Wiley
Journal of Food Processing and Preservation
Volume 2025, Article ID 9721088, 14 pages
https://doi.org/10.1155/jfpp/9721088

Research Article

Xylitol Production From Brewer's Spent Grain via *Pichia fermentans* Fermentation: Optimization, Scaling, and Isolation

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Received 8 January 2025; Accepted 12 February 2025

Academic Editor: Adadi Parise

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The primary aim of this study was to investigate the novel application of brewer's spent grain (BSG), a waste byproduct from the brewing industry, as sustainable and cost-effective feedstock for xylitol production using the yeast *Pichia fermentans*. The process encompassed fermentation optimization, scale-up, and then downstream processing to produce xylitol. Shake flask fermentation was employed to determine optimal conditions, evaluating key parameters including inoculum concentration (12.5%), feedstock (50%), pH (7.0), temperature (30°C), incubation time (96 h), and agitation speed (150 RPM) with a maximum xylitol production of 32.74 g/L. The yield of xylitol increased to 34.57 g/L by scaling up in an 8-L bioreactor within an incubation time of 72 h. Downstream processing, including centrifugation, charcoal treatment, and ethanol purification was performed successfully, recovering xylitol crystals with a purity of 85.90%. Characterization using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and high-performance liquid chromatography (HPLC) confirmed the purity and composition of crystals. This research highlights the economic and environmental advantages of utilizing BSG for xylitol production, offering a sustainable route over conventional substrates.

Keywords: brewer's spent grain (BSG); downstream processing; fermentation; *Pichia fermentans*; valorization; xylitol

1. Introduction

Brewer's spent grain (BSG), the abundant byproduct of brewing processes, poses environmental challenges due to its sheer volume and limited valorization pathways. It has garnered increasing interest because BSG is mostly utilized as a base for biofuel manufacturing, which offers a sustainable energy alternative that can lessen our need for fossil fuels [1]. Moreover, it is a high-fiber substance that readily transforms into sugars, which can then be fermented to produce ethanol, animal feed, fertilizer, and building materials [2]. As a lignocellulosic material, BSG consists primarily of 25% hemicellulose, 25% cellulose, and 25% lignin [3]. Of particular interest is the hemicellulosic fraction, rich in xylose, a pentose sugar that serves as a precursor for the microbial production of xylitol. This bioconversion technique not only addresses waste disposal concerns but also helps to produce sustainable and environmentally friendly

alternatives to standard sweeteners [4]. Its low caloric content, anticariogenic properties, and suitability for diabetics drive its demand [5]. Xylitol's increasing popularity in foods, pharmaceuticals, and personal care products necessitates the development of sustainable and cost-effective production methods.

Utilizing BSG as a xylitol feedstock offers a dual-pronged solution, that is, mitigating the environmental impact of BSG disposal while creating a valuable commodity [6]. BSG's xylose-rich composition makes it well-suited for microbial xylitol production [7]. Effective pretreatment procedures are crucial for the release of fermentable xylose from BSG with most studies identifying optimal conditions involving a combination of water bath heating followed by acid hydrolysis [8]. Building upon these findings, similar pretreatment conditions for xylose liberation, which involved a combination of water bath heating followed by acid hydrolysis, were used. Our prior work demonstrated that the combined approach of pretreatment of BSG for 30 min each resulted in maximum xylose yield. This study is aimed at optimizing xylitol production from BSG by investigating key fermentation and bioprocessing parameters, including substrate pretreatment, optimization, and scale-up from lab-scale to bioreactor system, followed by purification steps in the xylitol production, obtaining crystal formations.

The traditional xylitol production process is based on the chemical hydrogenation of purified xylose from hardwood hemicellulose or corncobs, which is expensive in raw materials and energy [9]. On the other hand, the use of BSG as a feedstock provides a low-cost, sustainable alternative by utilizing an abundant industrial by-product. Bioconversion of BSG through microbial fermentation does not require expensive chemical catalysts and lowers the processing cost [1]. Though supplementary purification steps would be needed in order to produce pharmaceutical-grade xylitol, the cost will be reduced by less expensive substrates and potential integration with industrial brewery wastewater treatment facilities; thus, BSG-derived xylitol is a viable and competitive cost option [7].

The rising demand for xylitol in the food and pharmaceutical industries, coupled with concerns about traditional production methods from hardwoods, underscores the urgency of developing sustainable and efficient alternatives [10]. By optimizing xylitol production from BSG, this research can contribute to the following: (a) using *Pichia fermentans*, a promising microbial strain, to produce xylitol efficiently, (b) developing and demonstrating a scalable xylitol production from lab-scale optimization to pilot-scale production [11], and (c) potentially improving the economic feasibility of xylitol production through the utilization of an inexpensive and readily available feedstock. While research on xylitol production from BSG exists, recent studies emphasize the importance of customizing processes for specific feedstock characteristics and local conditions [12]. This study's unique contribution is its comprehensive approach to xylitol synthesis from BSG, integrating upstream and downstream processing to overcome key obstacles in commercial use. This study contributes to the biorefinery concept which maximizes resource utilization.

2. Materials and Methods

The BSG was procured from a local brewery and distillery in Kasauli, Himachal Pradesh, India. It was subjected to a drying and preparation process to obtain BSG powder stored in an airtight container and was further subjected to proximate analysis to determine its nutritional composition. A combined approach of water bath heating at 100°C for 30 min followed by acid hydrolysis using 1% (*v*/*v*) sulfuric acid at 100°C for 30 min was used to release fermentable sugars. This process yielded a hydrolysate containing up to 48 g/L xylose, which served as the starting material for subsequent xylose-to-xylitol bioconversion in the present study.

2.1. Yeast Strain and Culture Conditions. Pichia fermentans (obtained from Cranfield University, United Kingdom) was routinely cultured on yeast extract–peptone–dextrose (YPD) agar plates every 4 weeks. The modified YPD agar [13] consisted of 10 g/L yeast extract, 20 g/L peptone, 20 g/L dextrose, and 25 g/L agar (adjusted to pH 4.5) for optimal growth. Following incubation on YPD agar plates, a representative sample of yeast colonies was examined microscopically to assess cell morphology and confirm the absence of bacterial contamination.

For inoculum preparation, the growth medium was formulated with 10 g/L yeast extract, 20 g/L peptone, 30 g/L xylose, 0.5 g/L KH_2PO_4, 0.5 g/L $(NH_4)_2SO_4$, and 0.5 g/L $MgSO_4$, with the final pH adjusted to 7.0. The inoculum was cultivated in 100 mL Erlenmeyer flasks containing 25 mL of the above medium and inoculated with a single colony of *Pichia fermentans*. Cultivation was carried out at 30°C in a rotary incubator shaker at 120 RPM for 24 h until an optical density (OD_{600}) of 1.0 was achieved using an ultraviolet–visible (UV-Vis) spectrophotometer (Thermo Fisher Scientific Evolution 201) at a wavelength of 600 nm with no dilution factor, facilitating the calculation of the yeast growth rate per hour [13, 14].

