable feedstocks because these are abundant renewable and much cheaper. Hemicelluloses are the second most abundant biopolymers in lignocellulosic materials primarily accounting for regions of cellulose and hemicellulose together as well as lignin xylan the polysaccharides that are present in large amount in lignocellulosics having a great percentage of hemicellulose content is the main precursor for XOS production. Various kinds of agricultural by products and residues such as corn cobs wheat straw rice straw barley husk and sugarcane bagasse have been vigorously studied as xylan rich substrates for production of XOS (Akpınar et al., 2009; Carvalho et al., 2008).

Among the different lignocellulosic feedstock's banana pseudostem (*Musa spp.*) now emerging as an attractive but unexplored biomass for value added bioconversion processes. Banana is cultivated all over the world and annual production records greater than 120 million tons which leads to lots of pseudostem waste after harvesting. This biomass is frequently disposed in landfills or composted in wells pits and heaps in the field which creates waste management problems and environmental impact. The banana pseudostem has significant amounts of cellulose and hemicellulose (20–30%) making it a potential feed stock for biorefinery applications (Happi Emaga et al. 2007). Nevertheless the xylooligosaccharide production potential is relatively under investigated considering this advantageous composition and global availability along with a lack of systematic studies aimed at optimizing the related processing strategies. XOSs are usually produced from lignocellulosic biomass through a hybrid process of physicochemical pretreatment and enzymatic hydrolysis. Pretreatment is the most important step to tackle the persistent recalcitrance of the lignocellulosic matrix by breaking down lignincarbohydrate complexes and increasing xylan accessibility. Different pretreatment technology have been evaluated such as dilute acid alkaline steam explosion hydrothermal processing microwave assisted and ionic liquid based systems with variations in efficiency cost and environmental impact (Moure et al., 2006). The extreme pretreatment circumstances can cause the era of inhibitory compounds for example furfural and hydroxyl methyl furfural turn out to be dangerous to enzyme interest and the best of downstream merchandise.

Then the enzymatic hydrolysis with endo- β -1,4-xylanases in co action with accessory debranching enzymes can selectively depolymerize xylan to their XOS containing a predefined degree of polymerization and characterized structures. Enzymatic alternatives avoid many of the problems associated with chemical hydrolysis including lower specificity and formation of undesired by products (Vázquez et al., 2000) in addition to the fact that they can operate under milder pressures and temperature conditions. However challenges such as expensive enzymes heterogeneous substrates low hydrolysis efficiency and poor scalability in process scale up still hinder industrial scale utilization.

Thus banana pseudostem presents a unexploited opportunity as green feedstock material for the sustainable production of XOS. The present study is aimed to bridge gaps in current research by assessing its bioconversion potential using improved pretreatment schemes and enzymatic hydrolysis conditions. This all encompassing aim would guarantee maximum valorization of agricultural residues and the establishment of circular bioeconomy frameworks dedicated on sustainable production of high value functional ingredients.

2. Critical Research Gap and Study Objectives in XOS Production:

The xylooligosaccharides (XOS) production from lignocellulosic biomass has been extensively studied most studies have focused on traditional agricultural residues including corn cobs wheat straw rice straw and sugarcane bagasse. Such feedstocks were well studied in terms of pretreatment optimization enzymatic hydrolysis efficiency XOS yield DP distribution and inhibitor formation leading to the establishment of process knowledge (Akpınar et al. 2009; Carvalho et al. 2008; Moure et al. 2006). The banana pseudostem has been studied leaves with much less

attention towards XOS production and carrier agents in detail exploring the systematic bioconversion of cellulose.

The published investigations on banana pseudostem in particular seem scarce and focus primarily either on general analyses of chemical composition or more ambitious biomass valorization pathways than just XOS production steps. Specifically there is limited and or comparative data available on important parameters including xylan accessibility pretreatment efficiency enzyme catalyzed hydrolysis performance XOS yield and distribution of DP species and inhibitory compounds compared to traditional lignocellulosic feedstock. Additionally coupled experiments of optimizing pretreatment followed by enzymatic hydrolysis and product characterization on banana pseudostem are still limited. Hence the topmost research gap is the lack of systematic data driven studies assessing banana pseudostem as a potential feedstock for XOS production against known biomass sources specifically through process optimization along with yield efficiency and product quality parameters. This limitation prevents a clear abstraction of its actual industrial potential and restricts validation as an economic substrate for XOS production at the commercial scale. This gap necessitates comprehensive experimental and comparative studies emphasizing the integration of pretreatment enzymatic hydrolysis standardization in the assessment of yield purity and structural features of XOS from banana pseudostem to classify it as an industrial XOS biomaterial.

Objectives

1. To evaluate the potential of banana pseudostem as a lignocellulosic feedstock for xylooligosaccharides (XOS) production by analyzing its compositional features and comparing it with other agricultural residues.
2. To optimize pretreatment and enzymatic hydrolysis strategies for improving xylan conversion efficiency XOS yield and degree of polymerization (DP) using banana pseudostem biomass.

