PRODUCTION OF LOW-CALORIE SWEETNER XYLITOL FROM SUGARCANE BAGASSE

Submitted by

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VIVEKANAND COLLEGE, KOLHAPUR

(AN EMPOWERED AUTONOMOUS INSTITUTE)

YEAR 2025-2026

"Dissemination of education for Knowledge, Science and culture"

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VIVEKANAND COLLEGE, KOLHAPUR

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DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE

OF

RESEARCH PROJECT COMPLETION

This is to certify that Ms. **SAKSHI D. KOLI** studying in M. Sc. part II, Sem-IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled **"PRODUCTION OF LOW-CALORIE SWEETNER XYLITOL FROM SUGAECANE BAGASSE"** during academic year 2025-26.

Dr. Komal K. Bhise	24.4.26	**Dr. T. C. Gaupale**
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INTRODUCTION

The global over-consumption of refined sugars such as sucrose and high-fructose corn syrup has increasingly been linked with the rising prevalence of obesity, type 2 diabetes, and dental caries. These health challenges have created a strong impetus for developing sugar substitutes that are both safe and beneficial for human health (World Health Organization, 2021). Among the alternatives, the sugar-alcohol (polyol) Xylitol has attracted particular attention due to its sweetness being comparable to sucrose while contributing markedly fewer calories, and offering additional potential health-benefits (Parajó et al., 2020).

Xylitol has been shown to exert minimal effect on post-prandial glucose and insulin responses, making it especially promising for people with impaired glucose tolerance or diabetes (Livesey,2013; Serra-Majem et el., 2018) Moreover, xylitol exhibits evidence of anti- cariogenic activity by reducing levels of cariogenic bacteria and lowering incidence of dental decay when used appropriately (Soderling, 2012; Makinen 2010). The combination of low glycaemic impact, reduced caloric value and oral health advantage thus positions xylitol as a natural safe alternative to refined sugar in the context of metabolic and dental health.

Simultaneously, the sugar-industry generates large volumes of lignocellulosic waste – in particular the fibrous by-product Sugarcane Bagasse. In India alone, the bagasse output is estimated to reach tens of millions of tonnes annually, yet only a fraction is used beyond cogeneration for heat or power (Bello et al., 2021; Saini et el., 2015). Much of the waste remains under-utilised, imposing environmental burdens such as open-burning or disposal issues. Converting this abundant agro- residue into a value-added bioproduct therefore offers a dual benefit: waste valorisation plus production of a health-promoting sweetener.

From a biotechnological perspective, the conversion of lignocellulosic biomass (such as bagasse) into sugar-alcohols like xylitol (via hydrolysis, microbial fermentation, product recovery) still faces significant technical and economic challenges (Mussatto & Roberto, 2004; Dasgupta et al., 2021). Key obstacles include: the recalcitrance of lignocellulose to hydrolysis, formation of inhibitory compounds during pretreatment (e.g., furans, phenolics) that impede enzymatic breakdown and microbial fermentation, microbial tolerance issues under complex hydrolysate

conditions, and the high cost of enzymes, process steps and downstream purification (Kumar et al., 2020). For the process to be commercially viable, it is essential to identify robust microbial strains (for example locally adapted yeast or Candida sp.) capable of efficiently converting complex hydrolysates from bagasse under minimal resource conditions, with good tolerance inhibitors and high xylitol yield (Chandrasekhar et al., 2018; Zhang et al., 2023).

Given these dimensions, the present research addresses a timely and integrated problem: developing a sustainable and health-relevant sugar substitute (xylitol) from locally abundant agro-waste (bagasse) via biotechnological conversion, thus contributing simultaneously to public health (by offering an improved alternative to refined sugar) and to industrial- environmental sustainability (by valorising an under-utilised waste stream) (Dasgupta et al., 2021; Mussatto & Roberto, 2004). This research will focus on isolating and characterising locally adapted strains of *Candida tropicalis* with high tolerance to hydrolysate inhibitors and sufficient metabolic capacity for xylitol production, optimizing process parameters to minimize costs and to improve overall viability (Chandrasekhar et al., 2018; Zhang et al., 2023).

Xylitol is a naturally occurring five-carbon sugar alcohol that has gained widespread attention for its unique properties as a low-calorie sweetener and for its dental health benefits (Mäkinen, 2010; Livesey, 2013). It is found naturally in small amounts in fruits, vegetables, and certain hardwoods (Serra- Majem et al., 2018). However, its industrial significance stems from the ability to produce it from xylose-rich lignocellulosic biomass (Dasgupta et al., 2021). Historically, xylitol was first isolated in 1890 by German chemist Emil Fischer and his assistant Rudolf Stahel, who extracted it from beech wood. Fischer named the compound "xylitol" after the Greek word "xylon," meaning wood (Fischer & Stahel, 1890, as cited in Mäkinen, 2010). Although this discovery marked an important milestone, the compound remained largely a laboratory curiosity for several decades due to the lack of efficient industrial production methods. Early xylitol production relied on hardwoods such as birch and beech, but these materials were expensive and not sustainable for large-scale manufacturing (Mussatto & Roberto, 2004; Dasgupta et al., 2021).

In the mid-20th century, the demand for alternative sweeteners increased, particularly during periods of sugar shortages, such as World War II. This led to

exploration of more abundant and cost-effective substrates for xylitol production. Agricultural residues, especially sugarcane bagasse, began to attract interest due to their wide availability in sugar-producing countries like India, Brazil, and Thailand. Bagasse, which is the fibrous residue left after extracting juice from sugarcane, is rich in hemicellulose—constituting approximately 20– 30% of its dry weight. Hemicellulose can be hydrolysed to release xylose, which serves as the main precursor for xylitol production. Early experiments in the 1990s demonstrated that chemical hydrolysis using acids or enzymatic treatment could effectively extract xylose from sugarcane bagasse, providing a promising alternative to hardwood-derived xylose (Shaji et al., 2022).

Initially, industrial production of xylitol from bagasse relied on chemical hydrogenation processes. This method involved hydrolysing hemicellulose from bagasse to release xylose, purifying the xylose, and then subjecting it hydrogenation using a nickel catalyst under high temperature and pressure condition. While effective, this process had several limitations, including high energy requirements, long processing times, and the need for extensive purification steps to obtain food-grade xylitol. Furthermore, chemical methods generated significant environmental concerns due to the use of acids and other chemicals during hydrolysis. These limitations prompted the search for more sustainable and economically feasible approaches.

The advent of biotechnological methods in the early 21st century revolutionized xylitol production from lignocellulosic biomass. Microorganisms such as *Candida tropicalis, Candida guilliermondii,* and *Debaryomyces hansenii* demonstrated the ability to convert xylose extracted from sugarcane bagasse into xylitol with high efficiency. These yeasts utilize specific enzymes to reduce xylose into xylitol under controlled fermentation conditions. Biotechnological production has several advantages over chemical hydrogenation, including lower energy consumption, milder operating conditions, and the potential for integrating production into existing sugar mill operations. Studies have shown that *Debryomyces hansenii*, in particular, can achieve xylitol yields exceeding 70% from bagasse hydrolysates, highlighting the feasibility of large-scale microbial conversion (Prabhu et al., 2020).

Recent research has also focused on developing integrated biorefinery approaches that utilize sugarcane bagasse for the simultaneous production of xylitol and other value-

added products, such as ethanol, lactic acid, and lignin-derived materials. These approaches aim to maximize the economic potential of bagasse by creating multiple revenue streams and reducing waste. By implementing integrated systems within sugar mills, researchers have shown that xylitol production can be economically viable and environmentally sustainable. Shaji et al. (2022) conducted techno-economic analyses and life-cycle assessments demonstrating that integrating xylitol production into existing sugarcane processing facilities not only improves cost efficiency but also reduces the environmental footprint of the process.

These advances, challenges remain in large-scale xylitol production from sugarcane bagasse. One significant issue is the presence of inhibitory compounds such as furfural and hydroxymethyl furfural, which are generated during the hydrolysis of hemicellulose. These compounds can negatively affect microbial growth and reduce xylitol yields. Furthermore, downstream purification of xylitol to meet industrial or food- grade standards remains costly and labor-intensive. Ongoing research is focused on optimizing fermentation conditions, developing robust microbial strains tolerant to inhibitors, and improving purification techniques to enhance overall process efficiency.

The history of xylitol production from sugarcane bagasse demonstrates a clear evolution from early chemical extraction methods using hardwoods to modern biotechnological approaches that employ renewable agricultural residues. This transition has been driven by the need for sustainability, cost reduction, and environmental considerations. By leveraging the hemicellulose content of bagasse, modern processes not only provide a valuable alternative sweetener but also contribute to the utilization of agricultural waste, aligning with global bioeconomy goals. As research continues, further improvements in microbial strains, process integration, and hydrolysis techniques are expected to enhance the commercial viability of bagasse-derived xylitol, establishing it as a cornerstone of sustainable sweetener production in the 21st century (Prabhu et al., 2020; Shaji et al., 2022).

The development of xylitol production has witnessed remarkable progress over the past several decades, transitioning from conventional chemical methods to advanced biotechnological and integrated production systems. Xylitol, a five-carbon sugar alcohol, is valued for its sweetness comparable to sucrose, low-calorie content,

and positive effects on dental health, which have made it a highly sought-after sugar substitute in food and pharmaceutical industries. The early industrial production of xylitol primarily relied on the chemical hydrogenation of xylose derived from hardwoods such as birch, beech, and oat straw. This process involved the hydrolysis of hemicellulose to obtain xylose, which was subsequently purified and hydrogenated using a nickel catalyst under high temperature and pressure. While this chemical method was effective in producing xylitol, it posed several challenges, including high energy consumption, the requirement for expensive raw materials, and extensive purification steps to achieve food- grade xylitol.

Moreover, the use of strong acids during hydrolysis and chemical hydrogenation created environmental concerns, limiting the sustainability and economic viability of the traditional production process (Arcaño et al., 2020; Umai, 2022). These limitations spurred the search for alternative, more efficient, and environmentally friendly production methods, particularly utilizing abundant agricultural residues. Among various agricultural residues, sugarcane bagasse emerged as a highly promising substrate due to its widespread availability in sugar-producing regions and its high hemicellulose content, which can constitute up to 30% of its dry weight.

The utilization of bagasse as a raw material marked a significant development in xylitol production, as it allowed the conversion of an otherwise low-value byproduct into a high- value industrial product. Early development efforts focused on optimizing hydrolysis techniques to maximize the release of xylose from bagasse. Acid hydrolysis and enzymatic hydrolysis were both explored, with enzymatic methods demonstrating higher specificity and reduced formation of inhibitory compounds such as furfural and Hydroxymethyl furfural. These inhibitors, if present in high concentrations, can hinder subsequent fermentation and reduce xylitol yield. Efficient hydrolysis is therefore a critical step in the development of xylitol production processes, and extensive research has been conducted to enhance xylose yield while minimizing inhibitor formation (Shaji et al., 2022).

The transition from chemical hydrogenation to microbial fermentation marked a major advancement in the development of xylitol production. Microorganisms such as *Candida tropicalis, Candida guilliermondii, Pichia fermentans* and

Debryomyces hansenii have demonstrated high efficiency in converting xylose into xylitol under controlled fermentation conditions. These yeasts employ specific enzymes, such as xylose reductase, to reduce xylose to xylitol, often achieving yields exceeding 70% when fermentation conditions are optimized. Microbial fermentation provides several advantages over chemical hydrogenation, including milder operating conditions, lower energy requirements, and the potential for integration into existing industrial operations, such as sugar mills. Moreover, microbial production is environmentally more sustainable and can be coupled with downstream processing to recover xylitol from fermentation broth efficiently (Prabhu et al., 2020). Optimization of fermentation parameters, including pH, temperature, aeration, and substrate concentration, has been a focus of research to maximize xylitol yield and productivity. Studies have also explored co-culture systems and genetic engineering of yeast strains to enhance xylose utilization and improve resistance to inhibitory compounds.

In recent years, the development of integrated biorefinery approaches has further advanced xylitol production from sugarcane bagasse. In such systems, bagasse is not only utilized for xylitol production but also for the generation of other value-added products, including ethanol, lactic acid, and lignin-derived chemicals. By creating multiple revenue streams from a single substrate, integrated biorefineries improve the economic feasibility and sustainability of xylitol production. Life-cycle assessments and techno-economic analyses have shown that integrating xylitol production within existing sugar mills can significantly reduce production costs and environmental impact, allowing for the efficient utilization of residual bagasse. This approach aligns with the principles of the circular bioeconomy and sustainable industrial practices, as it minimizes waste and maximizes resource utilization (Shaji et al., 2022).

Despite these technological advances, several challenges remain in the development of xylitol production systems. Inhibitory compounds generated during hydrolysis, including furfural and hydroxymethylfurfural, can negatively affect microbial growth and reduce xylitol yield. Downstream purification of xylitol to achieve high food- grade quality remains complex and costly. Additionally, large-scale microbial fermentation requires careful monitoring and control to maintain optimal conditions for yeast growth and xylose conversion. Current research is focused on developing genetically engineered yeast strains with improved tolerance to inhibitors,

optimizing fermentation conditions for higher productivity, and improving purification methods to produce xylitol in a cost- effective and sustainable manner.

The development of xylitol production reflects a continuous evolution from energy- intensive chemical methods to environmentally friendly, biotechnological processes that utilize renewable agricultural residues. Modern developments in hydrolysis techniques, microbial fermentation, and integrated biorefinery systems have collectively enhanced the efficiency, sustainability, and economic viability of xylitol production. Sugarcane bagasse, in particular, has emerged as a valuable feedstock, transforming a low-value byproduct into a commercially important sweetener. As research continues to refine microbial strains, fermentation processes, and downstream recovery methods, xylitol production is expected to become increasingly sustainable and industrially scalable, reinforcing its role as a key component of the bio-based sweetener industry and contributing to global efforts toward a circular and sustainable economy (Arcaño et al., 2020; Umai, 2022; Prabhu et al., 2020; Shaji et al., 2022).

- **Properties**

1.Physical properties
2.Chemical properties
3.Biological properties

1. Physical Properties of Xylitol

Xylitol is a naturally occurring sugar alcohol that exhibits a range of physical properties that make it particularly valuable in the food, pharmaceutical, and dental industries. From a structural perspective, xylitol is a five-carbon polyol (pentitol) with five hydroxyl groups, which contribute significantly to its solubility, sweetness, and hygroscopic characteristics. The physical properties of xylitol influence not only its functionality in formulations but also its stability during processing and storage.

I. Appearance and Form:-

Xylitol typically appears as a white, crystalline solid that is odorless and has a clean, sweet taste. Its crystalline structure contributes to its ability to form stable granules or powders, which are widely used in chewing gums, candies, and oral care products. The granular form of xylitol allows for uniform mixing with other ingredients, providing consistent texture and taste in food products. Additionally, its crystalline form ensures a

slow dissolution rate, which can enhance the cooling sensation perceived when consumed. This cooling effect, known as the endothermic dissolution effect, is a unique property that distinguishes xylitol from other sweeteners and sugar alcohols (Kaur et al., 2021).

II. Solubility

One of the most important physical properties of xylitol is its high solubility in water. At room temperature, xylitol can dissolve in water at approximately 64–68 g per 100 mL, and its solubility increases with rising temperature. This high solubility allows xylitol to be easily incorporated into liquid formulations such as syrups, beverages, and pharmaceutical suspensions. Unlike sucrose, xylitol does not crystallize as readily in solution under normal storage conditions, making it particularly useful in confectionery where smooth texture and stability are desired. The solubility also plays a role in xylitol's sweetness perception; as it dissolves in saliva, it triggers taste receptors, providing a sensation similar to sucrose but with lower caloric content (Mäkinen, 2010).

III. Sweetness and Taste Profile:-

Xylitol has a sweetness level comparable to that of sucrose, often rated at 90– 100% of table sugar's sweetness. However, it is perceived differently due to its cooling effect during dissolution. This cooling effect results from an endothermic reaction when xylitol dissolves in saliva, absorbing heat and creating a refreshing sensation in the oral cavity. The taste profile is clean, with minimal aftertaste, making xylitol a preferred sweetener in sugar-free chewing gums and dental products. The combination of sweetness and cooling effect not only enhances sensory acceptance but also contributes to oral comfort, particularly in therapeutic applications such as lozenges for dry mouth or dental hygiene products (Salliet al., 2019).

IV. Melting Point and Thermal Properties:-

Xylitol has a melting point of 92–96°C, which is relatively lower than that of sucrose (approximately 186°C). This lower melting point allows xylitol to be processed at moderate temperatures without significant degradation, making it suitable for various heat-sensitive applications. Additionally, xylitol exhibits hygroscopic behavior, meaning it can absorb moisture from the surrounding environment. This property can

be advantageous in maintaining moisture in certain food formulations but requires careful storage conditions to prevent clumping or caking. Its thermal properties are also crucial during extrusion or baking, where controlled melting and heat stability are required to maintain product integrity (Kaur et al., 2021).