2.2. Lab-Scale Fermentation Optimization. This study is aimed at optimizing the key fermentation parameters to enhance xylitol production using *Pichia fermentans* in shake flask cultivation. The parameters optimized for experimentation included the effect of inoculum concentration on xylitol production by varying it within a range of 11%–13.5% (*v*/*v*). The concentration of BSG in the fermentation medium varied from 10 mL to 60% (*v*/*v*). The pH optimization of culture conditions was done by varying the pH of the production medium from 3.0 to 8.0. Temperature variations within the range of 28°C–38°C were studied to assess their influence on microbial growth kinetics and xylitol biosynthesis. The duration of the incubation period for culture conditions varied across a range from 24 to 120 h. The effect of agitation on xylitol production was investigated by varying the agitation speed from 120 to 200 RPM in 250 mL Erlenmeyer flasks with 100 mL working medium. Optimal pH, temperature, and RPM ensure effective enzyme activity, microbial growth, and nutrient availability, while ideal incubation time and BSG concentration balance substrate utilization and fermentation performance [15]. Adjusting inoculum

concentration helps establish culture rapidly and maximize production rates. The findings are aimed at advancing xylitol bioprocessing strategies [16]. The amount of xylitol produced was firstly estimated by the spectrophotometric method (Thermo Fisher Scientific Evolution 201) at 600 nm employing the Bial's reagent method [17]. A standard curve was plotted with absorbance versus known xylitol concentrations, and the xylitol content in the fermented BSG extract was calculated using the derived equation, considering a 10× dilution factor. The sample with the greatest xylitol concentration was confirmed with high-performance liquid chromatography (HPLC) (Agilent 1260 Infinity) and a chromolith reversed-phase column. The column was kept at 18°C, with acetonitrile (HPLC grade) as the mobile phase at a flow rate of 1.0 mL/min. The UV detector was adjusted to 190 nm with an 11 μL cell volume. The system pressure was maintained at 160 psi [18]. Xylitol was detected by comparing retention periods to a standard, and quantitative analysis was done in triplicate for statistical validation.

2.3. Scale-Up Bioreactor Fermentation. The objective of this study was to translate the optimized xylitol production conditions from shake flasks to a large-scale bioreactor for efficient scale-up and high-level xylitol production. To achieve large-scale xylitol production, the optimized shake flask conditions were transitioned to an 8 L bioreactor Bioferm-LS2TV bioreactor (SciGenics India Pvt. Ltd.), and the following parameters were monitored: RPM, pH drop-off, xylose residual, and cell growth. The fermentation during the whole process was maintained by a constant pH of 7.0 (optimal for yeast growth and xylitol production) by using NaOH every 24 h of monitoring, and the agitation speed was increased to 300 RPM in the bioreactor to balance xylitol production and cell growth while mitigating foam formation, which was controlled using 1 mL of a silicone-based antifoam agent. Cell growth was monitored via OD at 600 nm, additionally, a constant flow rate of 1 (liters per minute) of oxygen was supplied using a sparger for dissolved oxygen concentration during the entire fermentation but not monitored regularly. Xylitol production final yield was determined via HPLC by the same methodology listed in Section 2.2 [18, 19]. Overall, xylitol detection was done in triplicate for statistical validation.

2.4. Down Streaming and Purification of Xylitol Crystals. After fermentation, the broth containing xylitol was separated from yeast cells and impurities through centrifugation at 10,000 × g for 1 h at 4°C, resulting in a clear, residue-free solution. This was then purified in multiple steps. To begin, a 100 mL aliquot of centrifuged broth was heated to 60°C–70°C for 1 h with constant stirring to concentrate the supernatant while avoiding scorching or excessive evaporation [20, 21]. A portion of the cleared broth was then subjected to a multistep purification process as mentioned to isolate xylitol crystals. Firstly, the solution was mixed with food-grade activated charcoal (100–200 mesh) and agitated for 30 min to absorb colored impurities before filtering to remove the charcoal and insoluble residues. Additional purification was performed by adding 100% ethanol (1:1 v/v) and agitating for 30 min to eliminate residual organic contaminants. The xylitol crystals were then separated by vacuum filtration or decantation, rinsed with cold ethanol, and dried in a desiccator to eliminate any excess solvent and moisture [22]. The final refined xylitol crystals were kept in an airtight container for future analysis using various techniques (X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), HPLC) to confirm their structure, composition, and high purity (the detailed experimental setup is provided in the Supporting Information section (available here)).

3. Results and Discussion

The present study involved BSG as a substrate for fermentation at both lab-scale (100 mL) and scale-up of xylitol production using a fermenter (8 L) using *Pichia fermentans* (Figure 1). The results of lab-scale optimization followed by scale-up fermenter are discussed in the following sections:

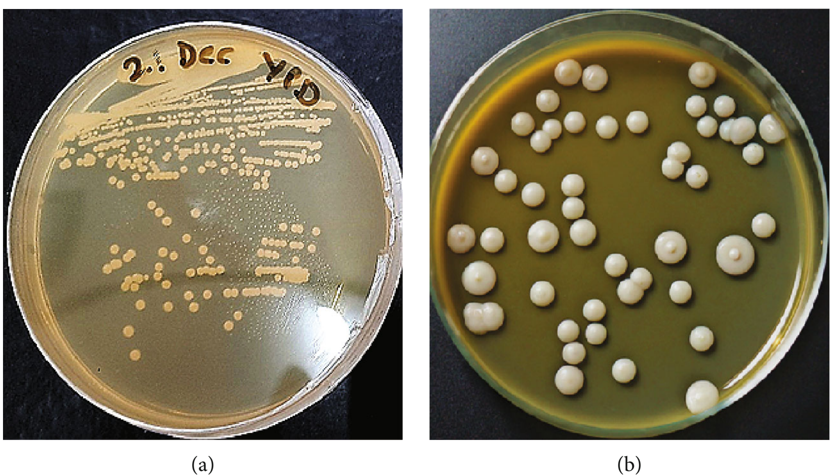
3.1. Yeast Strain Growth and Maintenance Outcomes. The YPD agar medium provided an optimal nutritional environment for *Pichia fermentans*, and dextrose served as the principal carbon source [13]. The pH was kept at 4.5 to promote yeast growth while preventing contamination. Microscopic examination revealed characteristic yeast morphology and the absence of bacterial contamination (Figure 1).