2. Lignocellulosic Biomass for XOS Production:

Lignocellulosic biomass has one of the most plentiful renewable and sustainable natural roots for biofuel performances biochemicals and high value products like xylooligosaccharides (XOS). It is primarily composed of three main polymers cellulose hemicellulose and lignin. Hemicellulosic polysaccharides are usually 20–35% of the total dry biomass and an important raw material for XOS production (Carvalho et al., 2008). In hemicelluloses xylan is the key polysaccharide and plays a leading role in the biosynthesis of (XOS). While structurally xylan comprises β -(1 $\rightarrow$ 4)-linked xylose units with corresponding side-chain substitutions affecting the physicochemical and biological properties of XOS (Ebringerová & Heinze, 2000). XOS offer many beneficial properties including prebiotic and antioxidant potentials as well as health-promoting characteristics; thus, these polysaccharides are increasingly used in food pharmaceutical and nutraceutical industries (Moure et al., 2006). Inexpensively available and environment friendly (Samanta et al., 2015); hence agricultural residues and agro-industrial wastes have been widely explored for XOS production The generation of xylooligosaccharides (XOS) requires the accessibility of xylan which can be approached by effective pretreatment and enzymatic hydrolysis to efficiently disrupt the rigid lignocellulosic structure containing cellulose hemicellulose and lignin (Kumar & Satyanarayana, 2015).

2.1 Chemical Composition of Major Biomass Sources:

Lignocellulosic biomass from agricultural residue and agro industrial waste which have higher hemicellulose and xylan contents (Carvalho et al., 2008) has promising for XOS production. Typical sources of biomass are corn cobs wheat straw rice straw barley husk and sugarcane bagasse. Typically cellulose counts for 35–50% of biomass makeup hemicellulose 20–35% and lignin ~10-25% (Sun & Cheng, 2002). The corn cobs has approximately 30–35% xylan that makes it especially qualified for XOS production (Samanta et al., 2015) in comparison with wheat straw and rice straw

containing around 20–30% hemicellulose (Santos DTLB et al., 2008). The high annual availability and the lower processing cost of sugarcane bagasse make this by product also an attractive substrate (Moure et al., 2006). They are also environmentally friendly as biomass materials are cheap biodegradable and renewable. Moreover using agricultural wastes solves disposal issues that would otherwise lead to environmental pollution from open field burning (Carvalho et al., 2008). Since lignocellulose has a complex and refracted structure its pretreatment by alkaline steam explosion or enzymatic treatments is required to facilitate access of enzyme for generating higher XOS yield (Kumar & Satyanarayana, 2015).

2.2 Composition and Specific Features of the Banana Pseudostem:

Banana pseudostem is an abundant renewable lignocellulosic biomass and has been widely recognized as a favorable biomass due to its carbohydrate composition (Sabiha-Hanim et al., 2015). Banana cultivation produces large amounts of pseudostems biomass since each plant bears fruit only once and is cut down afterwards creating a huge volume of agricultural waste. This biomass is commonly either left in the field to decompose or open burned which contributes towards environmental pollution and solid waste management challenges in several tropical and subtropical regions like India Pakistan Malaysia and Philippines.

The chemical composition of banana pseudostem contains about 35–45% cellulose 20–30% hemicellulose and 10–15% lignin (Sabiha-Hanim et al., 2015). The lower lignin content relative to many woody biomass feedstocks makes for easier pretreatment and greater enzymatic access. Among the hemicellulosic fraction xylan polymers found in this region are specifically important for xylooligosaccharide (XOS) production through selective hydrolysis into value added oligosaccharides (Moure et al., 2006). More over its high moisture content rapid biodegradability and cost effectiveness make it one of the best biological resources for bio refining.

As a whole banana pseudostem can be seen as an environmentally viable and economically attractive resource for biomass valorization which offers these benefits in terms of waste reduction the development of bioeconomy and the possibility to use it as a substrate for producing XOS (Kumar & Satyanarayana 2015).

Biomass Source	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Xylan Content (%)	Key Features / Suitability for XOS Production
Corn cobs	35–45	25–35	15–20	30–35	High xylan content; widely studied; excellent XOS yield potential
Wheat straw	35–45	20–30	15–20	20–25	Abundant residue; cost-effective and renewable
Rice straw	30–40	20–30	10–15	20–25	Widely available in Asia; moderate hemicellulose content
Barley husk	30–40	20–25	15–20	20–25	By-product of cereal processing; moderate xylan source
Sugarcane bagasse	35–50	20–30	10–15	20–30	High availability; strong biorefinery potential
Banana pseudostem	35–45	20–30	10–15	18–25 (approx.)	Underutilized; high moisture; promising but underexplored XOS feedstock