V. Crystallization and Particle Size

The crystalline nature of xylitol allows for precise control over particle size, which directly affects its dissolution rate, mouthfeel, and processing behavior. Fine powders dissolve rapidly, providing quick sweetness, while larger crystals can provide texture and gradual sweetness release. In industrial applications, particle size distribution is carefully controlled to achieve desired product characteristics, whether in chewing gum, candies, or pharmaceutical tablets. The crystallization process can also impact the hygroscopicity and flow properties of xylitol, which are important considerations during formulation and packaging (Mäkinen, 2010).

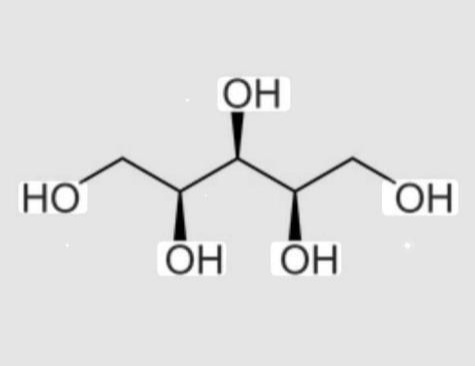
VI. Hygroscopicity and Storage Stability:-

Xylitol is moderately hygroscopic, meaning it tends to absorb moisture from humid environments. This property affects both its handling and storage. To maintain physical stability, xylitol should be stored in airtight containers in cool, dry conditions. In formulations such as powdered beverages or dry blends, controlling moisture content is critical to prevent lump formation and to maintain consistent sweetness. Despite its hygroscopic nature, xylitol remains stable under normal storage conditions and does not undergo significant chemical changes over time, which is a key factor in its widespread use in long shelf-life products (Salli et al., 2019).

VII. Miscellaneous Physical Properties:-

Other notable physical characteristics include its low viscosity in aqueous solutions, compatibility with other polyols and sweeteners, and resistance to Maillard browning reactions due to the absence of a reducing sugar moiety. This makes xylitol ideal for use in heat-processed foods where color and flavor preservation are essential. Its density, bulk flow properties, and non-stick behavior in processing equipment further enhance its industrial a pplicability (Kaur et al., 2021).

2. Chemical Properties of Xylitol



Xylitol is a five-carbon sugar alcohol (pentitol) that exhibits distinct chemical properties, which define its stability, reactivity, and functional applications in food, pharmaceutical, and dental industries. Its chemical characteristics are primarily determined by the presence of five hydroxyl (- OH) groups attached to the carbon backbone, which not only confer sweetness but also influence solubility, hydrogen bonding, and resistance to microbial metabolism. Understanding these chemical properties is crucial for researchers and industrial practitioners in optimizing xylitol-based formulations.

I. Molecular Structure:-

Chemically, xylitol is classified as a polyol with the molecular formula $C_5H_{12}O_5$. The molecule contains five hydroxyl groups, one attached to each carbon atom in the linear five-carbon backbone. This arrangement makes xylitol highly polar, contributing to its solubility in polar solvents such as water. The hydroxyl groups can form hydrogen bonds with water molecules, which explains xylitol's high solubility and its ability to influence the viscosity and texture of aqueous solutions. The molecular structure also ensures that xylitol is chemically stable under normal conditions, which is a desirable property for storage and processing (Mäkinen, 2010).

II. Non-Reducing Sugar Alcohol:-

Unlike reducing sugars such as glucose or fructose, xylitol does not have a free aldehyde or ketone group. This non-reducing nature prevents it from participating in Maillard reactions, which are chemical reactions between reducing sugars and amino acids that typically lead to browning in foods. Consequently, xylitol is suitable for heat-processed products where color preservation is critical. Its non-reducing property also

contributes to its low cariogenic potential, as it is not metabolized by oral bacteria in the same way as glucose or sucrose (Salli et al., 2019).

III. Stability and Resistance to Fermentation:-

Xylitol exhibits high chemical stability under a wide range of temperatures and pH values. It is resistant to enzymatic fermentation by most oral bacteria and does not undergo significant degradation during typical food processing operations. This property is particularly important for its application in sugar-free products where microbial stability and non-fermentability are required. The resistance to microbial fermentation also contributes to its ability to maintain low- calorie content while providing sweetness (Kaur et al., 2021).

IV. Hydrogen Bonding and Solubility:-

The five hydroxyl groups in xylitol enable extensive hydrogen bonding, both with water and with other hydroxyl-containing compounds. This property enhances solubility, contributes to a high boiling point relative to molecular weight, and influences the physical properties such as viscosity and texture in formulations. The hydrogen bonding capability also affects crystallization behavior, allowing xylitol to form stable granules and powders suitable for confectionery and oral care applications. These interactions are important when xylitol is combined with other sweeteners, polyols, or pharmaceutical excipients, as they can alter dissolution rate,sweetness perception, and overall stability (Mäkinen, 2010).

V. Chemical Reactivity:-

Xylitol is generally considered chemically inert under normal processing conditions, which is advantageous for maintaining product integrity. It does not react with common food additives or pharmaceutical excipients, and it remains stable under moderate heating, allowing it to be used in baked goods and syrups. However, under extreme oxidative or acidic conditions, xylitol can undergo degradation to form smaller polyols or carbonyl compounds, though such reactions are rare in typical industrial processes. Its chemical inertness ensures that it retains sweetness and functional properties throughout processing and storage (Salli et al., 2019).

VI. Redox Behavior:-

In biochemical systems, xylitol can be oxidized by xylitol dehydrogenase to D-xylulose, which then enters the pentose phosphate pathway. Then they goes into the basic

alternative of their reactivity is exploited in industrial xylitol production using microbial or enzymatic processes. Chemically, however, xylitol is stable in the absence of enzymes or strong oxidizing agents, which highlights its inertness in food and pharmaceutical formulations (Kaur et al., 2021).

VII. Interaction with Other Chemicals:-

Xylitol's chemical properties enable it to interact synergistically with other polyols and sweeteners. It can form eutectic mixtures, which lower the melting point of combined sweeteners and improve texture in confectionery products. Its chemical compatibility with other compounds also allows for co-crystallization in multi-sweetener systems, contributing to product stability and controlled sweetness release. Additionally, xylitol's chemical stability makes it suitable for incorporation into acidic beverages, lozenges, and chewable tablets without risk of degradation or off- flavor formation (Mäkinen, 2010)

VIII. Implications for Industrial Applications:-

The chemical properties of xylitol—non-reducing nature, resistance to fermentation, hydrogen bonding capability, chemical stability, and inertness— make it highly versatile in industrial applications. In the food industry, it is used as a sugar substitute in sugar-free candies, chewing gums, and baked goods. In pharmaceuticals, it acts as an excipient in tablets, syrups, and lozenges, providing sweetness without promoting microbial growth or chemical degradation. Its chemical characteristics ensure that xylitol can maintain efficacy, sweetness, and stability even under diverse processing conditions, enhancing its value as a functional ingredient (Salli et al., 2019) .

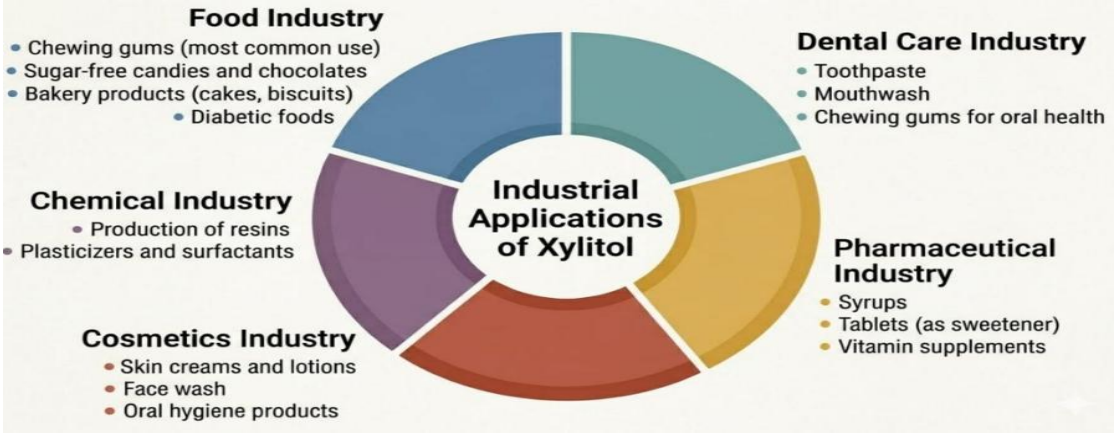


Fig. No. 1 – Industrial Applications of Xylitol

3. Biological Properties of Xylitol

Xylitol, a naturally occurring sugar alcohol, exhibits a variety of biological properties that contribute to its wide-ranging applications in health, dental care, and metabolism. These biological characteristics make xylitol not only a non- caloric sweetener but also a functional ingredient with therapeutic benefits. The biological properties of xylitol arise from its unique chemical structure, non- fermentable nature, and interactions with microbial and human metabolic systems.

I. Non-Cariogenic Property:-

One of the most well-documented biological properties of xylitol is its non- cariogenic nature. Unlike sucrose and other fermentable sugars, xylitol is not metabolized by *Streptococcus mutans* and other cariogenic oral bacteria. This prevents the production of acids that typically lead to dental caries. Regular consumption of xylitol reduces the adhesion of harmful bacteria to tooth surfaces and inhibits their growth, thereby decreasing the risk of cavities. This property has made xylitol a common ingredient in sugar-free chewing gums, toothpaste, and lozenges aimed at promoting oral health (Mäkinen, 2010).

II. Antimicrobial and Anti-Adhesive Effects:-

Xylitol also exhibits antimicrobial properties, particularly in the oral cavity. Studies have shown that it can reduce the growth and adhesion of pathogenic bacteria, including *Streptococcus mutans and Candida albicans*. Xylitol interferes with bacterial metabolism by being taken up into bacterial cells but not fully metabolized, resulting in an energy deficit that reduces bacterial proliferation. Furthermore, xylitol inhibits bacterial adhesion to enamel surfaces, preventing plaque formation. These combined effects enhance oral hygiene and reduce the incidence of dental infections and periodontal diseases (Salli et al., 2019).

III. Low Glycemic Index and Insulin Response:-

Another significant biological property of xylitol is its low glycemic index (GI). Unlike glucose or sucrose, xylitol is absorbed slowly in the small intestine and does not cause rapid spikes in blood glucose or insulin levels. This makes xylitol suitable for individuals with diabetes or those requiring controlled blood sugar levels. Its low-calorie nature also contributes to weight management and metabolic health. By reducing postprandial

glycemic and insulinemic responses, xylitol supports overall energy balance and glucose homeostasis (Kaur et al., 2021).

IV. Prebiotic-Like Effects and Gut Health:-

Emerging research suggests that xylitol may exert prebiotic-like effects in the gastrointestinal tract. Xylitol can influence the composition of gut microbiota by promoting the growth of beneficial bacteria such as Bifidobacteria while inhibiting pathogenic species. These effects improve gut health, enhance nutrient absorption, and may contribute to immune system regulation. Although xylitol is not a fermentable sugar in the oral cavity, it can undergo partial fermentation in the colon, producing short- chain fatty acids (SCFAs) that support colonocyte health and overall intestinal function (Salli et al., 2019).

V. Bone Health and Calcium Absorption:-

Some studies have reported that xylitol can positively affect bone health. Xylitol intake has been associated with increased calcium absorption in the intestine, possibly due to its influence on gut microbiota and SCFA production. Enhanced calcium absorption contributes to bone mineralization and strength, making xylitol potentially beneficial in preventing osteoporosis and supporting skeletal health. Although more clinical studies are required to fully elucidate this effect, it represents an additional biological advantage of xylitol consumption (Kaur et al., 2021).

VI. Safety and Metabolic Tolerance:-

Xylitol is generally recognized as safe (GRAS) for human consumption, and its biological effects are well-tolerated when consumed in moderate amounts. Excessive intake, however, may lead to osmotic diarrhea due to its slow absorption and partial fermentation in the colon. This side effect is dose-dependent and can be minimized by gradual introduction into the diet. The high metabolic tolerance and low toxicity of xylitol make it a suitable sugar substitute for children, adults, and diabetic patients (Mäkinen, 2010).

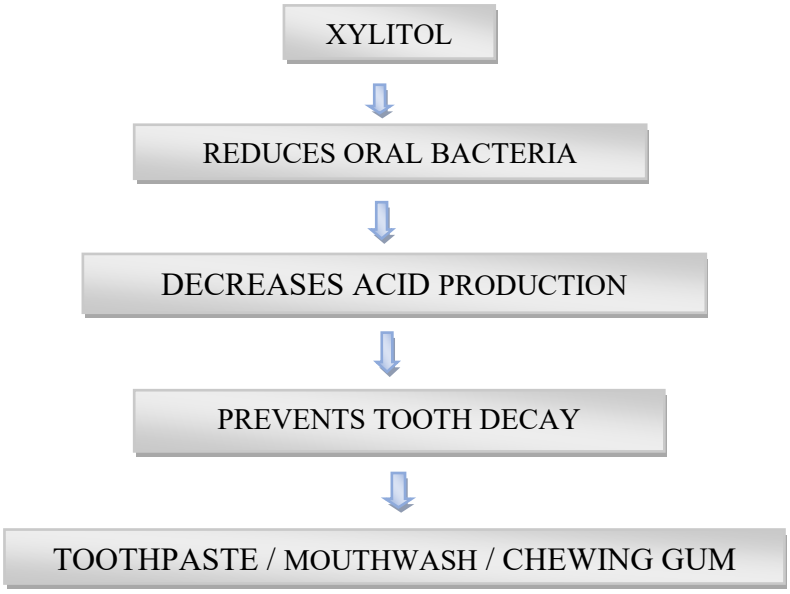
VII. Immunomodulatory Effects:-

Recent studies indicate that xylitol may have immunomodulatory properties. By modulating the activity of oral and gut microbiota, xylitol indirectly supports host immune defense mechanisms. Its role in reducing bacterial load in the oral cavity can lower inflammation and prevent infections, while its influence on gut microbiota can enhance

systemic immune responses. These highlight another xylitol's potential as a functional ingredient beyond mere sweetness, contributing to overall health maintenance (Salli et al., 2019).

VIII. Implications for Dental and Health Products:-

The combination of non-cariogenic, antimicrobial, low glycemic, and prebiotic-like properties makes xylitol a versatile ingredient in health-promoting products. In dental care, xylitol-containing chewing gums, toothpaste, and mouthwashes are widely used to prevent caries and maintain oral hygiene. In the food and pharmaceutical sectors, xylitol's biological properties support sugar-free products suitable for diabetic patients and health- conscious consumers. Its multifunctional biological activity enhances product efficacy and consumer health outcomes, making it an increasingly important ingredient in functional foods and therapeutic formulations (Kaur et al., 2021; Salli et al., 2019).



Flowchart No. 1 – Role of Xylitol in Oral Care

REVIEW OF LITERATURE

Review of literature

1. Xylitol

Xylitol is a naturally occurring sugar alcohol, also called a polyol, used as a sugar substitute. It has the sweetness of sugar but fewer calories and is non-cariogenic (does not cause tooth decay). It can be found in fruits, vegetables, and some plant fibers.

❖ Key points:

- Sweetener alternative to sugar
- Low calorie (~2.4 kcal/g)
- Safe for diabetic consumption due to low glycemic index
- Prevents cavities

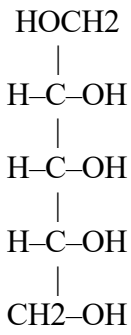
Chemical Nature

Chemical formula: $C_5H_{12}O_5$

Molar mass: 152.15 g/mol

Structure: Linear 5-carbon chain with five hydroxyl (-OH) groups

Linear structural formula:



(Lehninger, A L., Nelson, D. L., & Cox, M.M. 2017.)

- **Properties (Structure)**
    - Multiple hydroxyl groups → water soluble
    - Sweet taste similar to sucrose
    - Stable under heat → suitable for cooking

2. Natural Occurrence of Xylitol

Xylitol is a naturally occurring sugar alcohol belonging to the polyol family. It contains five carbon atoms ($C_5H_{12}O_5$) and is derived from the reduction of the pentose sugar xylose. In its pure form, xylitol appears as a crystalline, white, odorless substance with a sweetness equivalent to sucrose but with 40% fewer calories (Ur- Rehman et al., 2015). Though primarily produced industrially, xylitol is also found naturally in a variety of plants, fruits, and even animal metabolic pathways. Its natural presence highlights the role of xylitol as both a biological intermediate and a renewable bioproduct precursor.