To prepare the inoculum, a complex medium was used containing xylose as the principal carbon source and inorganic salts (KH_2PO_4, $(NH_4)_2SO_4$, and $MgSO_4$) to support metabolic stability. Cultivation at 30°C and 120 RPM increased oxygen availability, with an OD_{600} of 1.0 indicating a high viable cell concentration. The observed growth rate constant (K) is $0.0382\ h^{-1}$. This formulation reduced the lag phase, increased biomass yield, and boosted xylitol synthesis efficiency [14, 23].

3.2. Optimization of Xylitol Production via Shake Flask Cultivation. A shake flask optimization of xylitol production was carried out by varying the production parameters as per the methodology, and the results of this study were firstly estimated by Bial's assay and then confirmed by HPLC. The effects of these parameters on xylitol yield are discussed in Figures 2, 3, 4, 5, 6, and 7):

3.2.1. Effect of Inoculum Concentration. The production medium [13] was added with inoculum concentrations of 11%–13.5% (*v*/*v*). The maximum xylitol output (23.11 ± 0.19 g/L) was found at a 12.5% (*v*/*v*) inoculum concentration (Figure 2). Lower inoculum levels (e.g., 11.5%) resulted in longer lag phases and lower xylitol synthesis (15.59 ± 0.15 g/L), but increasing the concentration to 13.5% resulted in a drop (17.88 ± 0.21 g/L), possibly due to substrate competition and metabolic inefficiencies [24]. The use of Bial's assay for xylitol quantification was validated through a standard graph [25].

3.2.2. Effect of Feedstock Concentration. BSG concentrations were adjusted according to the approach, resulting in 50% (*v*/*v*) BSG in the production medium resulting in the maximum xylitol production (26.28 ± 0.11 g/L). Increasing the concentration to 60% (*v*/*v*) resulted in lower xylitol yield



(a) (b)

Figure 1: (a, b) Growth of *Pichia fermentans* colonies on YPD agar plates.

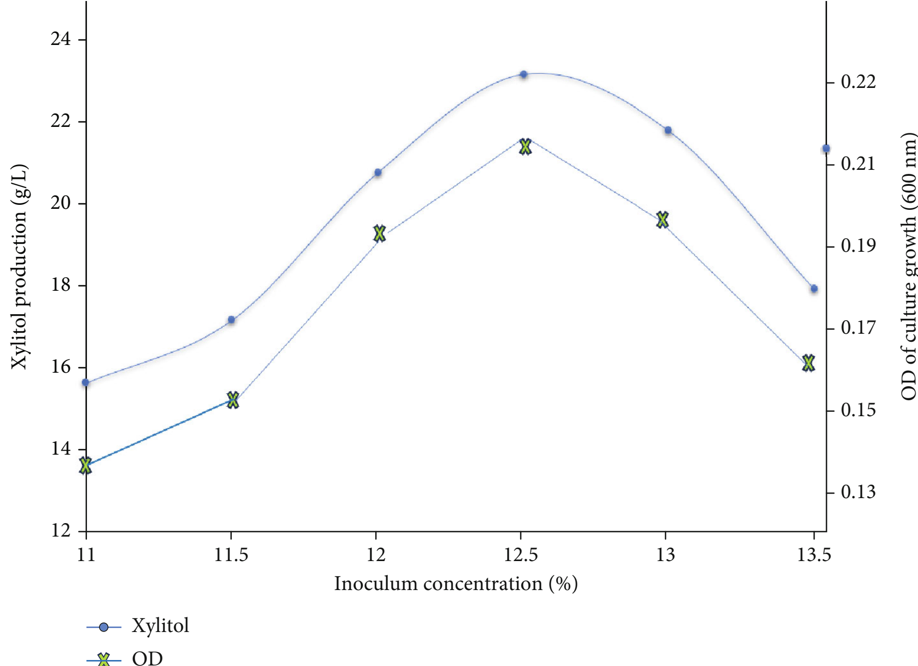


Figure 2: Effect of inoculum concentration on xylitol production using *Pichia fermentans* showing optimum result at 12.5%.

(25.73 ± 0.09 g/L), possibly due to substrate inhibition or osmotic stress.

3.2.3. Effect of pH. Xylitol production was investigated at pH levels ranging from 3.0 to 8.0, with neutral pH (7.0) resulting in the maximum production (26.29 ± 0.11 g/L). Production dropped at pH 3.0 (19.84 ± 0.14 g/L) and pH 8.0 (25.37 ± 0.12 g/L). A neutral pH promotes microbial metabolism, enzyme activity, and sugar alcohol production [26, 27].

3.2.4. Effect of Temperature. Xylitol production was determined at incubation temperatures ranging from 28°C to 38°C. The ideal temperature of 30°C resulted in the maximum xylitol yield (26.29 ± 0.11 g/L), while temperature changes reduced yield. The data imply that *Pichia fermentans* demonstrate the maximum enzymatic efficiency and metabolic activity at an ideal incubation temperature, consistent with earlier reports on yeast-based xylitol fermentation [13].

3.2.5. Effect of Incubation Time. The impact of incubation time was investigated by altering the fermentation duration from 24 to 120 h. The greatest xylitol yield (32.06 ± 0.19 g/L) was obtained at 96 h, after which the yield plateaued or decreased due to substrate depletion and potential product inhibition. An incubation time of 96 h is required for adequate biomass accumulation and substrate conversion to xylitol aligning with findings in previous studies [16]. The optimal BSG concentration provided a sufficient carbon source without inhibiting cell growth, as reported by Pabbathi et al. [6].

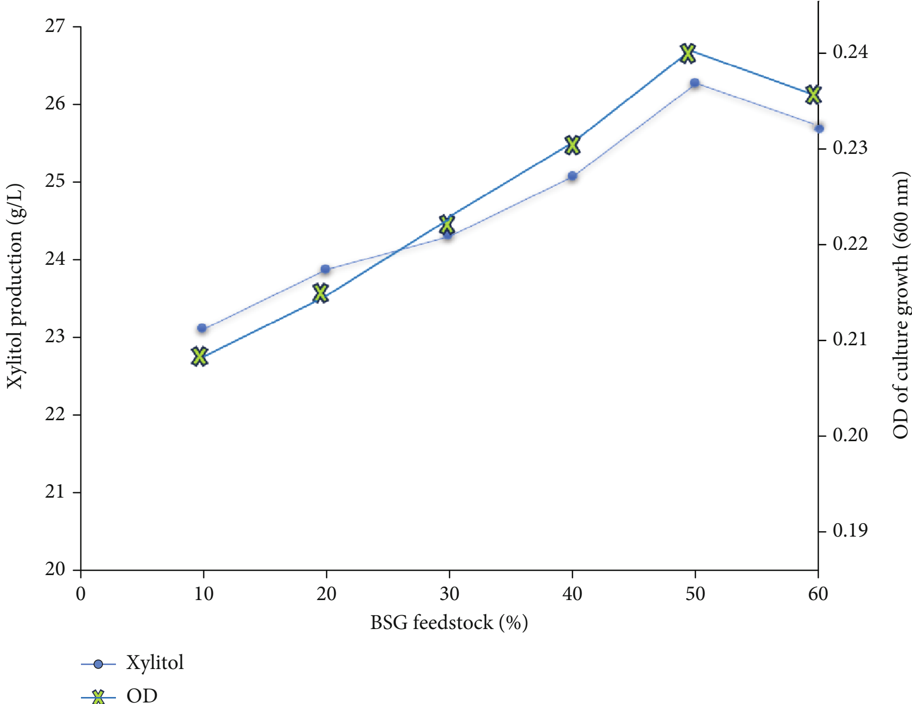


Figure 3: Effect of BSG feedstock on xylitol production using *Pichia fermentans* showing optimum result at 12.5% inoculum concentration at 50% (*v*/*v*).