Table 1. Comparative composition of lignocellulosic biomass for XOS production

2.3 Comparative Importance Use of Banana Pseudostem:

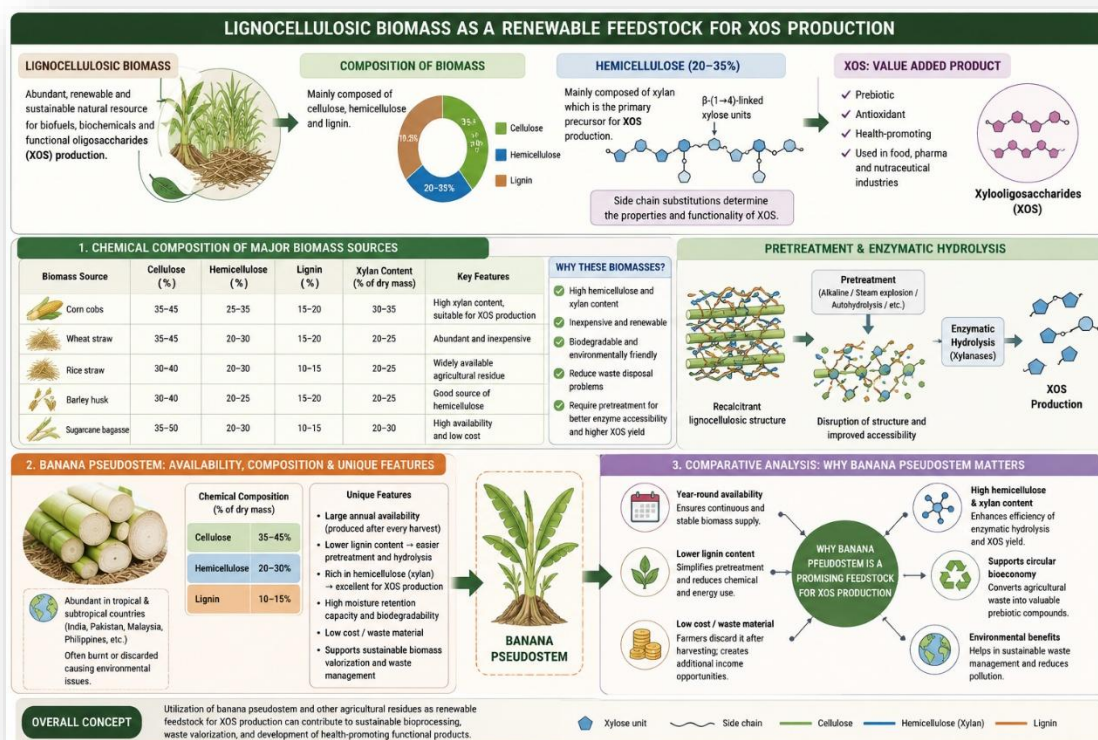
Banana pseudostem has emerged as an important potential lignocellulosic feedstock for xylooligosaccharide (XOS) production because of its quantifiable range of hemicelluloses and large-scale availability from agro industrial industries worldwide. Banana pseudostem for example has been reported to consist of roughly 35–45% cellulose 20–30% hemicellulose and 10–15% lignin (dry weight; Sabiha-Hanim et al., 2015). Xylan is a direct polysaccharide fraction of hemicellulose and can be enzymatically hydrolyzed into XOS if processing conditions are managed (Moure et al., 2006).

Nonetheless those compositional values vary widely according to cultivar type harvesting season geographic origin moisture level and sample pretreatment method. This kind of variability causes compositional variation that can change hemicellulose yield by approximately 10–20% in different studies so directly affects the XOS production efficiency in downstream processes. Moreover the high water content of banana pseudostem (often between 80–90% fresh weight) is a key limitation for storage stability and enhances transport and preprocessing costs before it can be bioconverted. Based on comparative studies of several lignocellulosic biomass suggested that their relative higher xylan accessibility scores for corn cobs and sugarcane bagasse are attributed to lower moisture content and a more uniform structural identity which finally leads to improved enzymatic hydrolysis efficiency at similar levels of pretreatment conditions (Carvalho et al., 2008). Banana pseudostem harbors comparatively lower lignin content (~ 10–15% lignin) compared to the above mentioned inedible biomass which could possibly decrease the severity for pretreatment or requirements of chemicals during delignification (Kumar & Satyanarayana, 2015). This lower lignin barrier has greater enzyme accessibility but no increase in XOS yield without appropriate process conditions.

In addition some seasonal residues are available intermittently (rice straw and wheat straw) while banana pseudostem production remains constant throughout the year because banana plants are harvested perennially. However this has not ensured the stability of industrial feeds as degradation kinetics at high moisture can be exploited and spoil rapidly with microbial action within a few days if left unprocessed or protected (Mohapatra et al., 2010).

Physico mechanically hemicellulose-containing biomass is generally converted to xylooligosaccharides (XOS) by harmless destroying and chemotactic hydrolysis with endo- β -1,4-xylanases in a Compact State. XOS yields from optimized lignocellulosic systems usually fall between 20% and 60% of the initial xylan content depending on pretreatment severity enzyme loading, and reaction conditions (Samanta et al., 2015). However with regards to banana pseudostem the literature is limited to systematic benchmarking of yield degree of polymerization (DP) profiling and inhibitor quantification we considered most studies focusing on compositional analysis but not process optimization.

Thus although both theoretical potential of banana pseudostem as XOS feedstock based on its high hemicellulose contents and low lignin structure is highly promising, overall industrial applicability lacks further validation in terms of quantitative process data established comparisons between pretreatment approaches as well as large-scale techno-economic analyses.



3. Pre-treatment Strategies for Xylan Extraction:

Pretreatment is a vital process in which the original lignocellulosic matrix consisting of recalcitrant hemicellulose and xylan is destroyed to enable subsequent hydrolysis of the hemicellulose ultimately leading to Xylooligosaccharides (XOS) production. The structural component of lignocellulosic biomass is due to the strong interactions between cellulose hemicellulose and lignin cross-linked with each other which restricts enzyme access during XOS extraction, resulting in lower yield of XOS. Thus an efficient pretreatment strategy should achieve high xylan extraction and low formation of degradation products and inhibitors (Carvalho et al., 2008).