I. Xylitol in Nature

Xylitol is widely distributed in the plant kingdom but occurs only in trace amounts. It was first identified in birch wood and later found in many fruits and vegetables such as plums, strawberries, raspberries, cauliflower, lettuce, oats, mushrooms, and carrots (Mäkinen, 2010). These natural sources contain very low concentrations—typically ranging from 0.01% to 0.2% of their fresh weight (Dasgupta et al., 2017). Due to its low concentration, it is impractical to extract xylitol directly from natural fruits and vegetables on a commercial scale. Nonetheless, its presence confirms that xylitol is a naturally biosynthesized metabolite involved in plant carbohydrate metabolism.

II. Occurrence in Fruits and Vegetables

Several fruits accumulate xylitol naturally during ripening and fermentation processes. This accumulation results from the reduction of D-xylose, which occurs through enzymatic pathways similar to those used by microorganisms in xylitol fermentation. For example, strawberries, raspberries, and plums exhibit detectable amounts of xylitol due to their high pentose sugar content and natural enzymatic activity

(Mäkinen & Söderling, 2010). Vegetables such as cauliflower and mushrooms also show xylitol traces, as these plants use it as an osmoprotectant—a molecule that helps balance osmotic pressure and maintain cell turgor under environmental stress conditions like drought or high salinity (Dasgupta et al., 2017).

In fruits, the concentration of xylitol increases as the fruit matures or undergoes mild fermentation. This explains why overripe fruits or naturally fermented fruit juices often yield xylitol-producing microorganisms. Hence, decayed or fermented fruits are often used in laboratory studies for isolating yeasts such as Candida species, which are capable of converting D-xylose into xylitol.

III. Xylitol in Woody Plants and Agricultural Residues

A major natural source of xylitol precursors is woody biomass and agricultural residues, which contain hemicellulose (xylan). Hemicellulose, after hydrolysis, releases D-xylose, which can be reduced to xylitol chemically or biologically. Hardwoods such as birch, beech, and oak contain 20–30% xylan, while softwoods have slightly less. These trees are traditional sources for industrial xylitol production, especially in European countries where birch wood hydrolysate was first used for xylitol extraction (Sene et al., 2011).

In tropical and agricultural regions, crop residues provide a sustainable alternative to wood- based raw materials. Materials such as corn cobs, rice husk, wheat bran, sugarcane bagasse, and sorghum stalks are rich in xylan and readily available as by-products of agricultural processing (Dasgupta et al., 2017). Among these, sugarcane bagasse has gained significant importance because it is a renewable, low-cost, and abundantly available by-product of the sugar industry. Its hemicellulosic fraction typically contains 25–35% xylan, which can be hydrolyzed to obtain D- xylose for xylitol fermentation (Sene et al., 2011). Utilizing such residues helps in waste valorization, reducing environmental pollution while generating value-added bioproducts.

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V. Natural Production in Animals and Humans

Xylitol is not only found in plants but also produced metabolically in animals and humans. It serves as an intermediate in the pentose phosphate pathway (PPP), which is essential for carbohydrate metabolism and energy production. In this pathway, D-xylulose-5-phosphate is reduced to xylitol through the enzyme xylose reductase (Ur-Rehman et al., 2015). In humans, xylitol is synthesized mainly in the liver, but also in small amounts in other tissues like the kidneys and adipose tissue. The average human

body produces approximately 5 to 15 grams of xylitol per day as part of normal glucose metabolism (Mäkinen, 2010). However, this endogenously produced xylitol is rapidly converted to D-xylulose and utilized for energy, leaving only a negligible amount in the bloodstream.

This metabolic presence explains why xylitol is considered a natural and safe sweetener, compatible with human metabolism and non-toxic even when consumed in moderate quantities. Furthermore, it plays a role in anti-cariogenic properties (preventing tooth decay) by inhibiting the growth of *Streptococcus mutans* in the oral cavity (Ur-Rehman et al., 2015).

VI. Natural Role and Function in Biological Systems

In biological systems, xylitol acts as an osmoprotectant and stress-related metabolite. In plants, xylitol and other polyols accumulate to stabilize proteins and cellular membranes under environmental stress. It helps maintain osmotic balance and protects cells against oxidative damage. In microorganisms such as yeasts, xylitol often accumulates as a by-product of xylose metabolism, especially under conditions where cofactor ($NADH/NAD^+$) imbalance occurs during fermentation. This metabolic feature has been harnessed for industrial xylitol production using microbial fermentation systems.

3.Lignocellulosic Biomass: A Sustainable Treasure for Xylitol Production

Introduction

Lignocellulosic biomass, comprising cellulose, hemicellulose, and lignin, is one of the most abundant renewable resources globally. Among its components, hemicellulose is particularly significant because it contains D-xylose, the principal sugar substrate for xylitol biosynthesis (Li et al., 2020). Its widespread availability, low cost, and eco-friendly nature make it an ideal feedstock for industrial xylitol production.

Importance of Lignocellulosic Substrates

Abundance and Economic Feasibility: Agricultural residues such as sugarcane bagasse, corn cobs, wheat straw, and rice straw are generated in large quantities and are often underutilized. Utilizing these wastes not only reduces disposal problems but also provides a low-cost source of fermentable sugars (Chen et al., 2018).

Sustainability: Unlike pure sugars, lignocellulosic biomass does not compete with food crops, making it a renewable and sustainable substrate (Zhang et al., 2019).

Xylose-Rich Nature: Hemicellulose constitutes 20–35% xylose, an essential sugar for microbial conversion to xylitol, which ensures a higher production yield when properly processed (Saha, 2003).

• **Table no. 1 : Common Lignocellulosic Substrates for Xylitol Production.**

Sr. No.	Substrate	Xylose content (%)	Pretreatment method	Common microbial Strains
1	Sugarcane bagasse	25-30	Acid hydrolysis / Enzymatic hydrolysis	*Candida tropicalis, Candida guilliermondii*
2	Corn cobs	30-35	Dilute acid hydrolysis	Candida tropicalis
3	Rice straw	20	Alkali /acid hydrolysis	*Candida tropicalis*
4	Wheat straw	20-25	Steam explosion / Dilute acid	*Candida tropicalis, Pichia stipitis*
5	Hardwood chips	20-30	Strong acid / Enzymatic hydrolysis	Lignin-tolerant yeast strain

These substrate require pretreatment to break down lignin barriers and release fermentable sugars for efficient xylitol production (Li et al, 2020; Chen et al, 2018).

❖ **Pretreatment Strategies**

- Acid Hydrolysis: Dilute or concentrated acids are used to hydrolyze hemicellulose into xylose. While effective, it can produce inhibitors such as furfural and hydroxymethylfurfural (HMF) (Zhang et al., 2019).
- Alkali Treatment: Sodium hydroxide or ammonia removes lignin, enhancing sugar accessibility and reducing fermentation inhibitors.
- Enzymatic Hydrolysis: Xylanases selectively hydrolyze hemicellulose into xylose under mild conditions, minimizing inhibitor formation (Saha, 2003).

❖ **Microbial Fermentation**

- Yeasts: *Candida tropicalis, Candida guilliermondii, Pichia stipitis*
- Conditions: pH 5–6, temperature 28–32°C, microaerophilic environment
- Mechanism: D-xylose is reduced by xylose reductase in yeasts to form xylitol (Li etal.,2020).

❖ **Advantages**

- Cost-effective and widely available
- Eco-friendly utilization of agricultural residues
- High xylose content ensures better xylitol yield

❖ **Limitations**

- Formation of inhibitors (furfural, HMF) during pretreatment can reduce fermentation efficiency
- High lignin content in some residues requires extensive processing
- Microbial strain optimization is necessary for maximum yield

3. Microorganisms Involved in Xylitol Production

Introduction

Xylitol is a five-carbon sugar alcohol produced primarily through the microbial reduction of D-xylose. While chemical methods exist, microbial fermentation is preferred for its eco-friendliness, selectivity, and mild reaction conditions (Saha, 2003). Various microorganisms, including yeasts, bacteria, and fungi, have been

reported to produce xylitol naturally from xylose-rich substrates.

I. Yeasts

Yeasts are the most widely used microorganisms for xylitol production due to their high xylose uptake, efficient xylose reductase activity, and tolerance to inhibitors formed during lignocellulosic hydrolysis.

Table No. 2: Characteristics of Yeast used for xylitol production

Sr.no.	Yeast	Characteristics	Reference
1.	*Candida tropicalis*	High xylitol yield, tolerant to inhibitors like furfural; widely used in industrial-scale production	Li et al., 2020
2.	*Candida guilliermondii*	Efficient xylose-to-xylitol conversion; requires controlled oxygen levels	Saha, 2003
3.	*Candida shehatae*	Thermotolerant strain; effective in hemicellulose hydrolysate Fermentation	Chen et al., 2018
4.	*Pichia stipitis*	Can ferment xylose under microaerophilic conditions; moderate xylitol yield	Zhang et al., 2019

Mechanism of xylitol production in Yeasts:

• D-xylose is transported into the cell.

• Xylose reductase (XR) reduces D-xylose to xylitol using NADPH as a cofactor.

• Xylitol can either accumulate in the medium or be further oxidized to D-xylulose by xylitol dehydrogenase (XDH), which requires NAD+ (Saha, 2003).

> Key note: High xylitol accumulation is favored under microaerophilic conditions, which limit NAD+ regeneration, slowing conversion to D-xylulose.

II. Bacteria

Some bacteria are capable of producing xylitol, though generally less efficient than yeasts. They are mainly of interest in research for genetically engineered pathways.

- **Examples**:

  - *Lactobacillus casei* – can convert xylose to xylitol under anaerobic conditions

  Bacillus subtilis – genetically modified strains have been studied for xylitol production from xylose.

- **Limitation:**

Bacteria often require strict pH and oxygen control and have lower xylitol yield compared to yeasts.

III. Filamentous Fungi

Filamentous fungi can metabolize lignocellulosic hydrolysates but are less commonly used due to slower growth rates and lower xylitol yields.

- **Examples:**

  - *Aspergillus niger* – capable of producing xylitol from xylose-rich substrates, often in combination with enzymatic hydrolysis of hemicellulose (Zhang et al., 2019).

- **Advantage**:

  - Some fungi produce xylanases that help release xylose from hemicellulose, integrating substrate hydrolysis and fermentation.

- **Factors Affecting Microbial Xylitol Production**

  - Oxygen Levels: Microaerophilic conditions favor xylitol accumulation in yeasts.
  - pH: Optimal pH for most xylitol-producing yeasts is 5–6.
  - Temperature: Usually 28–32°C for yeast fermentation.
  - Cofactors: XR uses NADPH/NADH; balance affects conversion efficiency.
  - Substrate Inhibitors: Furfural and HMF from pretreated lignocellulosic biomass can inhibit microbial growth (Li et al., 2020).

- **Industrial Relevance**

  - *Candida tropicalis* and *Candida guilliermondii* are commercially exploited for large-scale xylitol production using lignocellulosic hydrolysates.
  - Engineered strains of bacteria and yeasts are being developed to improve xylitol yield and productivity, making the process more cost-effective (Cao et al., 2018).

4. From Xylose to Xylitol: Conversion Mechanisms and Methods

Xylitol production involves the conversion of D-xylose, a five-carbon sugar, into xylitol, a value-added polyol widely used as a natural sweetener. This transformation can occur through chemical, microbial, or enzymatic routes, each with distinct mechanisms, efficiency, and sustainability (Granström et al., 2007). In recent years, the shift toward biotechnological approaches using renewable materials such as sugarcane bagasse has gained prominence due to environmental and economic advantages (Rafiqul & Sakinah, 2013).

I. Chemical Route: Catalytic Hydrogenation of D-Xylose

The chemical method is a conventional industrial approach in which pure D-xylose is hydrogenated under high pressure and temperature using metal catalysts such as Raney Nickel (Ni), Ruthenium (Ru), or Platinum (Pt). The reaction reduces the carbonyl group of xylose to a hydroxyl group, forming xylitol.

Reaction:

> D-xylose + H_2 → Xylitol

Operating Conditions:

- Temperature: 80–140°C
- Pressure: 40–50 atm ○ Catalyst: Raney Ni or Ru

Example:

- Industrial-scale xylitol production in Finland and China uses birchwood hydrolysate as the xylose source and Raney Ni catalyst for hydrogenation (Parajó et al., 1998).

Advantages:

- High yield (up to 90%)
- High purity product

Limitations:

- Requires pure xylose (expensive to obtain)
- High energy and equipment costs
- Not environmentally sustainable

Hence, although the chemical method dominates global xylitol markets, it is being gradually replaced by biotechnological production using waste materials like sugarcane bagasse.

II. Microbial Route: Biotechnological Fermentation Process

The microbial conversion of xylose to xylitol is an eco-friendly alternative that uses yeasts or fungi under mild conditions. This process mimics the natural enzymatic reduction of xylose inside microbial cells and is suitable for lignocellulosic hydrolysates such as sugarcane bagasse (Mussatto & Roberto, 2004).

Mechanism of Conversion

The key enzyme xylose reductase (XR) catalyzes the reduction of D-xylose to xylitol using NADPH or NADH as cofactors:

- D-xylose + NADPH + H^+ → Xylitol + $NADP^+$

Microorganisms Commonly Used

- *Candida tropicalis*
- *Candida guilliermondii*
- *Pichia stipitis*
- *Debaryomyces hansenii*
- *Kluyveromyces marxianus*

Fermentation Conditions

- Temperature: 30–35°C
- pH: 5.0–6.0

- Aeration: Microaerophilic (limited oxygen)
- Substrate: Hydrolyzed lignocellulosic biomass (e.g., bagasse, corn cobs, rice straw)

Example 1:

- *Candida guilliermondii* produced 0.75 g xylitol/g xylose from sugarcane bagasse hydrolysate under controlled oxygen conditions (Carvalho et al., 2004).

Example 2:

- *Candida tropicalis* achieved 80% xylitol yield from corn cob hydrolysate, highlighting its efficiency in utilizing xylose-rich substrates (Nigam, 2001).

Advantages

- Utilizes low-cost agro-wastes (bagasse, rice straw, etc.)
- Low energy requirement
- Environmentally sustainable
- Produces fewer by-products

Challenges

- Presence of inhibitors (furfural, acetic acid, phenolics) in hydrolysates
- Detoxification needed before fermentation
- Slightly lower yield than chemical route

Despite these limitations, microbial fermentation is preferred for eco-friendly xylitol production from renewable biomass, aligning with modern bioeconomy goals.

III. Enzymatic and Hybrid Routes: Emerging Biotechnologies

Recent developments have led to the use of purified enzymes or genetically engineered microbes for xylitol production. These enzymatic routes focus on direct conversion of xylose into xylitol using xylose reductase (XR) enzymes, often isolated from yeasts such as *Candida tropicalis* or *Pichia stipitis* (Granström et al., 2007)

Mechanism

- The xylose reductase enzyme catalyzes the conversion of xylose into xylitol in the presence of cofactors NADH/NADPH under mild reaction conditions.

Example:

- A recombinant strain of *Saccharomyces cerevisiae* expressing the xylose reductase gene from *Candida tropicalis* produced enhanced xylitol yield and productivity, demonstrating the potential of metabolic engineering (Granström et al., 2007).

Advantage

- High specificity and purity
- Operates under mild temperature and pH
- Reduced environmental impact

These methods are still under research and pilot-scale testing but hold promise for next- generation sustainable xylitol production from lignocellulosic sources.

5. Pretreatment Methods

Lignocellulosic biomass, such as sugarcane bagasse, is highly resistant to degradation because of the complex arrangement of cellulose, hemicellulose, and lignin. Pretreatment is therefore an essential step to break this structure and release fermentable sugars especially xylose for xylitol production. Several pretreatment techniques have been studied both globally and in India.

I. Dilute Acid Pretreatment

In this method, bagasse is treated with dilute sulfuric acid (0.5–2%) at 100–120 °C for 30– 60 minutes.The acid hydrolyzes hemicellulose into monomeric sugars, mainly xylose.It is a simple, economical, and widely used pretreatment method for xylitol production.

However, it can produce fermentation inhibitors such as furfural and hydroxyl methyl furfural (HMF), which reduce microbial growth and product yield (Chandel et

al., 2018; Bansal et al., 2019). Pillai et al. (2020) reported a 65% xylose recovery from sugarcane bagasse after dilute acid pretreatment, with enhanced xylitol yield upon detoxification. Similarly, Silva et al. (2020) observed comparable xylose yields using dilute acid pretreatment of sugarcane bagasse in Brazil, showing its global relevance.