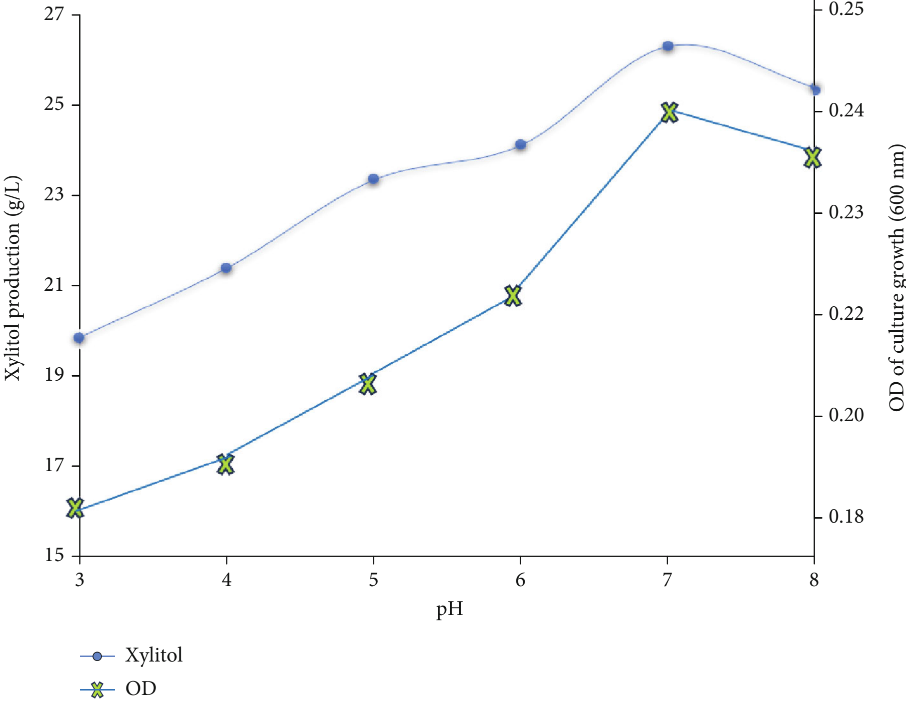


Figure 4: Effect of pH on xylitol production using *Pichia fermentans* showing optimum result at 12.5% inoculum concentration, 50% (*v*/*v*) at 7.0 pH.

3.2.6. Effect of RPM. After conducting the experiments, it was observed that 150 RPM was the optimal setting for xylitol production (33.03 ± 0.07 g/L). At this RPM, the balance between adequate mixing, oxygen transfer, and minimal shear stress was achieved, resulting in the highest xylitol yield. Lower RPM settings did not provide sufficient mixing

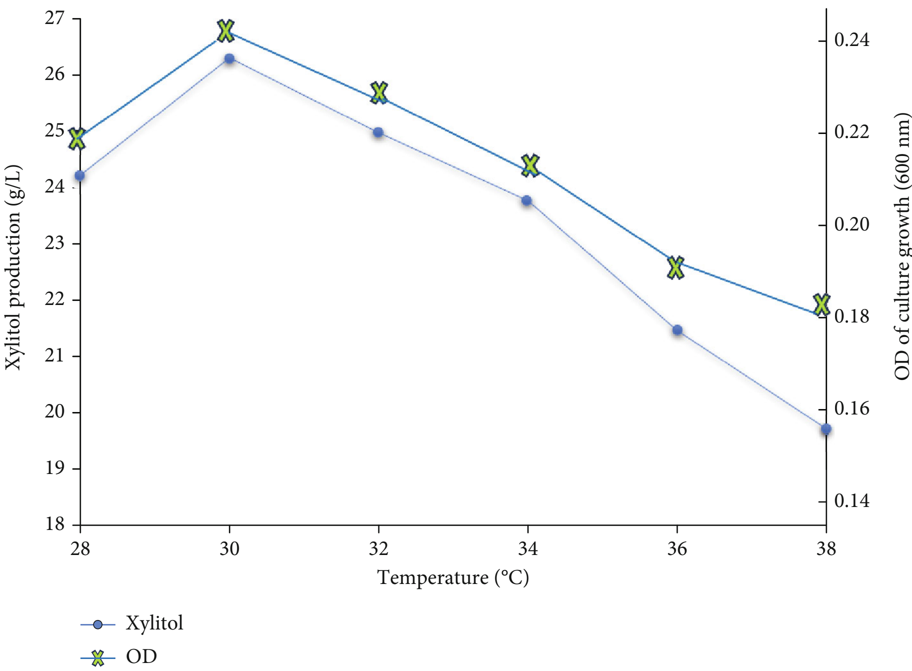


Figure 5: Effect of temperature on xylitol production using *Pichia fermentans* showing optimum result at 12.5% inoculum concentration, 50% (v/v),7.0 pH at 30°C.