Different physical chemical physicochemical and biological pretreatment methods have been investigated to enhance xylan availability. These mechanisms are based on the usage of biomass whose effectiveness relies on biomass composition, operational cost environmental impact and XOS properties (such as degree of polymerization (DP) and purity).

3.1. Hydrothermal and Auto-hydrolysis Pretreatment:

Hydrothermal or autohydrolysis pretreatment is one of the most commonly used environment-friendly methods for XOS production. During this process well water is used without adding chemicals and biomass is treated with hot compressed water. Severe temperature and pressure can break hemicellulose chains and liberate soluble xylooligosaccharides (Vegas et al., 2005). This approach is advantageous because it minimizes chemical inputs reduces pollution and yields lower concentrations of inhibitors. It should be noted that moderate DP of XOS suitable for prebiotic can also be generated by hydrothermally pretreatment. Though too high severity can degrade XOS into xylose and furfural which will lower product quality.

3.2. Dilute Acid Pretreatment:

The most common dilute acid pretreatment utilizes sulfuric or hydrochloric acid to hydrolyze hemicellulose and liberate xylan-derived oligosaccharides. This approach has been shown to produce high xylan solubilization and high biomass reduction at a rapid pace (Akpınar et al., 2009). However harsh acidic environments can subsequently

result in the production of byproducts that inhibit downstream enzymatic hydrolysis and purification (e.g., furfural hydroxymethylfurfural). Acid pretreatment may however have lower DP XOS due to extensive hydrolysis, thus limiting selectivity of functional oligosaccharide production.

3.3. Alkali Pretreatment:

Alkali pretreatment with sodium hydroxide potassium hydroxide or calcium hydroxide mainly focuses on the removal of lignin and increases the porosity of biomass. This allows enzymes to reach out efficiently which makes xylan easy to be extracted (Sun & Cheng 2002). Since alkali treatment destroys ester linkages between lignin and hemicellulose it is well established that this chemical treatment is effective for agricultural residues with high lignin content. Compared with acid pretreatment alkaline methods generate less degradation inhibitors and maintain higher molecular weight fractions of xylan. On the negative side high alkali consumption can result in the increase of operational costs and difficulties with chemical recovery and wastewater treatment.

4. Steam explosion pretreatment:

Steam explosion is a physicochemical pretreatment process including pressure steam treatment and rapid decompression. Rapid pressure release disrupts the structure of lignocellulose leads to partial removal of lignin and enhances xylan accessibility (Ballesteros et al., 2006). It is considered inexpensive and appropriate for industrial use

Step	Description	Methods & Conditions	References
Biomass Collection	Collection washing drying and grinding of banana pseudostem	Drying at 50°C, grinding to fine powder.	Vazquez et al. (2006)
Pretreatment	Alkaline acid and steam explosion treatments to enhance xylan.	Alkaline (NaOH 1-3%, 90-120°C), acid (H ₂ SO ₄ 1-2%, 120°C), steam explosion (160-200°C, 3-10 min).	Carvalho et al. (2008), Li et al. (2024), Yoo et al. (2022)
Enzymatic Hydrolysis	Hydrolysis using endo-xylanase to produce XOS.	pH 5-6, temperature 50°C enzyme concentration 0.5–2 U/g biomass.	Collins et al. (2005), Chen et al. (2024)
Optimization of Conditions	Optimization of enzyme concentration, temperature and pH.	Vary enzyme concentration (0.5–2 U/g), temperature (40–60°C), pH (5–6).	Zhang et al. (2020)
Characterization of XOS	Purity, degree of polymerization, and prebiotic activity.	HPLC TLC prebiotic assay (Bifidobacteria growth).	Kim et al. (2024)

due to the minimal input of chemicals needed with steam explosion. Nonetheless, under harsh processing conditions some hemicelluloses may be degraded and an array of inhibitory by-products are also produced. This calls for optimization of both temperature and residence time to achieve high yields of XOS whilst maintaining desirable DP distribution.

5. Organic Pretreatment:

Organic pretreatment employs organic solvents (e.g. ethanol, methanol, and acetone) with the addition of catalysts to solubilize lignin and isolate hemicellulose fractions from cellulose. This results in a cleaner xylan having enhanced enzymatic digestibility and reduced lignin contamination (Mesa et al., 2011). Although Organosolv

pretreatment improves the quality of XOS and reduces inhibitors generation, its high solvent cost and solvent recovery from the hydrolysate cannot favor commercial application.

6. Deep Eutectic Solvents and Ionic Liquids:

Deep eutectic solvents (DES) & ionic liquids have recently been proposed as novel green pretreatment methods for this process on lignocellulosic biomass. These solvents are good lignin solubilizers and also change biomass crystallinity, which is beneficial for xylose extraction (Xu et al., 2016). Pretreatments with DES and ionic liquid have the benefits of being selective, recoverable and environmental friendly. Additionally, they have the ability to maintain hemicellulose architecture as well. Moreover, these enzyme complexes can generate xylosyl oligosaccharides (XOS) with a closer DP distribution. Nevertheless, ionic liquids still have high costs and difficulties in solvent recovery, which remain a bottleneck to their implementation on an industrial scale.