II. Alkaline Pretreatment

Bagasse is soaked in 1–2% sodium hydroxide (NaOH) or calcium hydroxide ($Ca(OH)_2$) at 80–90 °C.This process removes lignin and increases hemicellulose accessibility to hydrolytic enzymes.It generates fewer inhibitors and can be carried out in a simple laboratory setup. The pretreated biomass needs neutralization before fermentation Alok et al. (2021) observed that alkaline pretreatment enhanced enzymatic digestibility and improved xylitol yield from sugarcane bagasse.

III. Steam Explosion

In this method, biomass is exposed to high-pressure steam (160–200 °C) for a short duration and then rapidly depressurized.The sudden release of pressure disrupts the lignocellulosic structure and increases xylose availability.It is an environmentally friendly process that does not require harsh chemicals.It requires specialized, pressure-controlled equipment.

Prajapati et al. (2023) reported increased xylose yield from sugarcane bagasse using steam explosion, followed by fermentation with Candida tropicalis.

IV. Enzymatic Hydrolysis (Supplementary Method)

After pretreatment, specific enzymes such as hemicellulases or xylanases are added to hydrolyze hemicellulose into xylose.This method is mild, eco-friendly, and produces fewer inhibitors compared to chemical pretreatments.

The main drawback is the high cost of enzymes, which can limit large-scale applications (Silva et al., 2020).

6. Optimization Strategies for Maximized Xylitol Output

Optimizing fermentation conditions such as temperature, pH, agitation rate, inoculum size, and xylose concentration is essential for efficient xylitol production.

Additionally, selecting suitable substrates and microbial strains can further enhance yields. These optimized conditions contribute to more sustainable and cost-effective xylitol production processes.

I. pH Optimization in Xylitol Production

The pH of the fermentation medium is one of the most critical factors influencing xylitol production. It affects the enzyme activity, transport of substrates, yeast metabolism, and overall cell growth, thereby directly impacting the yield of xylitol. Each microbial strain has its own optimal pH range for maximum productivity.

i) Role of pH in Xylitol Production

Enzyme Activity: The key enzyme responsible for converting xylose to xylitol is xylose reductase. Its activity is highly pH-dependent. Too low or too high pH can denature the enzyme or reduce its affinity for xylose, resulting in decreased xylitol yield.

Cell Membrane Transport: pH influences the transport of xylose into the yeast cells. An optimal pH maintains membrane integrity, ensuring efficient substrate uptake.

Metabolic Pathway Regulation: pH changes can shift the metabolic flux of xylose, leading either to xylitol production or further oxidation to xylulose and then into the pentose phosphate pathway. Maintaining optimal pH favors xylitol accumulation rather than complete metabolism to other by- products.

Table No. 3 : Optimal pH Ranges for Different Yeasts for xylitol production

Sr. no.	Yeast Strain	Optimal pH	Reference
1.	*Candida boidinii*	6.0	Jiang et al., 2009
2.	*Candida parapsilosis*	5.0	Chaudhary et al., 2015
3.	*Candida guilliermondii*	4.5	Chaudhary et al., 2015
4.	*Hansenula anomala*	4.5	Chaudhary et al., 2015

Research shows that most yeasts prefer a slightly acidic environment for xylitol production: As seen, the slightly acidic pH (4.5–6.0) favors xylitol accumulation.

Neutral or alkaline conditions reduce xylitol yield because the enzymes responsible for xylose reduction are less active, and yeast cells may experience stress.

iii) pH Control During Fermentation

During fermentation, xylitol production can lower the pH of the medium due to the release of acids or metabolic by-products. Continuous monitoring and adjustment using buffers (like phosphate or citrate buffer) or automated pH control in bioreactors ensures the pH stays within the optimal range. For instance, *Candida tropicalis* showed higher xylitol yield when the pH was maintained at 5.5 throughout the fermentation rather than allowing it to drop naturally.

iv) pH Interaction with Other Parameters

Aeration and pH: High aeration can sometimes raise pH due to CO_2 stripping, affecting xylitol yield. Temperature and pH: Enzyme activity is co-dependent on both pH and temperature, meaning suboptimal pH can reduce the effect of temperature optimization.

v) Practical Implications

Optimizing pH is strain-specific; thus, preliminary experiments with a small-scale shake flask culture are recommended. Slight deviations from optimal pH can significantly reduce xylitol yield. For example, in *Candida boidinii*, reducing pH from 6.0 to 4.0 decreased xylitol yield by almost 30%.

II. Temperature Optimization in Xylitol Production

Temperature is a critical factor affecting microbial growth, enzyme activity, and overall metabolic rate, which directly influences xylitol production. Both too low and too high temperatures can reduce yield, as they impact yeast viability and enzyme kinetics.

i) Role of Temperature in Xylitol Production

Enzyme Activity: The key enzyme in xylitol synthesis, xylose reductase, has an

optimal temperature range. Deviating from this range can decrease its activity, slowing the conversion of xylose to xylitol.

Yeast Metabolism: Temperature affects the general metabolic rate of yeasts. Higher temperatures can speed up metabolism but may also stress the cells, leading to lower xylitol accumulation. Lower temperatures slow metabolism, reducing productivity.

Membrane Fluidity: Temperature influences cell membrane properties, which can affect substrate uptake (xylose) and product export (xylitol).

Table No. 4 : Optimal Temperature Ranges for Different Yeasts for xylitol production

Sr.no.	Yeast Strain	Optimal Temperature	Reference
1.	*Candida tropicalis*	29–34°C	Frontiers, 2022
2.	*Candida peltata*	28°C	Jiang et al., 2009
3.	*Candida guilliermondii*	30°C	Chaudhary et al., 2015
4.	*Hansenula anomala*	30–32°C	Chaudhary et al., 2015

Most yeasts prefer a moderate temperature range of 28–35°C for optimal xylitol production. Temperature above 35°C often reduce yeast viability and enzyme stability.

iii) Impact of Temperature on Xylitol Yield

Maintaining the optimal temperature ensures maximum xylose reductase activity, promoting efficient conversion to xylitol. Deviations from optimal temperature can shift xylose metabolism toward by-products like xylulose or ethanol instead of xylitol.

Example: In *Candida tropicalis*, lowering the temperature from 32°C to 25°C reduced xylitol yield by nearly 20%, while increasing it to 37°C caused a 30% drop due to enzyme denaturation.

iv) Temperature Control During Fermentation

Shake Flask Cultures: Temperature is usually maintained using incubator shakers, ensuring uniform growth and xylitol synthesis.

Bioreactors: Advanced fermenters use automated temperature control systems to maintain the optimal range.

Combined Effects: Temperature interacts with pH and aeration. For instance, at higher temperatures, pH may need slight adjustment to maintain enzyme activity.

v) Practical Considerations

Each yeast strain should be tested in preliminary experiments to determine its strain- specific optimal temperature. Industrial fermentations must monitor temperature continuously, as even slight deviations can significantly impact productivity and yield. Temperature optimization is often combined with other factors like pH, substrate concentration, and aeration to achieve maximum xylitol output.

III. Substrate Concentration Optimization

The concentration of the sugar substrate, typically xylose or xylose-rich hydrolysates, plays a critical role in determining xylitol yield and productivity. Both too low and too high concentrations can negatively affect the process.

i) Role of Substrate Concentration Low Substrate Concentration:

- Limits the availability of xylose for the yeast.
- Slows down xylitol production rate due to substrate limitation.

High Substrate Concentration:

- Can cause substrate inhibition, reducing cell growth and enzyme activity.
- High osmotic pressure may stress yeast cells, decreasing productivity.

Optimal Concentration:

- Balances sufficient substrate for conversion without causing inhibition or osmotic stress.

Table No. 5 : Optimal Substrate Levels for Yeasts for xylitol production

Sr. no.	Yeast Strain	Optimal Xylose Concentration	Reference
1.	*Candida peltata*	50 g/L	Jiang et al., 2009
2.	*Candida boidinii*	71.1 g/L	MDPI, 2021
3.	*Candida tropicalis*	50–70 g/L	Frontiers, 2022

Initial xylose concentration around 50–70 g/L is generally favorable for high xylitol yield. Concentrations above 100 g/L often reduce xylitol yield due to osmotic stress and inhibition.

iii) Practical Considerations

Use controlled feeding strategies (fed-batch) to maintain optimal xylose levels and avoid inhibition. Monitor residual xylose; accumulation of unconverted sugar can indicate suboptimal conditions or enzyme limitation. Substrate optimization is often coupled with pH, temperature, and aeration adjustments for best results.

IV. Aeration Optimization

Aeration (oxygen supply) is crucial because xylitol production is an aerobic or microaerophilic process for most yeasts. Proper oxygen levels ensure high xylitol yield.

i) Role of Aeration

Oxygen Requirement:

Xylitol production requires some oxygen for cofactor regeneration (NADPH/NADH balance). Insufficient oxygen reduces xylose reductase activity and slows xylitol formation.

Excessive Aeration:

- Can lead to over-oxidation of xylitol to xylulose, reducing the final yield.
- May increase by-product formation (ethanol, CO_2).

Optimal Aeration:

- Maintains a microaerobic condition to favor xylitol accumulation

Table No. 6 : Optimal Aeration for Yeasts

Sr. no.	Yeast Strain	Oxygen Transfer Rate (OTR)	Reference
1.	*Candida parapsilosis*	6.1 mmol/L·h	Chaudhary et al., 2015
2.	*Candida guilliermondii*	5.7 mmol/L·h	Chaudhary et al., 2015

Optimal OTR ensures high xylitol yield without promoting further oxidation. Microaerophilic conditions are preferred in bioreactors for large-scale xylitol production.

iii) Practical Considerations

- In shake flasks, aeration is influenced by shaking speed and flask-to-medium ratio.
- In bioreactors, dissolved oxygen (DO) is monitored and maintained via controlled aeration and agitation.
- Aeration interacts with pH and temperature, so optimization requires approach

7. Xylitol Recovery and Production Efficiency

The recovery and production efficiency of xylitol are vital factors influencing the overall success and commercial viability of xylitol bioproduction. It involves multiple downstream processing stages designed to separate, purify, and crystallize xylitol from complex fermentation broth. Each step plays a critical role in maximizing yield and ensuring high purity suitable for industrial applications (Parajó et al., 1998).

I. Cell Separation

After fermentation, the first step in recovery is cell separation, where microbial biomass and other suspended solids are removed from the fermentation broth. This can be achieved using centrifugation, microfiltration, or membrane-based systems. Efficient cell separation prevents downstream contamination and facilitates further purification (Mussatto & Roberto, 2002). Centrifugation is particularly effective due to its ability to

rapidly separate dense yeast cells, while membrane filtration offers continuous processing and scalability (Kumar et al., 2018).

II. Clarification and Decolorization

The clarified filtrate often contains proteins, pigments, and other soluble impurities that must be removed. Clarification is performed using chemical coagulants or heat treatment, followed by decolorization with activated charcoal or ion-exchange resins. These methods enhance the color, clarity, and purity of the xylitol solution (Dasgupta et al., 2017).Activated carbon adsorption efficiently removes colored compounds, while ion-exchange resins help eliminate charged impurities and organic acids (Silva & Roberto, 2001).

III. Concentration of Xylitol Solution

After purification, the xylitol-rich filtrate is subjected to vacuum evaporation or reverse osmosis to concentrate the solution. This reduces the water content and increases the xylitol concentration before crystallization. The use of vacuum reduces energy consumption and prevents thermal degradation of xylitol, maintaining product quality (Saha, 2003). Optimization of temperature and pressure during this step enhances concentration efficiency and reduces process cost.

IV. Crystallization

Crystallization is the most important step for obtaining pure solid xylitol. Controlled cooling or addition of organic solvents (like ethanol) induces crystal formation from the concentrated solution. Parameters such as temperature, cooling rate, and seed crystal size influence crystal purity and yield (Parajó et al., 1998). Proper crystallization conditions yield xylitol of >99% purity, suitable for food and pharmaceutical use.

V. Drying and Final Recovery

The crystallized xylitol is then separated from the mother liquor and dried using hot air or vacuum dryers. Drying reduces residual moisture and improves the shelf stability of the product (Chandel et al., 2011). The final product is typically obtained as colorless, odorless, crystalline powder with high sweetness and cooling effect similar

to sucrose.

VI. Factors Affecting Production Efficiency

The efficiency of xylitol production depends on both bioconversion parameters and microbial strain performance. Factors such as substrate concentration, pH, aeration, temperature, and nitrogen source directly affect xylose-to-xylitol conversion. Yield values between 0.60–0.85 g xylitol/g xylose have been reported under optimized fermentation conditions (Saha & Bothast,1997). Genetic modification and cell immobilization techniques have further enhanced productivity and tolerance to inhibitors (Chandel et al., 2011).

VII. Process Optimization for Higher Efficiency

Recent developments in bioprocess optimization have improved overall recovery and yield. These include

- Immobilized cell reactors for continuous xylitol production
- Membrane-based separation for simultaneous fermentation and purification
- Use of low-cost substrates like bagasse hydrolysate to reduce raw material cost (Kumar et al., 2018)

Such advancements have made xylitol production more sustainable and economically feasible on an industrial scale (Dasgupta et al., 2017).

VIII. Overall Process Efficiency

The integration of optimized fermentation with efficient recovery techniques leads to higher overall process efficiency. Combining biotechnological advancements with modern downstream processes ensures improved yield, purity, and cost reduction. Continuous research into eco-friendly and scalable technologies promises to make xylitol production more competitive with conventional chemical synthesis (Silva & Roberto, 2001).

- **Summary of Key Optimization Strategies for Xylitol Production**

Based on the literature reviewed, the following table and list summarize the most effective strategies for optimizing xylitol yield and productivity.

Table No.7 - Summary of Key Optimization Strategies for Xylitol Production

Sr. No.	Strategy	Optimal Condition/Range	Purpose
1.	pH Control	4.5-6.0	Maintain enzyme stability and growth
2.	Temperature	28◦C - 35◦C	Ensure metabolic rate & yeast viability
3.	Xylose Conc.	50-70 g/L	Prevent osmotic stress & substrate inhibition.
4.	Aeration (OTR)	2.8 – 6.1 mmoL/L.h	Balance NADPH/NADH for cofactor conversion

Fed – batch Fermentation : Implementing controlled feeding of xylose to prevent substrate inhibition.

Cell Immobilization : Using immobilized cell reactors to allow for continuous production and cell recycling.

Integrated Downstream Processing : Coupling fermentation with membrane – based separation to improve overall effieciency.

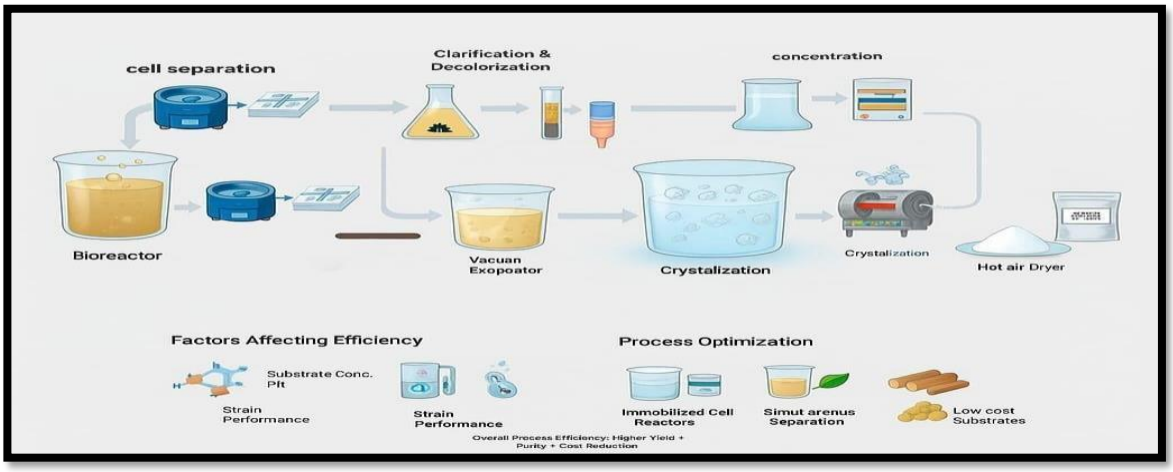


Figure no. 2 : Xylitol Recovery and Production Efficiency

8. Xylitol: Health, Industry, and Sustainability

I. Tooth Guardian: Oral Health Champion

Xylitol protects teeth by inhibiting *Streptococcus mutans*, reducing plaque formation and acid production. Its frequent use in gums, toothpaste, and oral care products helps prevent cavities and supports enamel remineralization. (Maguire et al., 2014).