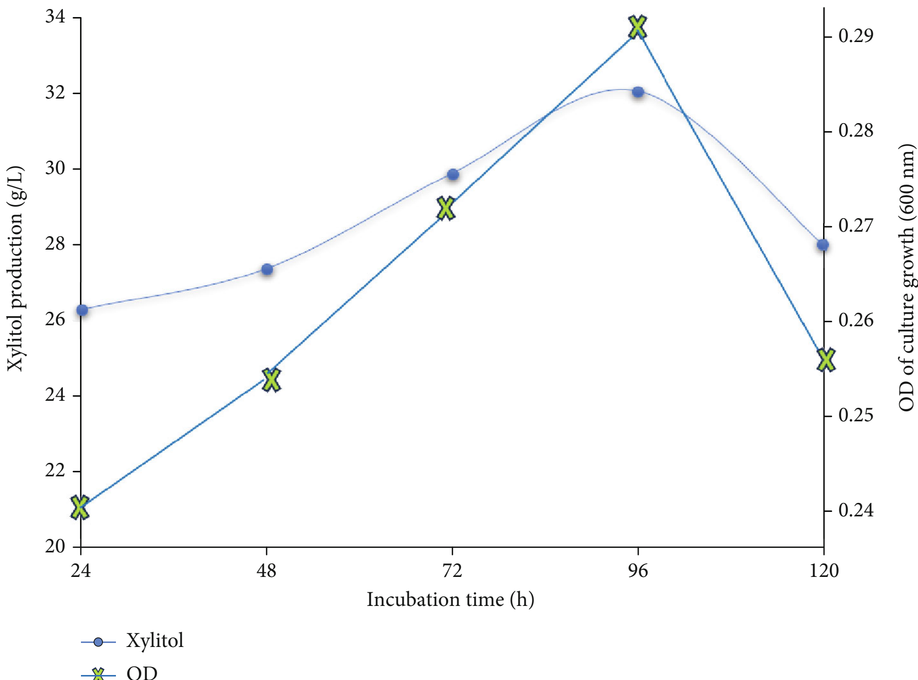


Figure 6: Effect of temperature on xylitol production using *Pichia fermentans* showing optimum result at 12.5% inoculum concentration, 50% (v/v),7.0 pH, 30°C at 96 h.

and oxygenation, while higher RPM settings caused increased shear stress, adversely affecting cell viability and product yield [28].

A maximum xylitol production of 32.74 g/L was achieved under optimized conditions quantified and confirmed by HPLC (Figure 8) (refer to the Supporting Information section for the detailed graph) through optimum values, that is, pH 7, temperature of 30°C, incubation time of 96 h, BSG concentration of 50% (v/v) agitation speed of 150 RPM, and inoculum size of 12.5% (Figure 9). These conditions were determined to be optimal for balancing cell growth, substrate utilization, and product formation [15].

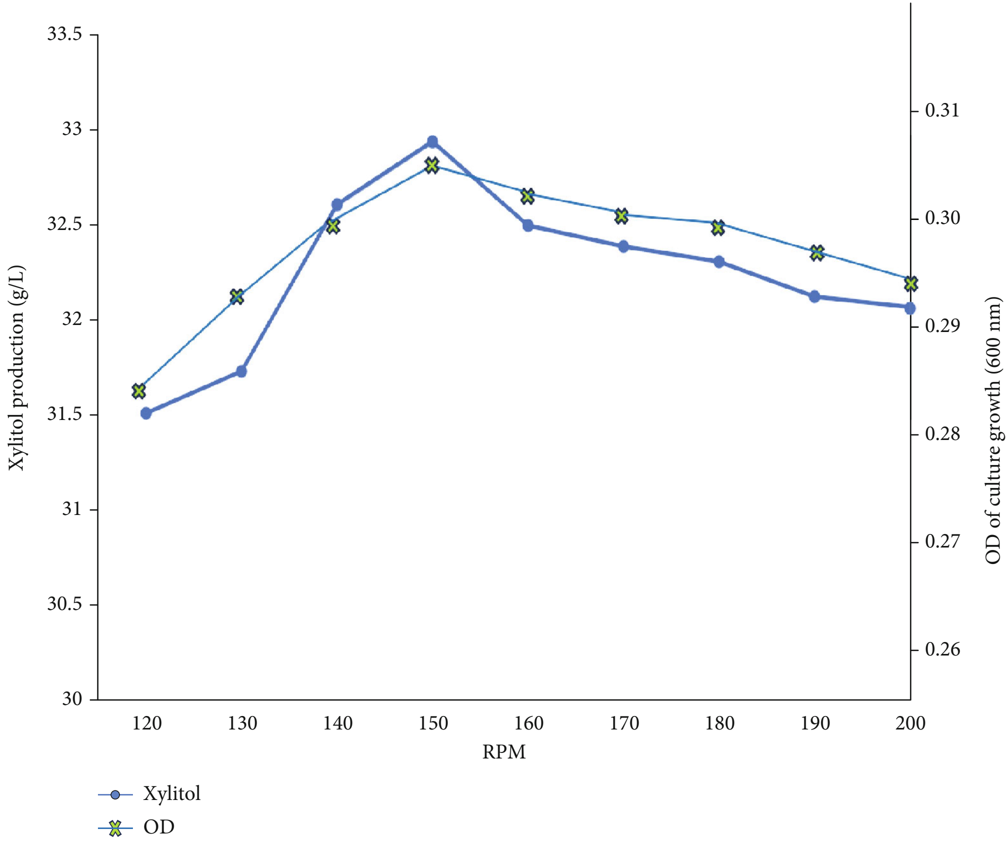


Figure 7: Effect of temperature on xylitol production using *Pichia fermentans* showing optimum result at 12.5% inoculum concentration, 50% (*v*/*v*),7.0 pH, 30°C, 96 h at 150 RPM.

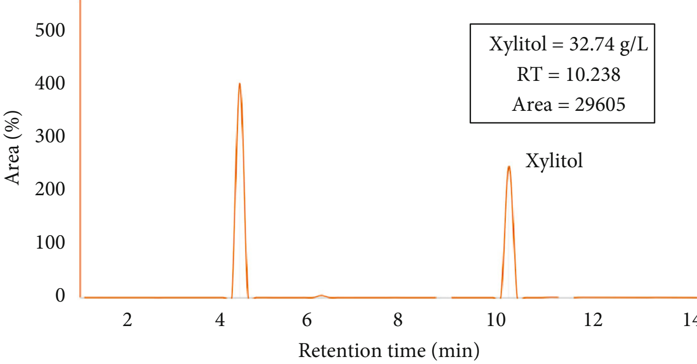


Figure 8: HPLC chromatogram of xylitol assay after its production under optimized flask shake conditions using *Pichia fermentans*.

A neutral pH of 7 is known to be favorable for microbial processes. The mesophilic temperature of 30°C ensured sufficient metabolic activity without inducing excessive stress on the cells [29].

Agitation at 150 RPM promoted adequate oxygen transfer and nutrient distribution, while preventing excessive shear stress. On the other hand, an inoculum size of 12.5% ensured rapid culture establishment and efficient substrate utilization. The resulting xylose utilization rate was reported at 68% (final xylitol ÷ initial xylose × 100), demonstrating the effectiveness of the optimized process in converting xylose to xylitol comparable to or exceeding yields reported in similar studies [7].