7. Microwave and Ultrasound Assisted Pretreatment:

The microwave-assisted and ultrasound-assisted pretreatments attract much attention due to their relatively low processing time and energy consumption. Internal heating effect of microwave led to the instant fracture of lignocellulosic structure speeding up hemicellulose solubilization and enzymatic accessibility (Chen et al., 2014). Ultrasound treatment because of cavitation effects, promotes mass transfer and disrupts the cell wall. This technology will improve the yield of XOS while decreasing its chemical needs. But those limitations currently make it expensive for them to be used at scale across the industry.

8. Biological Pretreatment

Biological pretreatment using white-rot fungi or ligninolytic bacteria to degrade the lignin layer enhancing xylan accessibility. It is environmentally friendly, energy-saving, and generates little toxic inhibitors at the same time (Wan & Li, 2012). Biological pretreatment is a process of interest for sustainable biorefinery applications due to the mild operating conditions it mandates. Nevertheless, its slow processing rates and slower hydrolysis efficacy compared to chemical methods render it less industrially attractive.

The relative efficiencies of pretreatment strategies are dependent upon both the composition of the biomass feedstock and desired XOS characteristics. The selection of pretreatment for banana pseudostem is specially critical for its high moisture content, high fiber structure and a variety of lignin composition makeup. Hydrothermal and alkaline pretreatments are often regarded as more promising for banana pseudostem since they can effectively solubilize hemicellulose while reducing the formation of degradation products. Alternatively however advanced methods such as DES microwave-assisted and biological pretreatments might contribute to improving sustainability and foreground XOS quality even more, but need further optimization before techno-economic evaluations could be performed.

In a more general sense, an optimal pretreatment method for producing XOS should be aimed at retrieving the majority of xylan while leaving an acceptably DP distribution that does not often form inhibitors and reduces environmental consequences to some extent while still remaining within satisfactory economic paths in industry.

9. Enzymatic Hydrolysis and Product Profile Control:

The enzymatic hydrolysis is the most important step of hemicellulosic xylan into Xylooligosaccharides (XOS) conversion, because it determines yield, degree of polymerization (DP), and functionality. Enzymatic systems have been also studied because they provide more specificity, milder reaction conditions, and better control of product profile when compared with chemical hydrolysis (Polizeli et al., 2005). The efficiency for this step is various by xylan structure, enzyme systems, reaction parameters and hydrolysis strategy design.

9.1 Xylan structure and xylanase systems:

Xylan is a complex polysaccharide with a β -(1 $\rightarrow$ 4)-linked xylose backbone that contains arabinose, glucuronic acid and acetyl substituents (Ebringerová & Heinze, 2000). The structural complexity influences access to the enzymes and their hydrolysis. The dynamic, yet coordinated action of several xylanolytic enzymes is needed for efficient XOS production; most important are endo- β -1,4-xylanases that cleave xylan chains in internal bonds to produce oligomers.

Accessory enzymes including α -L-arabinofuranosidase acetyl xylan esterase and feruloyl esterase, promote hydrolysis efficiency by cleaving side chain substitutions and lignin carbohydrate linkages to improve the access of enzymes to XR and XOS yield (Collins et al., 2005).

9.2 Enzyme sources and production:

Bacteria (*Bacillus* *Streptomyces*) and fungi (*Aspergillus* *Trichoderma*) produce the Xylanases however for industrial production of xylooligosaccharide (XOS), fungal enzymes are usually preferred because they can secrete a larger number of enzyme compared to bacteria and fungal enzymes show generally broader substrate specificity than bacterial ones (Polizeli et al., 2005). Enzymes are produced by conventional microbial fermentation with submerged or solid-state systems. Developmental advances in biotechnology allowed for the expression of xylanases with improved catalytic efficiency and reduced β -xylosidase activity that limits over-detoxification of XOS into xylose (Sharma & Kumar, 2013).

9.3 Process optimization (temperature pH enzyme/substrate ratio time):

Operational conditions have a significant impact on the efficiency of enzymatic hydrolysis. Most of the xylanases have been shown to exhibit maximum activity at 50–60°C and pH 5.0–6.0 (Collins et al., 2005). The reaction rate and the XOS yield are directly affected by enzyme/substrate ratio, where increasing of enzyme loading leads to higher hydrolysis efficiency but results in higher production cost. Hydrolysis has to be controlled because if it takes a long enough time, XOS can be degraded into an over-degree into monosaccharides (Samanta et al., 2015). As a result, optimizing yield, price and product selectivity helps obtain XOS profiles according to demand as well.

4.4 Regulation of XOS degree of polymerization and prevention of excessive hydrolysis

XOS bioactivity is strongly DP-dependent, thus controlling the DP becomes critical. Whether low-DP XOS (DP 2–3) exhibit higher prebiotic activity than higher oligomers is still a matter of debate (Aachary & Prapulla, 2011). Regulation is mainly achieved by:

- Low β -xylosidase contaminated endo-xylanases
- Controlling reaction time and temperature
- Using enzyme immobilization to stabilize the activity
- When designing enzyme cocktails, having balanced accessory enzyme activity

Excessive β -xylosidase activity is unfavorable since it hydrolyzes XOS into xylose, which results in the decrease of functional oligosaccharide yield (Vázquez et al., 2000). Hence the objective in this case is selective hydrolysis instead of complete saccharification.