II. Sugar Smart: Diabetic-Friendly Sweetener

With a very low glycemic index (~7), xylitol provides sweetness without causing rapid blood sugar spikes. This makes it ideal for people with diabetes or those managing blood glucose levels. (Umai et al., 2022). Frontiers in Sustainability

III. Bone Buddy: Calcium Helper

Xylitol can enhance calcium absorption in the intestine and reduce calcium excretion in urine. This can help maintain bone density and potentially reduce the risk of osteoporosis. (Healthline, 2023)

IV. Importance of Xylitol in Industry

Xylitol has gained considerable industrial importance due to its unique chemical properties, functional versatility, and health-promoting characteristics. It serves as a multi-functional ingredient in the food, pharmaceutical, cosmetic, and chemical industries. The demand for xylitol continues to rise globally as consumers increasingly prefer natural, low-calorie, and non- cariogenic sweeteners over synthetic sugars. Its industrial relevance extends beyond sweetening functions to include stabilizing, humectant, and texturizing roles in various formulations (Ur- Rehman et al., 2015).

i) Food Industry

The food industry is the largest consumer of xylitol, utilizing it as a low-calorie sweetener and functional additive. Xylitol has a sweetness intensity comparable to that of sucrose but provides 40% fewer calories (2.4 kcal/g), making it ideal for dietary and

diabetic food formulations (Dasgupta et al., 2017). Its low glycemic index ensures minimal impact on blood glucose levels, thus making it a suitable ingredient for sugar-free and health-oriented foods.

Xylitol's hygroscopic nature allows it to retain moisture, preventing products such as cakes, biscuits, and candies from drying out during storage. Moreover, it exhibits a cooling effect upon dissolution, which enhances the sensory quality of confectionery items like mints, chewing gums, and chocolates (Mäkinen, 2010). In addition, xylitol is heat-stable and can be used in baking and cooking without undergoing caramelization, unlike regular sugar. This property broadens its application range in baked goods, dairy products, and beverages. Thus, the food industry values xylitol not only for its sweetening ability but also for its role in improving texture, shelf life, and palatability in various food products.

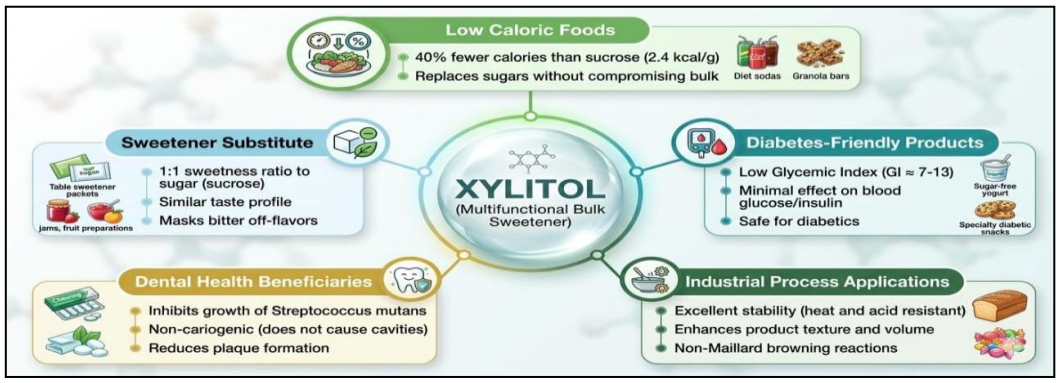


Figure No. 3. Xylitol Use in Food Industry

ii) Pharmaceutical Industry

In the pharmaceutical sector, xylitol is widely used as a non-cariogenic excipient in tablets, syrups, and chewable formulations. Its pleasant taste and non-fermentable nature make it an ideal sugar substitute for oral medications, cough syrups, vitamin tablets, and throat lozenges. Xylitol also acts as a stabilizer and moisture-retaining agent, ensuring consistent drug texture and preventing crystallization (Ur-Rehman et al., 2015). Xylitol-based oral formulations are beneficial for diabetic patients, as they do not interfere with blood glucose regulation. Moreover, due to its osmotic properties, xylitol solutions are used in intravenous therapy for energy supplementation and rehydration in

clinical settings (Dasgupta et al., 2017).It also plays a preventive role against respiratory and ear infections, as it inhibits bacterial adherence to mucosal membranes, particularly in *Streptococcus pneumoniae* and Haemophilus influenzae infections (Mäkinen & Söderling, 2010).

Hence, the pharmaceutical industry's reliance on xylitol extends from formulation enhancement to therapeutic applications, reflecting its biochemical compatibility and functional importance.

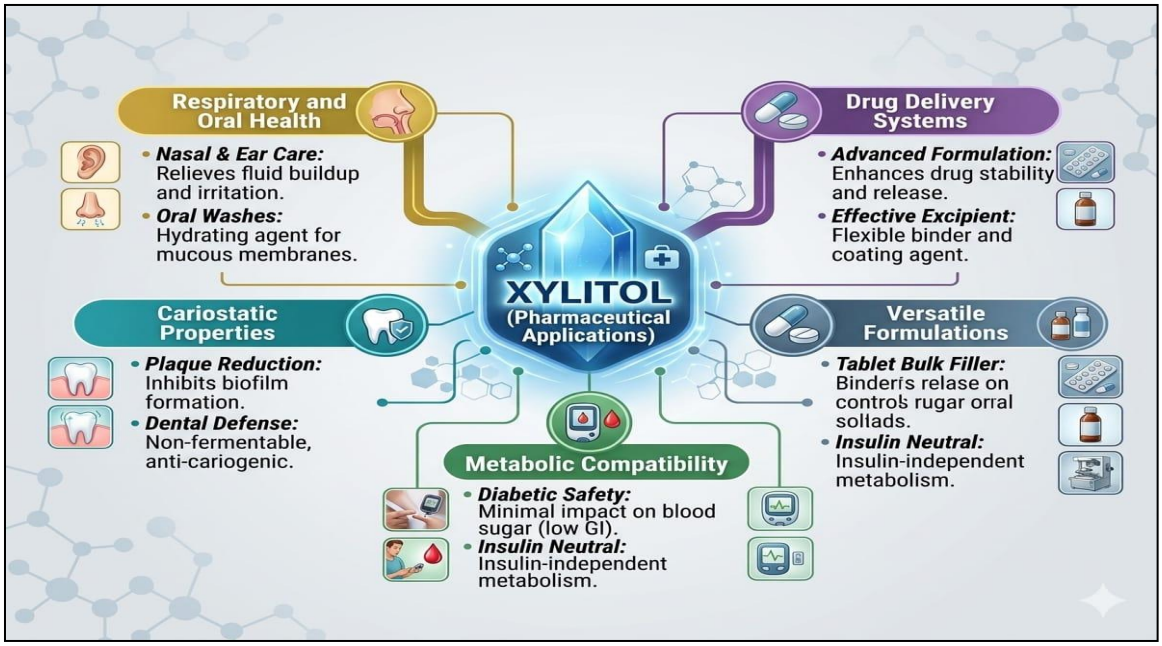


Figure no. 4. – Xylitol Use in Pharmaceutical Industry

iii) Cosmetic and Personal Care Industry

In the cosmetic and personal care industry, xylitol is valued for its moisturizing, conditioning, and humectant properties. It attracts and retains water, making it a key ingredient in skin creams, lotions, shampoos, and toothpaste. Xylitol-based formulations enhance skin hydration and improve barrier function by stimulating natural ceramide synthesis in the skin (Sene et al., 2011). In oral care cosmetics, xylitol helps maintain oral hygiene by inhibiting the growth of plaque-forming bacteria, reducing dryness, and refreshing breath. Its non-fermentable nature ensures that it does not contribute to acid formation in the mouth, thereby preventing dental erosion. Additionally, xylitol is used in lip balms and hair care products for its ability to smoothen texture and prevent

moisture loss, enhancing the overall sensory appeal of cosmetic goods.

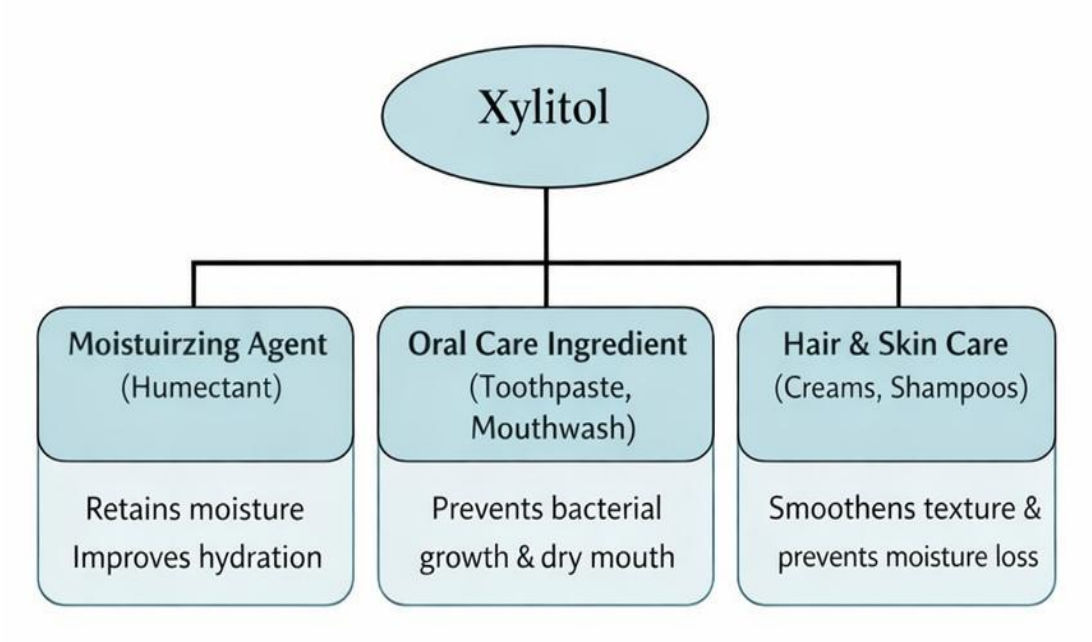


Fig. No. 5 – Xylitol in Cosmetic and Personal Care Industry

iv) Chemical and Biotechnological Industry

The chemical industry utilizes xylitol as an intermediate compound for synthesizing a variety of value- added chemicals such as xylitol esters, xylitol phosphate, and glycosides. It serves as a platform chemical in the biochemical and polymer industries, where it can be converted into xylitol-based surfactants, biodegradable plastics, and emulsifiers (Dasgupta et al., 2017). Furthermore, xylitol is used as a precursor in the synthesis of xylitol-derived polyesters and resins, which have potential applications in coatings and packaging materials. With the growing demand for bio-based materials, xylitol has become an important raw material in the biorefinery concept, where renewable resources like sugarcane bagasse are used to produce not only xylitol but also other biochemicals and biofuels. Its use in biotechnology extends to fermentation industries, where microbial strains such as *Candida tropicalis*, *Debaryomyces hansenii*, and *Candida guilliermondii* convert D-xylose (from lignocellulosic biomass) into xylitol efficiently.

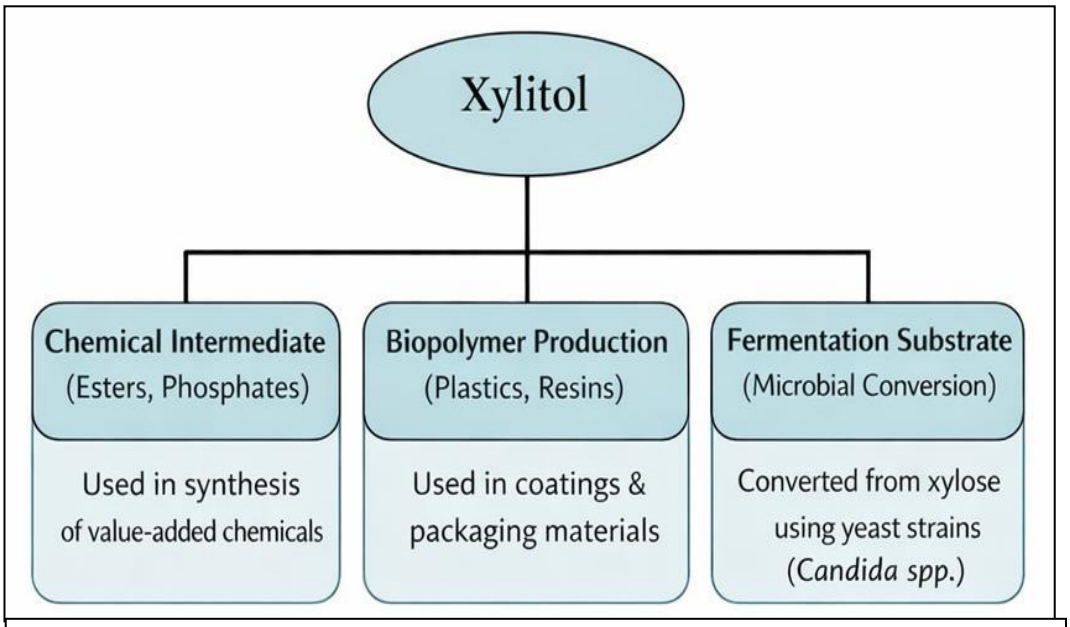


Fig. No. 6 – Xylitol in Chemical and Biotechnological Industry

The present study focuses on the production of xylitol from sugarcane bagasse through microbial fermentation. Sugarcane bagasse is an abundant agro-industrial by-product generated from the sugar industry and is mainly composed of lignocellulosic components such as cellulose, hemicellulose and lignin. Among these components, hemicellulose contains a significant amount of xylose which can be utilized as a substrate for the microbial production of xylitol. The utilization of such agricultural residues not only provides a low-cost raw material for biotechnological processes but also contributes to effective waste management and environmental sustainability.

In the present work, yeast strains were isolated from natural sources such as spoiled fruits including apple, orange and banana, and these isolates were identified as *Candida* species based on their morphological and biochemical characteristics. In addition, organism isolated from sugarcane bagasse was identified as *Debaryomyces hansenii* based on cultural, morphological and biochemical study. The isolated microorganisms were further used for fermentation studies.

Prior to fermentation, sugarcane bagasse was subjected to alkaline pretreatment followed by acid hydrolysis in order to break down the complex lignocellulosic structure and release fermentable sugars, particularly xylose. The obtained bagasse hydrolysate served as a suitable medium for microbial growth and fermentation.

The selected yeast strains were inoculated into the fermentation medium where they utilize the available xylose and converted it into xylitol during the fermentation process.

After completion of fermentation, the product was subjected to further analysis and confirmation. The formation of crystals indicated the recovery of the product, and further confirmation of xylitol production was carried out using Thin Layer Chromatography (TLC). The results obtained in this study demonstrate that sugarcane bagasse can serve as a potential and economical substrate for the microbial production of xylitol. Thus, the present study highlights the importance of converting agro-industrial waste into valuable products through sustainable biotechnological approaches.

Objectives.

Objectives of the Study

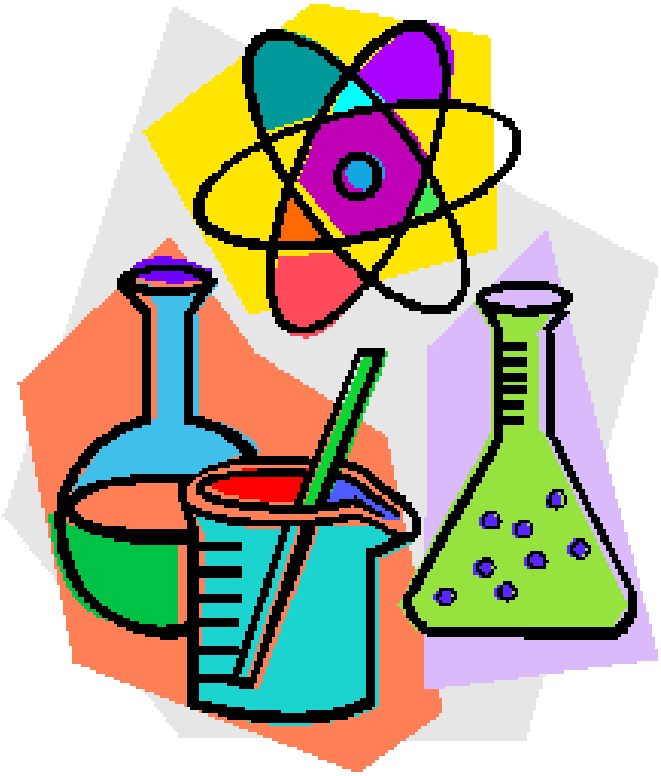
1. To isolate, characterize and identify efficient yeast strains which are capable of converting xylose into xylitol.

2. To utilize sugarcane bagasse as a cost-effective and renewable lignocellulosic substrate for the production of xylitol.