3.3. Scale-Up of Xylitol Production Using Bioreactor. The xylitol yield achieved in flask shake laboratory trials suggests strong potential for implementation in scale-up production systems. In this experimental setup, further scale-up studies were carried out using an 8-L fermenter Bioferm-LS2TV bioreactor (SciGenics India Pvt. Ltd.), using optimized parameters from shake flask cultivation. Xylitol production was assessed at an interval of 24 h using Bial's reagent assay,

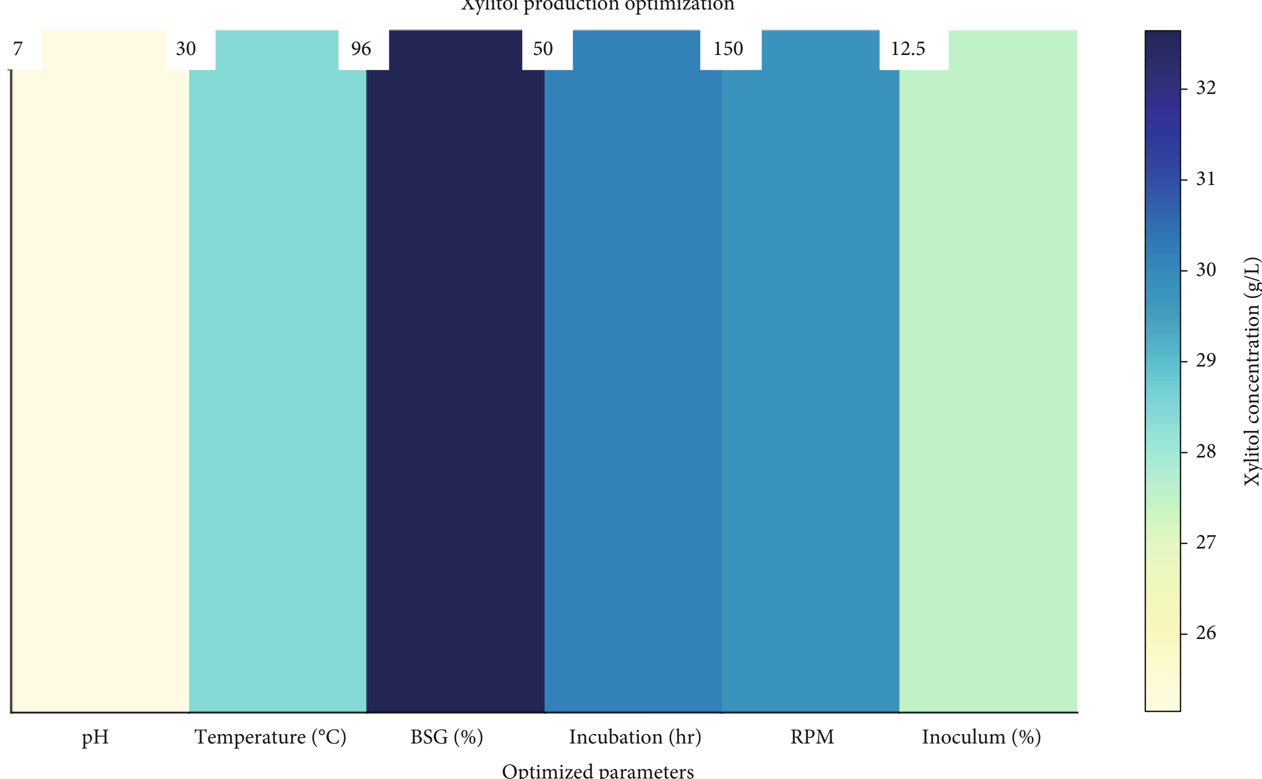


Figure 9: Heat map of maximum xylitol production with optimized parameters in shake flask fermentation.

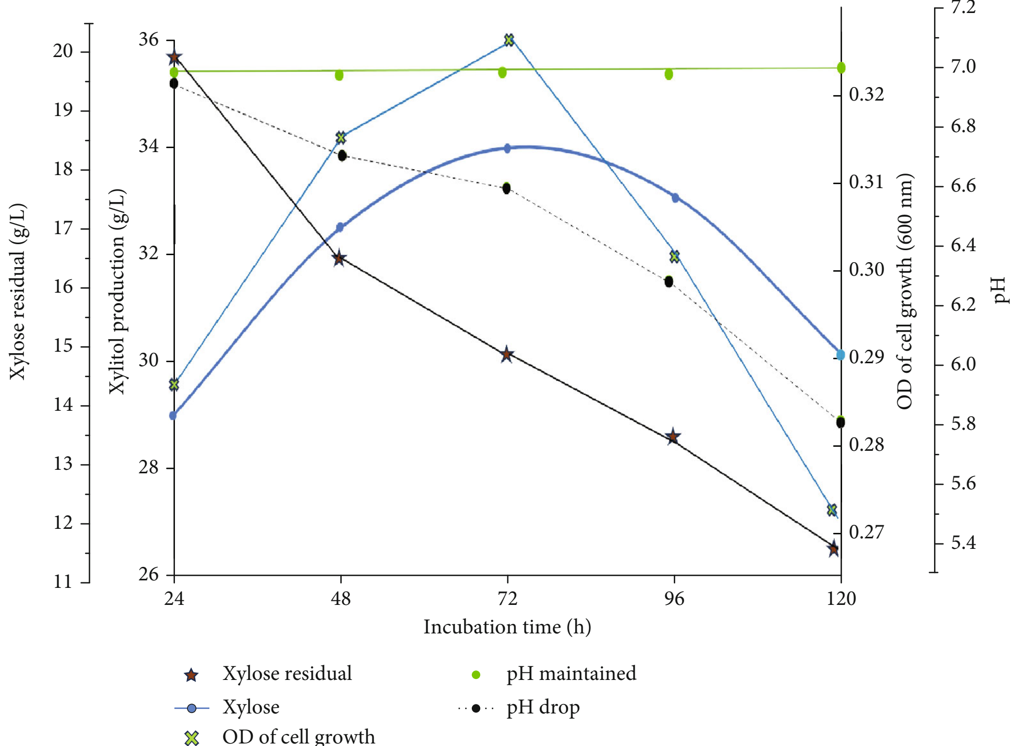


Figure 10: Monitoring of cell growth and its correlation with xylose utilization, pH, and xylitol production during bioreactor fermentation by *Pichia fermentans* at 300 RPM, 30°C.

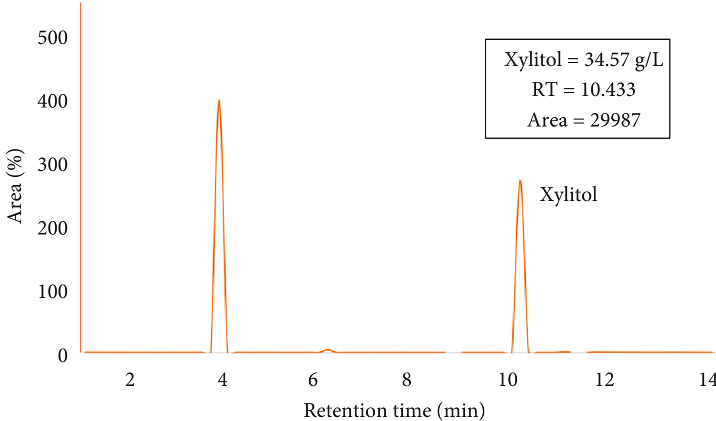


Figure 11: The chromatogram represents xylitol produced by bioreactor cultivation, confirming its quantification.