4.5 Enzymatic hydrolysis of pre-treated banana pseudostem

Tailored xylanase systems for enzymatic conversion of pre-treated lignocellulosic biomass to XOS is still a new research field. Pretreatment enhanced enzyme accessibility in Banana pseudostem (BP) probably by breaking lignin carbohydrate complexes which enables efficient hydrolysis of hemicellulose fractions.

Pretreatment of banana pseudostem (bs) with hydrothermal and alkali treatments increases its susceptibility to endo-xylanase attack leading to higher yields of XOSs DP 2–5 as dominant products (Kumar et al., 2021). While it is known that the cell wall lignin also contains a residual amount of phenol-type compounds after enzymatic hydrolysis, variation in metabolism gives rise to inconsistent product profiles and this

evolution cost prohibits one from producing large quantities of such proteins. Enzymatic cocktail optimization and mild hydrolysis conditions have been recently reported to increase XOS selectivity while reducing xylose generation. Banana pseudostem enzymatic hydrolysis thus appears to provide a genuine independent avenue for the sustainable production of XOX but additional optimisation is needed with respect to process efficiency scalability and product standardisation.

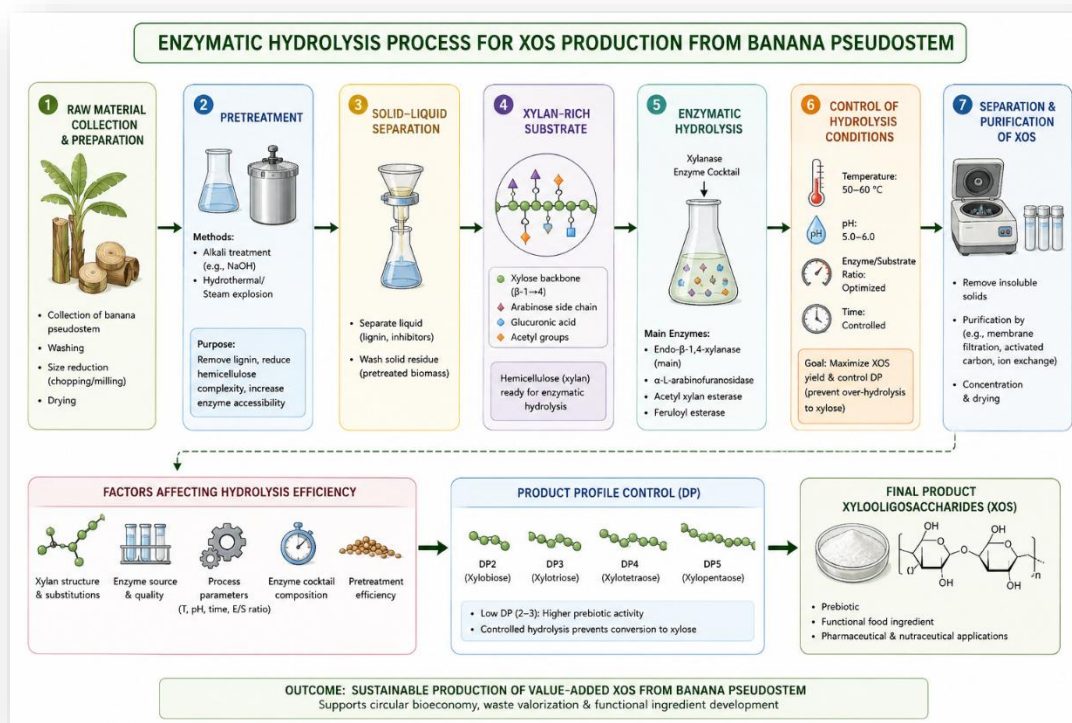


Figure 1 Enzymatic Hydrolysis Process (Banana Pseudostem)

5. XOS Recovery Purification and Characterization and Functional Evaluation

Downstream processing is a critically important step for producing Xylooligosaccharides (XOS) with desired final product purity, structural integrity and biological functionality. Following enzymatic hydrolysis of lignocellulosic biomass, the crude hydrolysate almost always consists of a complex mixture of XOS, monosaccharides, by-products from the lignin degradation and potential inhibitors. This calls for important separation, purification, and characterization strategies to deliver high-value functional xylan-oligosaccharides (XOS) with a predetermined degree of polymerization (DP) and prebiotic activity (Gullón et al., 2011; Vázquez et al., 2005).

5.1 Separation and concentration methods:

The first stage of XOS recovery from hydrolysates generally depends on physical separation techniques such as ultrafiltration, nanofiltration, ethanol precipitation and membrane-based fractionation. Membrane processes are more commercially attractive as they are solvent-free, scalable and economical. Ultrafiltration eliminates impurities and low-molecular-weight structures, with nanofiltration fractionating oligosaccharides with a lower degree of polymerisation (DP), while obtaining low-DP XOS fractions (DP2–DP6) efficiently (Nabarlatz et al. These operations will increase the amount of oligosaccharides but decrease monosaccharides and fermentation inhibitors.

5.2 Purification techniques:

Food grade XOS with high purity requires purification. Typically employed systems are based on activated carbon adsorption ion exchange chromatography gel filtration

and novel purification system based on advanced green materials. Chromatography methods allow for high resolution separation based on differences in molecular size and charge allowing for precise fractionation of the XOS moieties associated with a given DP value (Carvalho et al., 2008). Although these are effective means they tend to be costly and not so suitable for larger scale production.