3. To prepare and optimize the hydrolysate obtained from sugarcane bagasse for maximum xylose availability.

4. To evaluate the efficiency of selected yeast strains in converting xylose to xylitol under controlled conditions.

5. To analyze and confirm xylitol production using techniques such as paper chromatography and calculation of Rf values.



MATERIAL AND METHODOLOGY

MATERIALS AND METHODOLOGY

1. Collection of Sample

Rotten fruit samples such as sweet lime, pomegranate, apple and banana along with jaggery and raw sugarcane bagasse were collected for isolation of yeast. Fruits and sugary materials are known to contain naturally occurring yeast because they provide suitable nutrients for their growth.

The fruit samples and jaggery were collected in clean containers and brought to the laboratory for further processing. Raw sugarcane bagasse used in this study was obtained from a local sugar processing industry in Kolhapur. Approximately 1–2 kg of bagasse was collected and transported to the laboratory.

2. Preparation of Sample Suspension

Samples were prepared for isolate microorganisms from the collected fruits such as sweet lime, pomegranate, apple and banana along with jaggery and raw sugarcane bagasse. Small pieces of rotten fruits were cut using a sterile blade and transferred into sterile test tubes containing 10 ml of sterile distilled water. The tubes were gently shaken so that microorganisms present on the fruit surface could mix with the water. This mixture formed the microbial suspension, which was used for yeast isolation.

3. Isolation of Yeast From Rotten Fruit Samples

In this study, yeast was used because many yeast species are able to ferment sugars and convert them into valuable products. Certain yeasts can utilize xylose and convert it into xylitol through metabolic processes and they are also easy to maintain and grow in laboratory conditions. (Parajo et al., 1998)

The microbial suspension prepared from rotten fruits and jaggery was used for isolation of yeast isolates. This yeast is selected because it has strong ability to convert xylose into xylitol during fermentation and is widely used in research studies for microbial xylitol production. This yeast possesses enzymes involved in pentose metabolism.

4. Preparation of Culture PDA Medium

The detailed quantitative formulation of the Potato Dextrose Agar (PDA).

Table No. 8 – Composition of PDA Medium

Sr. No.	Components	Quantity
1.	Potato Dextrose Powder	2 g
2.	Agar	2.5 g
3.	Distilled Water	Up to 100 ml

A loopful of the suspension was taken with a sterile inoculating loop and streaked on Potato Dextrose Agar (PDA) plates using the streak plate method. The plates were then incubated at room temperature for 24–48 hours.

After incubation, colonies showing yeast-like growth were selected and subcultured to obtain pure cultures. These purified cultures were transferred onto PDA agar slants and stored for further analysis (Atlas,2010)

- **Isolation of yeast From Sugarcane Bagasse**

Isolation of yeast isolates was carried out using raw sugarcane bagasse small pieces of bagasse were transferred into test tubes containing distilled water. These tubes were heated at 60°C for about 10 minutes to help release microorganisms attached to the bagasse fibers.

A loopful of this suspension was then streaked onto YPX agar medium containing NaCl, which supports the growth of halotolerant yeast. The plates were incubated at room temperature and the colonies obtained were transferred onto YPX agar slants for maintenance of the culture (Kurtzman et al., 2011).

- **Composition of Yeast Producing Xylitol (YPX) Medium**

Yeast Extract Peptone Xylose (YPX) agar medium was used in the present study for the cultivation of yeast strains capable of metabolizing xylose. This medium provides a balanced nutritional environment for yeast growth and allows selective growth of

microorganisms capable of utilizing pentose sugars.

In addition to the standard components of YPX medium, sodium chloride was added to facilitate the selective growth of yeast isolate*s*, which is known to be a halotolerant yeast species.

Table no.9.- Composition of YPX Medium (YPX)

Sr. No.	Components	Quantity
1.	Yeast extract	1 g
2.	Peptone	2 g
3.	Xylose	2 g
4.	NaCl	5 g
5.	Agar	1.5 g
6.	Distilled water	100 ml
7.	pH	5.5 – 6.0

Gram Staining & Motility

Gram staining was performed to observe the microscopic morphology of the yeast isolates. A small amount of culture was placed on a clean glass slide and a smear was prepared. The smear was heat fixed and stained with crystal violet, followed by Gram's iodine. The slide was then decolorized with alcohol and counterstained with safranin.

Finally, the slide was observed under a microscope using the oil immersion objective (Prescott et al., 2017). Motility of the yeast isolates was examined using the hanging drop method. A drop of yeast suspension was placed on a cover slip and inverted over a cavity slide. The preparation was observed under the microscope to determine whether the cells showed any motility (Aneja, 2012).

- **Biochemical Test**

Sugar fermentation tests were carried out to determine the ability of yeast isolates to ferment different carbohydrates.

The fermentation medium was prepared separately for each sugar. The sugars used for the test included glucose, xylose, galactose, sucrose, maltose, mannitol and lactose.

The yeast cultures were inoculated into fermentation tubes containing the respective sugars and incubated at 30°C for 48–72 hours (Pelczar et al., 2010)

Table no.10.- Composition of Peptone Water

Sr.no.	Components	Quantity
1.	Peptone	1 g
2.	NaCl	0.5 g
3.	Respective sugar	1 g
4.	Phenol Red Indicator	1 ml
5.	Distilled water	100 ml

- **Yeast Isolate From Sweet Lime**

The yeast isolate recovered from sweet lime was inoculated into peptone water containing different sugars and Phenol Red as a pH indicator. Each tube included an inverted Durham tube to capture gas. After incubation, fermentation was confirmed by the medium turning yellow (acid production) and the presence of gas bubbles in the Durham tubes

- **Yeast Isolate From Pomegranate**

The pomegranate isolate was subjected to sugar fermentation testing using a peptone water base. The metabolic activity was monitored via Phenol Red, which indicated acidification of the broth. The accumulation of CO_2 within the Durham tubes was recorded alongside these color changes to determine the isolate's fermentative capacity.

- **Yeast Isolate from Apple**

For the apple sample, the yeast isolate was evaluated for carbohydrate utilization. The isolate was introduced into fermentation tubes containing various sugars, Phenol Red, and Durham tubes. Post-incubation, the tubes were inspected for a shift from red to yellow and for gas displacement, indicating successful fermentation.

- **Yeast Isolate from Banana**

The banana isolate was inoculated into a peptone water fermentation medium. To detect the breakdown of specific sugars, Phenol Red was utilized to signal acid formation, while Durham tubes were employed to detect gas evolution. The results were recorded after observing the tubes under suitable incubation conditions.

- **Yeast Isolate from Jaggery**

The yeast isolate from jaggery was analyzed using a series of fermentation broths. Each setup consisted of peptone water, a specific sugar, and a Phenol Red indicator. The production of gas, trapped by the internal Durham tube, and the resulting acidity were documented as positive markers for fermentation.

- **Yeast Isolate from Orange**

Following the standard procedure, the orange isolate was tested in peptone water fermentation tubes. A loopful of culture was added to the media containing Phenol Red. The reaction was considered positive if the indicator turned yellow and gas was observed in the Durham tube after the required incubation period.

- **Yeast Isolate from Guava**

The guava-derived yeast was tested for its ability to ferment various carbohydrates in a peptone water medium. The fermentation reactions indicated by the acidification of the Phenol Red and the collection of gas in the Durham tubes were recorded to establish a biochemical profile for this isolate.

- **Yeast Isolate from Molasses**

After serial dilution and isolation, the molasses yeast was inoculated into fermentation tubes. These tubes contained peptone water, the target sugar, and Phenol Red. We monitored the tubes for gas collection in the Durham tubes and a color transition to yellow, signifying active fermentation of the substrate.

- **Yeast Isolate from Sugarcane Bagasse**

The sugarcane bagasse isolate was incubated at 30°C for 48–72 hours in peptone water sugar broths. The setup utilized Phenol Red to detect pH changes and Durham tubes to identify gas production. The final observations were recorded for all sugars tested.

5. Preparation of Sugarcane Bagasse

Sugarcane bagasse collected from the sugar industry was used as the raw material for xylitol production. Before using it for further experiments, the bagasse was cleaned and prepared properly.

Collection of Sugarcane Bagasse

↓

Washing with Water

(Removal of dust, soil particles and impurities)

↓

Drying in Hot Air Oven (60°C for 4–5 hours)

↓

Cooling of Dried Bagasse

↓

Weighing of Bagasse (Approximately 100 g)

↓

Bagasse Ready for Pretreatment

Flow Chart 2. – Preparation of Sugarcane Bagasse

The initial preparation of sugarcane is important to remove impurities and make the material suitable for further processing. Washing, Drying and Weighing ensures a uniform quantity is used for all experiments, which helps in maintaining consistency in further steps.

6. Alkaline Pretreatment of Bagasse

Pretreatment of lignocellulosic biomass is an important step because it helps in breaking down the complex structure of plant material and improves the availability of cellulose and hemicellulose for hydrolysis.

Weighed Sugarcane Bagasse

↓

Division into Two Portions (50 g each)

↓

Transfer into Conical Flasks

↓

Addition of NaOH Solution

(500 mL distilled water + 5 g NaOH ≈ 1% NaOH)

↓

Heating in Water Bath (80°C for 45 minutes)

↓

Breakdown of Lignin Structure

↓

Washing with Distilled Water (Removal of excess alkali)

↓

Drying of Pretreated Bagasse

↓

Pretreated Bagasse Ready for Acid Hydrolysis

Flow Chart 3 – Alkaline Pretreatment of Sugarcane Bagasse

After pretreatment, the bagasse was removed, washed thoroughly with distilled water to remove excess alkali and then dried before the next step (Sun & Cheng, 2002).

7. Acid Hydrolysis

Acid hydrolysis was performed to release fermentable sugars from the pretreated bagasse. Hydrolysis breaks down the hemicellulose present in lignocellulosic biomass into simple sugars such as xylose.

Pretreated Sugarcane Bagasse

↓

Preparation of Dilute Acid Solution

(32 mL concentrated HCl + 500 mL distilled water)

↓

Addition of Acid Solution to Pretreated Bagasse

↓

Heating in Pressure Cooker (Until one whistle)

↓

Further Heating (Low flame for 30 minutes)

↓

Hydrolysis of Biomass (Hemicellulose breaks down to sugars such as xylose)

↓

Cooling to Room Temperature

↓

Hydrolysate Ready for Filtration

Flow Chart 4.- Acid Hydrolysis of Pretreated Sugarcane Bagasse

Acid hydrolysis is performed to convert complex carbohydrates present in hemicellulose into simple sugars such as xylose. The use of dilute acid helps in breaking down the biomass structure efficiently. Heating under pressure accelerates the hydrolysis process, ensuring maximum sugar release. Cooling is necessary before further handling, as high temperatures may affect subsequent steps. (Taherzadeh & Karimi, 2007)

8. Filtration of Hydrolysate

After hydrolysis, the mixture contained both liquid hydrolysate and solid residues of bagasse.In order to separate these components, filtration was carried out.

Hydrolyzed Bagasse Mixture (Liquid hydrolysate + solid residues)

↓

Filtration Using Whatman Filter Paper

↓

Solid Residues Retained on Filter Paper

↓

Liquid Hydrolysate Collected

↓

Filtrate Containing Soluble Sugars

Flow Chart 5.- Filtration of Hydrolysate

Filtration is carried out to separate liquid hydrolysate from the solid residues of bagasse. The liquid portion contains soluble sugars released during hydrolysis which are essential for fermentation. Removing solid particles ensures a clear medium, which supports better microbial growth and prevents interference in downstream processes.

9. Detoxification of Hydrolysate

During acid hydrolysis, certain compounds may be formed that can inhibit microbial fermentation. Therefore, detoxification of the hydrolysate is necessary before using it for fermentation.

Hydrolysate Obtained After Filtration

↓

Addition of Calcium Hydroxide [$Ca(OH)_2$]

↓

Adjustment of pH to ~10

↓

Heating at 60°C for 30 minutes

↓

Precipitation of Inhibitory Compounds

↓

Filtration to Remove Precipitate

↓

Adjustment of pH to ~6

↓

Detoxified Hydrolysate Ready for Fermentation

Flow Chart 5.- Detoxification of Hydrolysate

Detoxification using calcium hydroxide helps in neutralizing these inhibitors and adjusting the pH to a suitable level for yeast growth. Detoxification using calcium hydroxide helps in neutralizing these inhibitors and adjusting the pH to a suitable level for yeast growth. Heating promotes precipitation of unwanted compounds, filtration removes these precipitates, resulting in a cleaner and more suitable fermentation medium. (Palmqvist & Hahn-Hägerdal, 2000).

10. Fermentation for Xylitol Production

The detoxified hydrolysate obtained after pretreatment and hydrolysis was used as the fermentation medium for xylitol production. (Parajó et al., 1998).

Detoxified Hydrolysate

↓

Inoculation of Yeast Culture (Yeast isolates)

↓

Incubation at 30°C (48–72 hours)

↓

Fermentation Process (Sugars converted into xylitol)

↓

Fermented Broth

Flow Chart 6.- Fermentation for Xylitol Production

11.Detection of Xylitol

11.1 Paper Chromatography

Paper chromatography was used to detect the presence of xylitol in the fermentation broth. Chromatography is a technique used to separate different compounds present in a mixture based on their movement along a stationary phase with the help of a solvent. (Harborne, 1998)

Fermented Broth Sample

↓

Spotting Sample on Chromatography Paper

↓

Spotting Standard Xylitol Solution

↓

Placement in Solvent Chamber

↓

Solvent Movement Along Paper

↓

Separation of Compounds

↓

Comparison of Sample Spot with Standard

↓

Detection of Xylitol

Flow Chart 7.- Detection of Xylitol by Paper Chromatography

11.2 Estimation of Xylitol

After confirming the presence of xylitol, the yield of xylitol produced during fermentation can be estimated.

The yield is generally calculated by comparing the amount of xylitol produced with the amount of substrate used.

The yield obtained in the study was 4.5 g/L of xylitol from sugarcane bagasse.

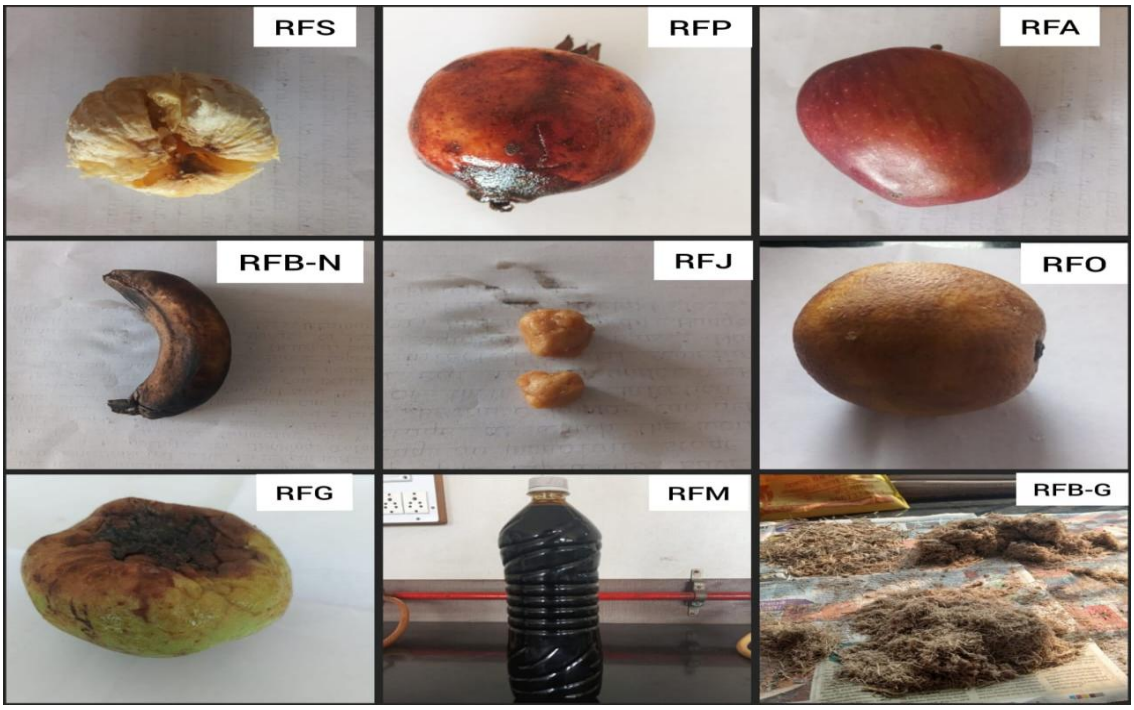

Results

RESULT

1. Collection of Samples

A total of 50 diverse natural samples were initially collected from local markets and environments in Kolhapur to ensure a broad scope for yeast isolation. These included various sugar-rich substrates such as fruits at different stages of decay, industrial byproducts, and traditional sweeteners. Following a preliminary screening for visible microbial activity, 9 representative samples were selected for detailed microbiological analysis and xylitol production studies.