Table 1: Comparison of xylitol production between lab-scale and bioreactor-scale fermentation systems.

Parameter	Lab-scale (100 mL)	Bioreactor scale (8000 mL)
Xylose production (g/L)	48 ± 0.05	48 ± 0.05
Xylitol production (g/L)	32.74 ± 0.08	34.57 ± 0.09
Xylose utilization (%)	68	72
Fermentation time (h)	96	72
Agitation rate (RPM)	150	300

Note: Values in the table are expressed as mean ± SD ($n = 3$).

as mentioned in Section 2.2. Results of scale-up study for xylitol production with *Pichia fermentans* are summarized in Figure 10.

Successful xylitol production using *Pichia fermentans* in a bioreactor was achieved by closely monitoring the key parameters: RPM, pH drop, and cell growth in the present study (Figure 10), correlated directly with xylitol production [19]. The optimized shake flask conditions (150 RPM, 8 L working capacity) in a bioreactor were used for the first attempt at scaling up the production of xylitol. However, as indicated by the considerable froth development, the fermentation process at this agitation speed led to poor mixing, which in turn resulted in inadequate oxygen and nutrient distribution. These restrictions resulted in 96 h of fermentation with little xylitol synthesis and cell development. To overcome these issues, the agitation speed was increased to 300 RPM, while maintaining a constant dissolved oxygen level (1 L/min). This modification produced ideal conditions for microbial activity, decreased froth formation, and greatly increased mixing efficiency, all of which led to an increase in xylitol output. Under controlled conditions at 300 RPM, the bioreactor fermentation yielded 34.00 ± 0.09 g/L xylitol within 72 h, compared to 33.08 ± 0.08 g/L in shake flask fermentation over 96 h measured by Bial's assay, spectrophotometer method.

Maintaining a constant pH of 7.0 was crucial for optimal yeast activity and xylitol yield (Figure 10), aligning with previous research [30]. Increased agitation in the bioreactor significantly reduced fermentation time from 96 to 72 h, likely due to improved mass transfer and oxygenation [31]. With peak cell density and maximum xylitol levels recorded at 72 h, OD measurements demonstrated a clear relationship between cell growth and xylitol production. The HPLC significantly proved (Figure 11) that scaling up the process to an 8-L bioreactor resulted in a higher xylitol yield (34.57 g/L) compared to lab-scale cultivation experiments (32.74 g/L). The xylose utilization rate of bioreactor was reported 72% (final xylitol ÷ initial xylose × 100) and the xylose residual was monitored (total initial xylose – xylitol production) every 24 h for better monitoring of results (Figure 8). This enhancement is attributed to the bioreactor's precise control over fermentation conditions, promoting optimal cell growth and xylitol production [32]. These findings demonstrate the successful scalability of the process and its potential for industrial application using BSG as a sustainable feedstock. This analysis delves into the key findings optimization of xylitol production from a lab-scale (100 mL) to a bioreactor (8000 ml) setup using *Pichia fermentans* culture as an inoculum and BSG as a substrate. The comparative results are summarized in Table 1.

The xylitol production achieved in this study was comparable to or higher than several previously reported values. For instance, Carneiro, Silva, and Almeida [33] reported a maximum xylitol concentration of 24.75 g/L using *Wickerhamomyces anomalus* 740 in sugarcane bagasse hydrolysate under oxygen-limited conditions. Similarly, another study found that a stirred-tank reactor (STR) bioreactor produced 29 g/L of xylitol over 120 h on corncob hydrolysate by *Debaryomyces hansenii* strain; however, the current study produced a higher output in a much shorter amount of time (72 h), indicating enhanced fermentation efficiency [34]. Moreover, Silva, Mussatto, and Roberto [35] reported the kinetic behavior of *Candida guilliermondii* FTI 20037 on rice straw hydrolysate, achieving a maximum xylitol concentration of 45.4 g/L after 45 h, although it is slightly higher xylitol content, but the current study obtained a comparable yield (34.57 ± 0.09 g/L) while maintaining a constant pH and effective substrate utilization. These comparisons emphasize

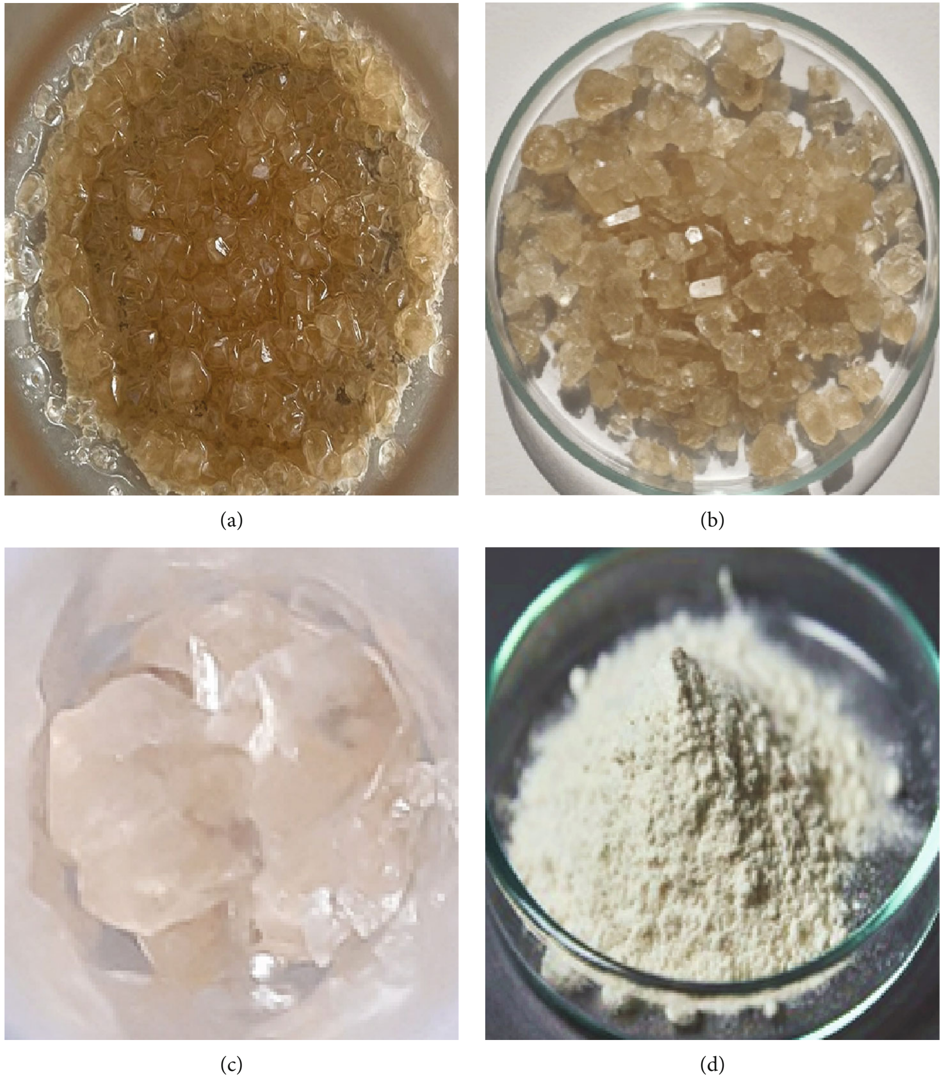


Figure 12: Purification of xylitol crystals obtained from fermented BSG broth, where (a) freshly formed xylitol crystals in the fermented BSG broth, (b) dried xylitol crystals after charcoal wash, (c) ethanol washed xylitol crystals, and (d) final white xylitol granulated product obtained after complete downstream processing.