This has led to the recent development of environmentally friendly purification techniques including graphene oxide adsorption (Sun et al., 2023) and membrane integrated processes with enhanced selectivity towards inhibitors such as furfural and acetic acid and XOS recovery efficiency (Sonkar et al., 2023). The cost-effectiveness aspect to achieve a high purity is still a bottleneck for industrial applications, despite the advances.

5.3 Structural characterization and DP analysis:

Information such as chemical structure of XOS and their function/biological activity is extremely important in order to study their physiology. Numerous methods have been employed to analyze their molecular weight distribution, linkage patterns and DP profiles such as high-performance liquid chromatography (HPLC) high-performance anion-exchange chromatography (HPAEC-PAD), mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy (Rastall, 2010; Samanta et al., 2015). For example DP determination (DP2–DP10) is usually performed by describing the proportion of units in each degree of polymerization, using methods such as HPLC and HPAEC-PAD since they are preferred for this type of analysis, whereas MS provides a detailed molecular mass profiling. In particular NMR spectroscopy can aid in validating glycosidic linkages (if they are linked to arabinose or acetyl groups) and thereby influence XOS functionality and stability.

5.4 Functional evaluation and prebiotic activity of XOS

The functional property of XOS is important thing to consider that determines its utilization as prebiotic activity by selective stimulation of beneficial gut microbiota like *Bifidobacterium* and *Lactobacillus*. Generally, the effects have been determined in vitro as well as using simulated gut models, and via in vivo animal studies. Lower DP (especially DP2–DP3) XOS have stronger prebiotic action as they are better utilized by microbes. Functional analysis also involves the quantification of short-chain fatty acid (SCFA) production potential, pathogen inhibition and immune response modulation. Studies confirm that purified XOS fractions can promote gut health by raising the levels of SCFA and balancing intestinal microbiota (Samanta et al., 2015; Gullón et al., 2011).

6.1 Cost drivers in XOS production

XOS cost drivers Factors that considerably affect the cost of producing XOS from lignocellulosic feedstocks and finally increase their commercial price involve feedstock procurement pretreatment enzyme production costs, downstream processing and wastewater treatment (Fig. 4). In the latter enzymatic hydrolysis is usually one of the most costly steps since commercial xylanase preparations are expensive (Polizeli et al., 2005). In addition how agricultural residues are collected, transported and stored (feedstock logistics) based on the characteristics of the feedstock itself will strongly impact total production cost. Depending on the process selected, pretreatment costs can differ chemical and organosolv methods require greater capital investment and chemical recovery systems than other types of methods while hydrothermal processes are more cost-efficient. The downstream purification (membranes or chromatography) increases the operational costs, especially in large scale (Carvalho et al., 2008).

6.2 Comparison of process configurations

The process configurations also affect the cost and efficiency of XOS production. Integrated biorefinery systems in which pretreatment, enzymatic hydrolysis and membrane separation are combined into a single process to complete the entire biomass utilization while minimizing waste production are believed to be more efficient than individual processes. At laboratory scale batch processes are widely utilized but suffer

from low productivity and high downtimes. On the other hand, continuous and semi-continuous systems provide better scalability, more effective use of enzymes and interchangeable operational costs. The use of immobilization to recycle the enzyme or membrane assisted systems help lower the cadre of enzymes needed for a process (Gullón et al., 2011). Methods of Green processing such as deep eutectic solvents (DES) and mild hydrothermal system are appealing alternatives for the reducing environmentally harmful effects toward chemical usage.

6.3 Life cycle assessment considerations

Life cycle assessment (LCA) is a common method used to evaluate the environmental sustainability of XOS production systems. Major indicators include energy use greenhouse gas emission water consumption and waste production. Because the production of enzymes and chemical pretreatment are generally energy intensive processes that require multiple chemicals inputs they have been shown to contribute most of the environmental burden prior to yeast cultivation (Mussatto & Dragone, 2010). Various sustainable XOS production strategies aim to reduce environmental impact and can be divided into green pretreatment approaches, enzyme engineering, and waste valorization methods. For instance agricultural residues like Banana pseudostem requires no food-based feedstocks and promotes the circular principles. Recycling process water (and recovering chemicals from pretreatment streams) can also have a major impact on sustainability performance. In techno economic and environmental assessments are complementary parts of the XOS production systems design process.

7. Future Perspectives

Xylooligosaccharides (XOS) synthesis from lignocellulosic biomass is anticipated to grow dramatically as a result of sustainable bioprocessing technology and the growing demand for functional food components. The primary goals of future research are to increase environmental sustainability, lower production costs, and improve process efficiency through enzyme engineering, enhanced pretreatment, and integrated biorefinery systems.

7.1 Novel pre-treatment technologies

Deep eutectic solvents (DES) ionic liquids plasma-assisted treatment and sophisticated hydrothermal processes are examples of more environmentally friendly and effective pretreatment techniques. By reducing inhibitor production and environmental impact these approaches seek to increase hemicellulose accessibility. Green solvents may selectively dissolve lignin while maintaining xylan structure, which enhances downstream XOS yield and quality as compared to traditional acid or alkali techniques (Xu et al., 2016).