Rotten fruits such as sweet lime, pomegranate, apple, and banana were chosen as primary substrates due to their natural ability to support yeast fermentation. Additionally, jaggery and molasses were included as concentrated sugar sources, while raw sugarcane bagasse was obtained from a local sugar industry to serve as lignocellulosic biomass



Fig, No.6 –Collection of Samples for Yeast Isolation

Figure 6 displays the nine primary samples such as : RFS (Sweet lime's rotten fruit), RFP (Pomegranate's rotten fruit sample), RFA (Apple's rotten fruit), RFB-N (Banana's rotten fruit), RFJ (Jaggery sample), RFO (Orange,s rotten fruit), RFG (Guava,s rotten fruit), RFM (Molasses) and RFB-G (Sugarcane bagasse).

2. Preparation of Sample Suspension

Microbial suspensions were successfully prepared from all the collected samples including sweet lime, pomegranate, apple, banana, orange, guava, jaggery, molasses and sugarcane bagasse. The suspensions appeared slightly turbid, indicating the presence of microorganisms released from the sample surfaces.These prepared suspensions were further used for isolation of yeast on suitable culture media.

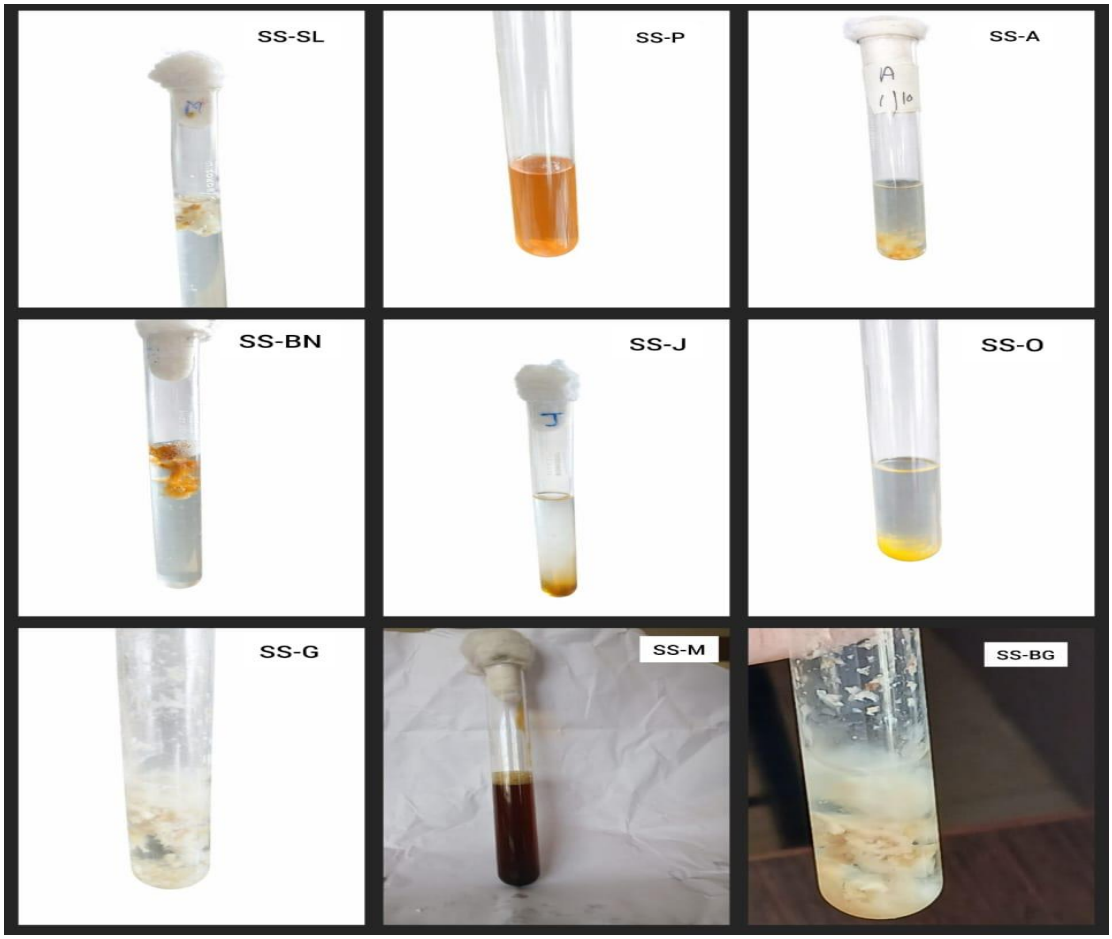


Fig. No.7 – Representative Collection of Sample Suspensions for Yeast Isolation

Figure 7 displays the nine processed suspensions prepared from the primary samples. Each tube contains the extracted microbial environment from: SS-SL (Sweet lime), SS-P (Pomegranate), SS-A (Apple), SS-BN (Banana), SS-J (Jaggery), SS-O (Orange), SS-G (Guava), SS-M (Molasses), and SS-BG (Sugarcane bagasse).

3. Isolation of Yeast From Rotten Fruits And Bagasse

Inoculated on PDA medium for isolation of yeast colonies. After incubation, visible colonies were observed on the plates.

The colonies showed typical yeast characteristics such as creamy white colour, smooth surface and circular colony morphology. These colonies were selected and sub cultured on PDA slants to obtain pure culture for further identification tests.

Similarly, yeast colonies were also observed on YPX agar plates prepared for the isolation of halotolerant yeast species. Selected colonies were transferred to YPX slants for maintenance.

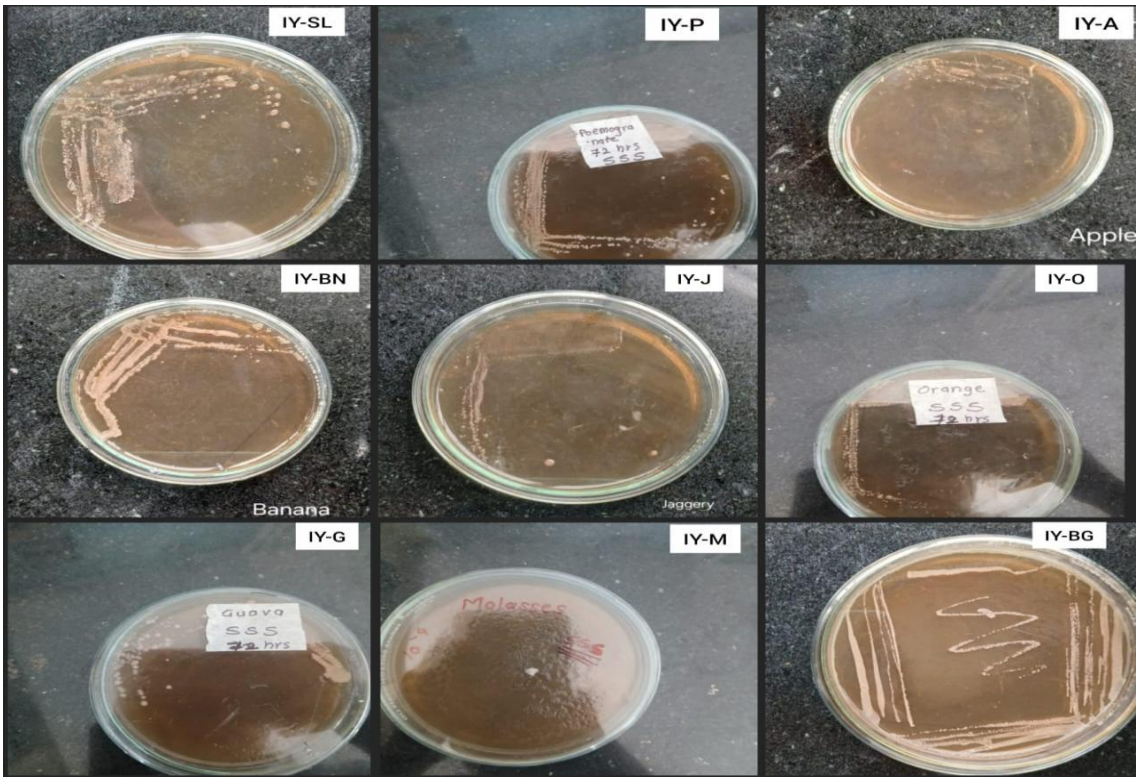


Fig. No.8 – Isolation of Yeast from Various Biomass Samples

Figure 8 illustrates the primary isolation of yeast colonies on agar medium. The isolates are coded as follows: IY-SL (Sweet lime), IY-P (Pomegranate), IY-A (Apple), IY-BN (Banana), IY-J (Jaggery), IY-O (Orange), IY-G (Guava), IY-M (Molasses), and IY-BG (Sugarcane bagasse). Distinct colony morphology characteristic of yeast was observed across all samples.

Preservation of Culture

- The microbial culture used for xylitol production was preserved at low temperature (4°C) to maintain its viability and metabolic activity.
- The culture was stored on appropriate agar slants/broth under refrigerated conditions to prevent contamination and genetic changes.
- Periodic subculturing was performed under aseptic conditions to maintain active growth.
- Before experimental use, a fresh culture was prepared by inoculating the preserved stock into sterile growth medium and incubating under optimal conditions.
- This ensured that the microorganism used in the study was active, pure, and capable of efficient xylitol production..

4. Colony Characteristics of Yeast Isolates.

Colomy characters of well isolated colony of Yeast grown on Potato Dextrose Agar and Yeast Producing Xylitol Media incubated at 37°C and room temperature for 24-48 hours.

Table 11. Colony Characters Of Yeast Isolates From Sweet Lime.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 12. Colony Characters Of Yeast Isolates From Pomegranate.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 13. Colony Characters Of Yeasts Isolates From Apple.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 14. Colony Characters Of Yeast Isolates From Banana.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 15. Colony Characters Of Yeast Isolates From Jaggery.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 16 Colony Characters of Yeast Isolates From Orange.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 17 Colony Characters Of Yeast Isolates From Guava.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 18 colony Characters Of Yeast Isolates From Molasses.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

Table 19 Colony Characters Of Yeast Isolates From Sugarcane Bagasse.

Size	Shape	Colour	Margin
1-2mm	Circular	Creamy White	Entire

Surface	Elevation	Consistency	Opacity
Smooth	Flat	Moist	Opaque

5. Gram Staining and Motility

Gram staining was performed to observe the microscope characteristics of the isolated organisms, under microscopic observation, the cell appeared oval to round in shape and showed Gram-positive staining. The cells were mostly observed singly or in budding form, which is a typical characteristic of yeast cells. The microscopic observation confirmed the presence of yeast-like organism in the isolated culture.

Motility of the yeast isolates was examined using the hanging drop method, under microscopic observation.

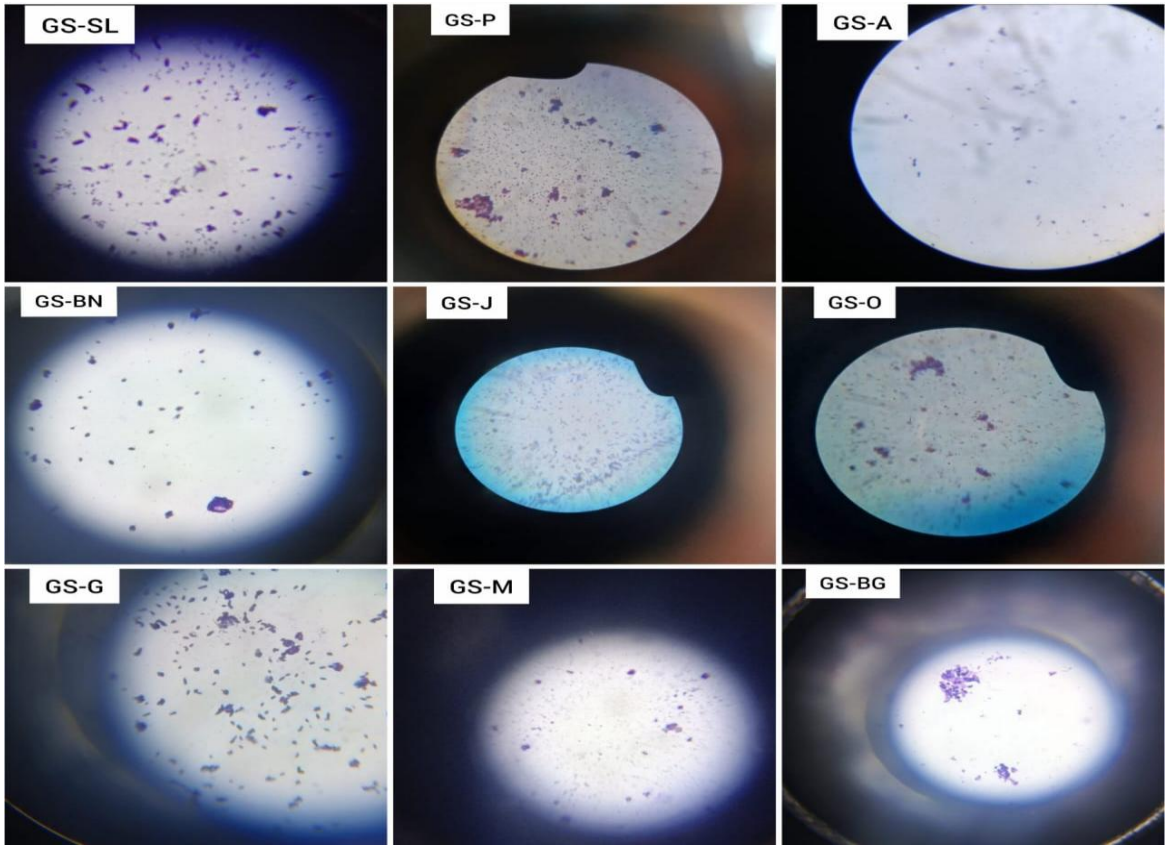


Fig. No.10 - Gram Staining and Microscopic Morphology of Yeast Isolates

Figure 10 illustrates the microscopic characteristics of the nine yeast isolates. The images show Gram-positive, ellipsoidal cells, confirmed as yeast-like organisms. The labels represent: GS-SL (Sweet lime), GS-P (Pomegranate), GS-A (Apple), GS-BN (Banana), GS-J (Jaggery), GS-O (Orange), GS-G (Guava), GS-M (Molasses), and GS-BG (Sugarcane bagasse).

6. Sugar Fermentation Tests of Yeast Isolates.

Sugar fermentation tests were carried out to determine the ability of the yeast isolates to utilize different carbohydrates. The sugars tested included glucose, xylose, galactose, sucrose, maltose, mannitol and lactose.

The results showed that the isolates were able to ferment several sugars such as mannitol, lactose and maltose, while some sugars such as galactose and sucrose showed negative reaction. A slight positive reaction was also observed for xylose fermentation.

Table No.20. Sugar Fermentation Tests of Yeast Isolates.

Sr. No.	Isolate Source	Glucose	Xylose	Galactose	Sucrose	Maltose	Mannitol	Lactose
1.	Sweet Lime	++	+	-	+	++	-	-
2.	Pomegranate	++	+	-	+	+	-	-
3.	Apple	+	+	-	+	+	+	-
4.	Banana	++	+	-	++	+	-	-
5.	Jaggery	++	++	-	++	++	+	-
6.	Orange	+	+	-	+	+	-	-
7.	Guava	+	+	-	+	+	-	-
8.	Molasses	++	++	+	++	++	+	-
9.	Sugarcane Bagasse	++	++	+	++	+	+	-

++ (Strong Positive) = Acid and gas formation
+ (Positive) = Acid formation only
-(Negative) = No acid or gas formation

7 Identification of Isolates

The identification of isolates was carried out following morphology, Gram staining and biochemical tests. On the basis of observed characteristics, both the organisms showed Gram-positive staining and appeared oval-shaped budding yeast cells under the microscopic examination. The biochemical tests, including carbohydrate fermentation and assimilation patterns, confirmed their ability to utilize carbohydrate fermentation and assimilation patterns, confirmed their ability to utilize sugars such as xylose. Based on these morphological and biochemical characteristics and the Gram-staining, the isolates were confirmed as *Candida tropicalis* and *Debaromyces hansenii.*

8.Preparation and Pretreatment of Bagasse

Sugarcane bagasse collected from the sugar industry was washed thoroughly to remove impurities and then dried in a hot air oven.

The dried bagasse was subjected to alkaline pretreatment using sodium hydroxide solution at 80°C for 45 minutes. This treatment helped in loosening the structure of the lignocellulosic biomass.