Due to their potential to lower processing time and energy usage, emerging hybrid technologies including ultrasound-enhanced pretreatment and microwave-assisted DES systems are also drawing interest.

7.2 Enzyme engineering and multi enzyme systems

Enzyme technology is essential to increasing the effectiveness of XOS synthesis. In order to prevent excessive hydrolysis into xylose, future research will concentrate on genetically modified xylanases with increased specificity, thermostability, and decreased β -xylosidase activity (Polizeli et al., 2005). By working together to break down complex hemicellulose structures, multi-enzyme systems (enzyme cocktails) containing endo- β -1,4-xylanase, α -L-arabinofuranosidase, acetyl xylan esterase, and feruloyl esterase are anticipated to greatly increase hydrolysis efficiency. Emerging methods to enhance enzyme stability, reuse, and process economics include immobilized enzyme systems and nanobiocatalysts (Collins et al., 2005).

7.3 Process intensification and scale-up

The goal of process intensification is to lower production costs while increasing mass and energy efficiency. To replace traditional batch processes, integrated reaction-

separation units membrane bioreactors, and continuous reactor systems are being developed. Product yield and uniformity can be increased by using sophisticated process control techniques, such as real-time enzymatic activity and degree of polymerization (DP) monitoring. Maintaining enzyme efficiency, regulating viscosity in high-solid systems, and guaranteeing consistent heat and mass transmission are some of the issues associated with scaling up. For XOS production systems to be commercially successful these problems must be resolved (Gullón et al., 2011).

7.4 Integrated biorefinery approaches

The most environmentally friendly method of producing XOS in the future is through integrated biorefineries. This model fractionates lignocellulosic biomass into several value-added products, such as organic acids, bioethanol, lignin-derived compounds, and XOS.

In a circular bioeconomy paradigm, using agricultural wastes like banana pseudostem improves resource efficiency and lowers waste production. Better use of all biomass fractions is made possible by integrated systems, which enhance overall process economics and environmental performance. To maximize value creation from biomass resources, future biorefineries will probably integrate pretreatment enzymatic hydrolysis fermentation and product recovery into a single optimized platform.

Section	Key Focus	Importance / Future Benefit
Novel Pretreatment Technologies	DES, ionic liquids, hydrothermal and microwave-assisted treatments	Improve hemicellulose accessibility, reduce inhibitors, lower environmental impact, and enhance XOS yield
Hybrid Pretreatment Systems	Ultrasound and microwave-assisted DES methods	Reduce processing time and energy consumption
Enzyme Engineering	Modified xylanases with high specificity and thermostability	Increase hydrolysis efficiency and prevent excessive xylose formation
Multi-Enzyme Systems	Enzyme cocktails containing xylanase and accessory enzymes	Improve breakdown of complex hemicellulose and increase XOS production
Immobilized Enzymes & Nanobiocatalysts	Reusable and stable enzyme systems	Enhance enzyme stability, reuse, and reduce production cost
Process Intensification	Continuous reactors, membrane bioreactors, integrated systems	Increase production efficiency and reduce operational costs
Advanced Process Control	Real-time monitoring of enzyme activity and DP	Improve product consistency, yield, and quality control
Scale-Up Challenges	Heat transfer, viscosity control, enzyme stability	Essential for successful industrial and commercial production
Integrated Biorefinery Approach	Conversion of biomass into multiple value-added products	Maximizes biomass utilization and improves economic feasibility
Circular Bioeconomy	Use of banana pseudostem as agricultural waste	Supports sustainable waste management and environmental sustainability
Overall Conclusion	Banana pseudostem as feedstock for XOS production	Promising renewable source for functional foods,

Table 2. Future Perspectives and Importance of XOS Production from Banana Pseudostem Biomass

Conclusion

Banana pseudostem an agricultural residue with high annual availability has been increasingly studied as a potential lignocellulosic resource for xylooligosaccharide (XOS) production because of its appreciable hemicellulosic xylan containing fraction. Studies conducted so far imply that the composition of XOS as a xylan oligomer may favor enzymatic transformation into XOS provided that strategies are used to improve xylan degradability through appropriate pretreatment methodologies. Physicochemical treatment of lignocellulosic matrices followed by enzymatic hydrolysis with endo- β -1,4-xylanases has been shown in the literature to release XOS however efficiency is highly dependent on feedstock attributes and operational conditions. Recent findings also validate major factors like pH temperatures enzyme dosages and severity of pretreatment that strongly affect hydrolysis behavior along with the profile of XOS. However these data mainly come from work with other lignocellulosic biomass and similar systematic quantitative data have so far not been published for banana pseudostem. However comprehensive evaluations performing pretreatment optimization enzyme system specificity product DP distribution purification efficacy and functional behavior are particularly lacking. In conclusion banana pseudostem can be a potential feedstock for XOS production by Kombucha tea fermentation with substantial of studies are still needed to fully establish sustainable methodology from local sources until October 2023. Yet its fully industrial applicability is not clearly defined due to the absence of standardized comparative studies and techno-economic assessments. Moving forward integrated process optimization and performance benchmarking are needed to confirm feasibility within large scale, commercially relevant biorefinery platforms. Such improvements would enhance its new role in integrating circular bioeconomy strategies based on the valorization of agricultural wastes to high value functional ingredients.

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