Fig.No.11 – Dried Pretreated Bagasse.

This treatment helped in loosening the structure of the lignocellulosic biomass and softer texture of it. Which indicates the effective delignification and effective for further hydrolysis treatment.

9.Acid Hydrolysis

The pretreated bagasse was subjected to acid hydrolysis using dilute hydrochloric acid solution. The mixture was heated in a pressure cooker and further heated for 30 minutes to facilitate hydrolysis. After hydrolysis, the mixture was cooled to room temperature.

Fig.No.12 – Before Heating

Fig.No.13 – After Heating

This process shows the breakdown of hemicellulose and release of xylose rich fermentable sugars in liquid fraction as substrate for further detoxification and fermentation for xylitol production.

10.Filtration of Hydrolysate.

The hydrolyzed mixture contained both liquid hydrolysate and solid residues of bagasse. Filtration was carried out using Whatman filter paper to separate the liquid hydrolysate from the solid material.

The filtrate obtained contained soluble sugars released during hydrolysis.

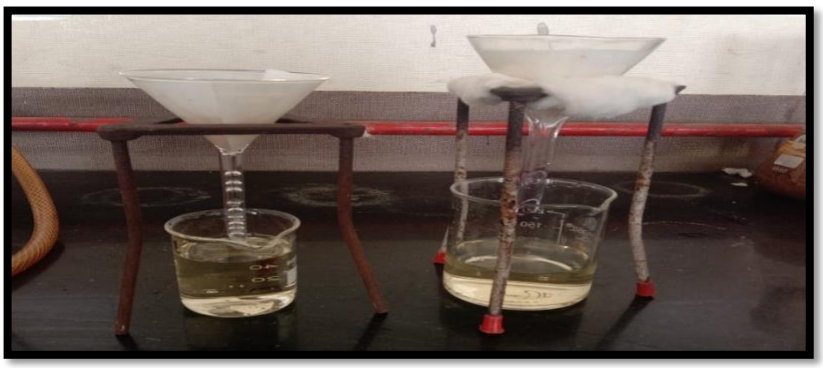


Fig.No.14 – Filtration of Hydrolysate.

11.Detoxification of Hydrolysate

The hydrolysate obtained after filtration was detoxified using calcium hydroxide. The pH of the solution was adjusted and the mixture was heated to remove inhibitory compounds formed during acid hydrolysis.

After detoxification, the hydrolysate was filtered again and adjusted to suitable pH for fermentation.

Fig.No. 15 – Hydrolysate Detoxification

This process increases in pH and results in precipitation of inhibitory compounds formed during hydrolysis. And leads to obtain clear hydrolysate which is suitable for for fermentation for production of xylitol

12.Fermentation for Xylitol Production

The detoxified hydrolysate was used as the fermentation medium. Yeast cultures were inoculated into the hydrolysate and incubated at 30°C for 48–72 hours. During incubation, the yeast organisms utilized the sugars present in the hydrolysate for metabolic activity.

Fig.No.16 – Fermentation of Detoxified Hydrolysate

The process confirmed the suitability of the hydrolysate as a substrate for the xylitol production.

Detection of Xylitol

Paper chromatography was performed to detect the presence of xylitol in the fermented broth. The fermented sample and standard xylitol solution were spotted on chromatography paper and placed in the solvent system. After solvent migration, spots were observed on the chromatogram. The presence of a spot corresponding to the standard xylitol indicated the formation of xylitol in the fermented sample.

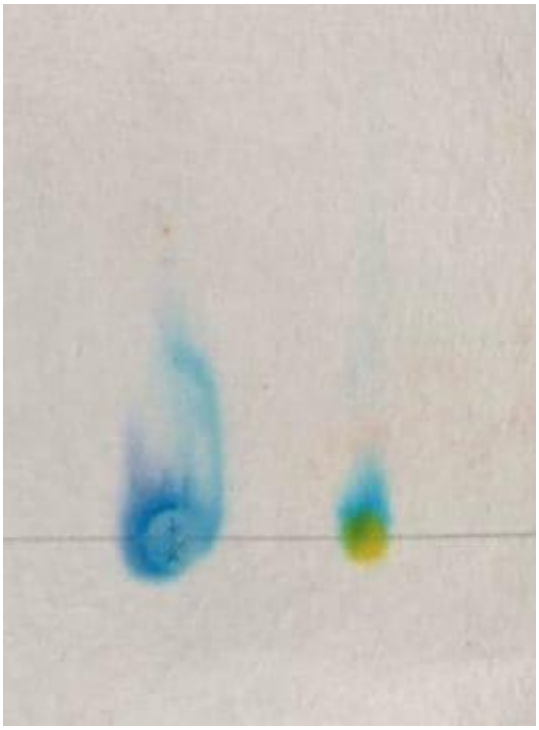


Fig. No. 17 – Paper Chromatography of Fermentation.

DISCUSSION

Discussions

Xylitol is a five-carbon sugar alcohol widely used in the food, pharmaceutical, and dental industries due to its low caloric value and anti-cariogenic properties (Granström et al., 2007). Sugarcane bagasse, a lignocellulosic agro-industrial waste, contains hemicellulose rich in xylose which can be utilized for xylitol production through microbial fermentation (Mussatto and Roberto, 2004). Therefore, using sugarcane bagasse as a substrate provides a cost-effective and sustainable method for xylitol production while also supporting agro-waste management (Silva and Roberto, 2001).

The identification of the isolated microorganisms was carried out through detailed morphological and biochemical characterization. The isolates obtained from spoiled fruit samples such as apple, orange and banana showed colony characteristics typical of yeast, including smooth, creamy, circular and convex colonies with a whitish to cream color on the culture medium. Microscopic observation revealed oval to round budding yeast cells, which are characteristic features of Candida species. The isolate obtained from sugarcane bagasse was identified as *Debaryomyces hansenii,* which also showed creamy, smooth and circular colonies with slightly raised elevation. Microscopically*, Debaryomyces hansenii* cells appeared oval to ellipsoidal in shape and reproduced by budding. Gram staining of both isolates showed Gram-positive yeast cells, which is a common characteristic of many yeast species. In addition to morphological observations, biochemical tests such as sugar fermentation and assimilation tests were performed to confirm the identity of the isolates. The results of these tests indicated the ability of the isolates to utilize different sugar substrates for growth. Similar observations have been reported in previous studies where *Candida* and *Debaryomyces* species were isolated from fruit wastes and agro- industrial residues and were found to possess potential for fermentation and biotechnological applications (Silva and Roberto, 2001; Granström et al., 2007).

In the present study, sugarcane bagasse was used as a substrate for xylitol production through microbial fermentation. Before fermentation, the bagasse was subjected to pretreatment in order to release fermentable sugars from its lignocellulosic structure. Pretreatment facilitates the breakdown of hemicellulose and leads to the release of xylose, which acts as an important substrate

for xylitol production by yeast species. Similar observations were reported by Parajó et al. (1998), who demonstrated that lignocellulosic materials such as sugarcane bagasse can be effectively pretreated to obtain xylose-rich hydrolysates suitable for microbial fermentation.

The fermentation medium prepared from bagasse hydrolysate was inoculated with selected yeast isolates including Candida species obtained from spoiled fruit samples and *Debaryomyces hansenii* isolated from bagasse. Inoculation is an essential step in fermentation as it introduces the microorganisms responsible for sugar utilization and metabolite production. In the present study, both yeast strains were able to grow in the fermentation medium and initiate the conversion of sugars present in the hydrolysate. Similar findings were reported by Silva and Roberto (2001), who observed that Candida species are efficient microorganisms for fermentative production of xylitol from lignocellulosic hydrolysates.

The fermentation process was allowed to proceed for a specific incubation period under controlled conditions in order to allow microbial metabolism and product formation. During this period, the microorganisms utilized the available xylose through metabolic pathways and converted it into xylitol as a fermentation product. Previous studies have also shown that yeast species such as Candida and Debaryomyces possess the ability to metabolize xylose through the pentose phosphate pathway and produce xylitol during fermentation. Similar results were reported by Saha and Bothast (1997), who demonstrated the potential of yeast strains for efficient xylitol production from xylose-containing substrates.

Overall, the results obtained in the present study indicate that sugarcane bagasse hydrolysate can serve as an effective fermentation medium for xylitol production using yeast isolates. The ability of the isolated Candida and Debaryomyces hansenii strains to grow in the hydrolysate and utilize xylose is consistent with earlier reports where these yeasts were successfully used for fermentation and biotechnological production of value-added products from agricultural residues (Granström et al., 2007).

After the completion of fermentation, the fermented broth was processed further for product recovery and standardization in order to confirm the formation of xylitol (Parajó et al., 1998). During this step, concentration and purification of the fermentation broth

resulted in the formation of crystals, which is considered an important preliminary indicator of xylitol production in microbial fermentation studies (Granström et al., 2007). The appearance of crystalline structures suggests that the xylose present in the bagasse hydrolysate was successfully converted into xylitol by the yeast isolates during fermentation (Saha and Bothast, 1997). Similar crystal formation after fermentation has also been reported in earlier studies where xylitol produced from lignocellulosic substrates was recovered through crystallization techniques (Parajó et al., 1998). In the present study, standardization of the obtained product was further carried out using Thin Layer Chromatography (TLC) analysis in order to confirm the presence of xylitol (Mussatto and Roberto, 2004). The TLC results showed the formation of a spot corresponding to the standard xylitol sample, indicating the successful production of xylitol by the isolated yeast strains (Granström et al., 2007). Similar TLC-based confirmation of xylitol production has also been reported in previous studies where microbial fermentation products were analyzed and confirmed through chromatographic techniques (Silva and Roberto, 2001). Therefore, the results obtained in the present study are consistent with earlier reports indicating that fermentation followed by crystallization and chromatographic analysis can effectively confirm the production of xylitol from lignocellulosic substrates such as sugarcane bagasse (Mussatto and Roberto, 2004).

Table 21. Price Comparison of Xylitol Production

Sr. No.	Production Method	Raw Material	Approximate Cost	Remarks
1	Chemical Synthetic Method	Purified xylose	Very High	Requires expensive chemicals and catalysts
2	Corn cob hydrolysate fermentation	Corn cobs	Moderate	Requires pretreatment and detoxification
3	Wood hydrolysate method	Hardwood residues	Moderate to High	Complex processing required
4	Sugarcane bagasse fermentation (Present study)	Sugarcane bagasse	Low cost	Agro-industrial waste, easily available

Table 22: Comparison of Xylitol Production Using Different Substrates

Sr.No.	Parameter	Corn Cob Hydrolysate Fermentation	Sugarcane Bagasse Fermentation (Present Study)
1	Raw material	Corn cobs	Sugarcane bagasse
2	Availability	Seasonal agricultural residue	Abundant agro-industrial waste
3	Raw material cost	Moderate	Very low / almost free
4	Pretreatment requirement	Acid hydrolysis required	Pretreatment required but simpler
5	Microorganism used	Yeasts such as Candida spp.	Candida spp. and *Debaryomyces hansenii*
6	Fermentation process	Microbial fermentation	Microbial fermentation
7	Economic feasibility	Moderate cost	More economical

The table compares xylitol production from corn cobs and sugarcane bagasse. Sugarcane bagasse is cheaper, more abundant, and requires simpler pretreatment than corn cobs. Both use yeast-based microbial fermentation, but bagasse is more economically feasible, making it a better substrate for xylitol production.

Conclusion

The present study successfully demonstrated the feasibility and potential of utilizing sugarcane bagasse as an effective and sustainable substrate for the microbial production of xylitol. Sugarcane bagasse, an abundant agro-industrial by-product generated by the sugar industry, is often underutilized or disposed of in ways that contribute to environmental pollution.(Sun & Cheng, 2022; Sarkar et al., 2012) This research highlights its value as a low-cost and renewable raw material that can be efficiently converted into high-value bioproducts through microbial fermentation, thereby supporting the concept of waste-to-wealth and circular bioeconomy.(Cherubini, 2010; Mussatto & Teixeira, 2010).

The isolation and identification of yeast strains played a crucial role in this study. Yeast strains were successfully isolated from spoiled fruits such as apple, orange, banana etc. were identified as belonging to the genus *Candida*, which is widely known for its ability to ferment xylose into xylitol (Parajo et al; 1998; Granstrom et al; 2007) In addition, *Debaryomyces hansenii* was isolated from sugarcane bagasse itself, indicating that lignocellulosic environments can harbor microorganisms capable of utilizing complex substrates.(Breuer & Harms, 2006).The identification of these yeast strains was carried out using morphological and biochemical characterization techniques, which are standard methods in microbial identification (Cappucino & Sherman, 2014). These findings emphasize the diversity of naturally occurring microorganisms and their adaptability to different ecological niches, making them valuable candidates for industrial biotechnology applications (Jeffries, 2006).

The pretreatment of sugarcane bagasse was a critical step in this study, as it enabled the release of fermentable sugars, particularly xylose, from the lignocellulosic matrix (Mosier et al., 2005). Lignocellulosic biomass is composed mainly of cellulose, hemicellulose, and lignin, and its complex structure requires appropriate pretreatment to make the sugars accessible for microbial utilization (Sun & Cheng, 2002). The hydrolysate obtained after pretreatment served as an efficient fermentation medium, supporting the growth and metabolic activity of the isolated yeast strains. This step underscores the importance of optimizing pretreatment methods to enhance sugar recovery while minimizing the formation of inhibitory by-products (Palmqvist & Hahn-Hagerdal, 2000).

The fermentation studies demonstrated that the selected yeast strains were capable of converting xylose into xylitol efficiently (Granstrom et al; 2007; Rafiqul & Sakinah, 2013). Xylitol is a value-added product widely used as a sugar substitute in food and pharmaceutical industries due to its low glycemic index, dental health benefits, and suitability for diabetic patients (Makinen, 2010). The ability of the isolated microorganisms to utilize lignocellulosic substrates for xylitol production highlights their industrial relevance (Parajo et al., 1998).

Furthermore, the study confirmed xylitol production through crystal formation and Thin Layer Chromatography (TLC) analysis, ensuring the reliability and validity of the experimental results (Stahl, 1969).

Another significant outcome of this study is the demonstration of cost-effectiveness and environmental sustainability associated with the use of sugarcane bagasse (Mussatto&Teixeira, 2010). Conventional methods of xylitol production involve chemical processes that are energy-intensive and environmentally unfriendly (Hyvonen et al., 1982) In contrast, microbial fermentation offers a greener alternative with lower energy requirements and reduced environmental impact (Rafiqul & Sakinah, 2013). By utilizing an agricultural waste product as a substrate, the overall production cost can be significantly reduced, making the process economically viable for large-scale applications (Cherubini, 2010).

Moreover, this study contributes to the growing field of industrial microbiology and biotechnology by showcasing the potential of microbial systems in converting renewable resources into valuable products (Cherubini,2010; Chandel et al., 2011). The integration of microbial fermentation with agro-industrial waste management can lead to sustainable industrial practices and reduce dependency on non-renewable resources (Mussatto & Teixeira, 2010). It also opens avenues for further research in optimizing fermentation parameters, improving yield, and scaling up the process for commercial production (Gnansounou & Dauriat, 2010).

Despite the promising results, there are certain limitations that need to be addressed in future studies. The efficiency of xylitol production can be further enhanced by optimizing factors such as pH, temperature, inoculum size, aeration, and fermentation time (Converti et al., 2000). Additionally, genetic and metabolic engineering approaches can be employed to

develop more efficient microbial strains with higher xylitol productivity and tolerance to inhibitors (Jeffries, 2006). The detoxification of hydrolysate to remove inhibitory compounds is another area that requires attention to improve fermentation efficiency (Palmqvist & Hahn-Hagerdal, 2000).

Future research can also explore the use of co-cultures or mixed microbial systems to enhance substrate utilization and product yield (Chandel et al., 2011). Advanced bioprocessing techniques, including immobilized cell systems and continuous fermentation, may further improve productivity and process efficiency (Rafiqul & Sakinah, 2013). Additionally, techno-economic analysis and life cycle assessment studies are essential to evaluate the feasibility of scaling up this process for industrial applications (Gnansounou & Dauriat, 2010).

In conclusion, this study successfully establishes sugarcane bagasse as a promising and sustainable substrate for microbial xylitol production (Sun & Cheng, 2002). The use of naturally occurring yeast strains capable of converting xylose into xylitol demonstrates the potential of microbial fermentation as an eco-friendly and cost-effective alternative to conventional production methods (Parajo et al., 1998). The findings of this research contribute to sustainable waste management, value addition to agro-industrial residues, and the advancement of green biotechnology. With further optimization and technological advancements, this approach has significant potential for industrial-scale production, thereby supporting both economic development and environmental sustainability.

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