the efficacy of the bioreactor scale-up procedure using *Pichia fermentans* in BSG, indicating a feasible alternative for long-term xylitol production.

3.4. Downstream Processing of Xylitol From Fermenter Scale-Up Production. Following centrifugation of the fermented brewer spent hydrolysate broth, a 100-mL sample was taken in a flask and was subjected to downstream processing for xylitol crystal recovery as mentioned in the methodology. The crystals were formed within a 24-h timeframe (Figure 12), and initially, the xylitol crystals displayed a brown color likely due to cocrystallization of residual-colored compounds from BSG (melanoidins from Maillard's reactions) and caramelization of residual sugars during heating [36, 37]. Subsequent treatment with charcoal and ethanol improved the crystal purity.

Using food-grade activated charcoal (100–200 mesh) and agitated for 30 min to absorb colored impurities charcoal first acts as an adsorbent, effectively removing colored impurities like melanoidins. After charcoal wash ethanol was used as it is a good solvent for xylitol but not for many colored compounds. This further purifies the crystals by dissolving and removing residual sugars (small amounts of xylose, arabinose, and glucose) and salts like sulfates and phosphates [38]. These residual sugars originate from incomplete fermentation or hydrolysate composition and can impact xylitol purity by contributing to unwanted crystallization or affecting the final product's taste and stability [39]. Salts, on the other hand, can be obtained from the pretreatment chemicals or the inherent mineral content of BSG, which could have an impact on the production and crystallization process [40]. The final product of xylitol after complete downstream processing was further analyzed for its structure composition and purity using XRD, FTIR, and HPLC. The results of this study are shown in Figures 13, 14, and 15. XRD analysis confirmed the crystalline structure of the recovered xylitol, similar to the standard but with minor impurities (Figure 13).

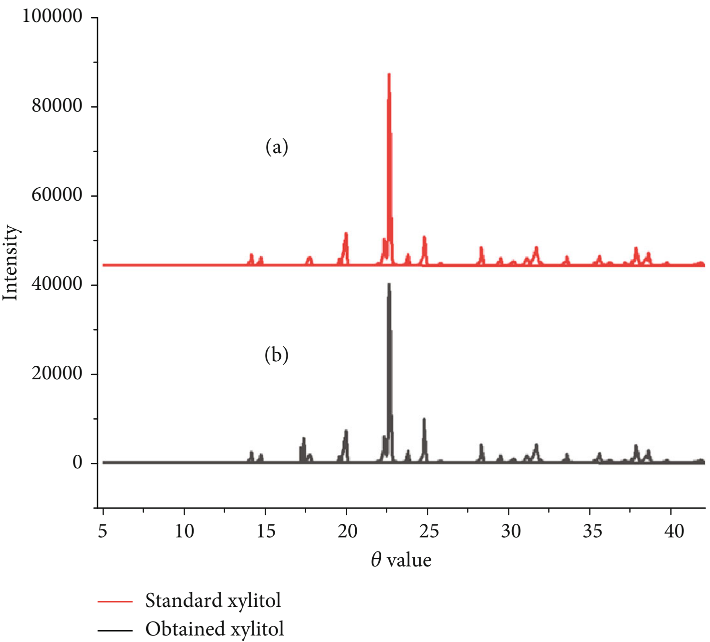


FIGURE 13: XRD peaks of both (a) standard and (b) obtained xylitol crystals produced from *Pichia fermentans* after fermenter scale-up showing peak positions matched within 0.1° 2θ.

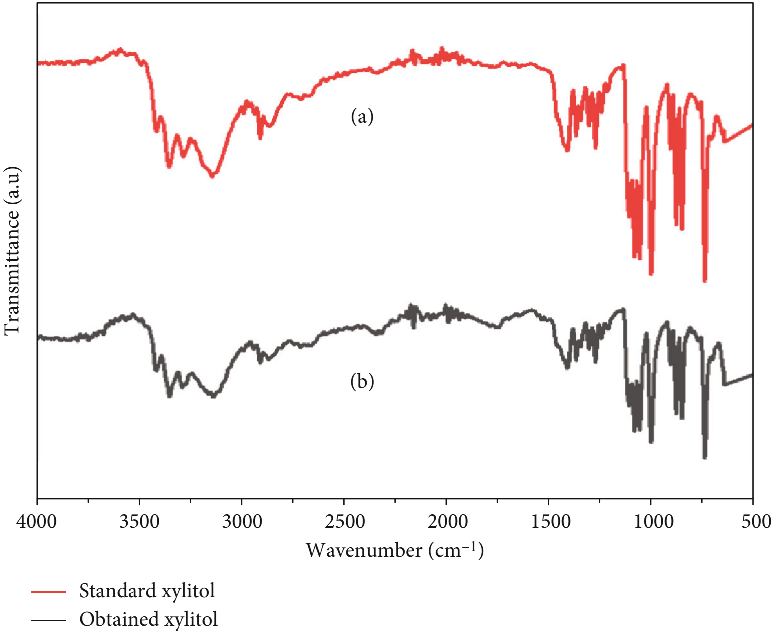


FIGURE 14: FTIR study of (a) standard and (b) crystals obtained from *Pichia fermentans* after fermenter scale-up showing peak positions matched within the same transmittance (a.u).

A well-defined crystalline structure is indicated by the most prominent peak at 20.5° θ value, with an intensity of 45000 units. However, the XRD pattern of the fermented broth xylitol crystals (Figure 13) reveals a peak pattern comparable to the standard xylitol, indicating that the crystalline structure was mostly preserved after extraction and purification. Fermented xylitol has a significant peak at 20.6° θ with a similar intensity (42,000 units) to regular xylitol (45,000 units), demonstrating crystallization. Both the regular and fermented broth xylitol have comparable XRD patterns, especially in the position and strength of the main peaks [41].

Further, FTIR spectroscopy also verified the presence of characteristic xylitol functional groups (Figure 14), indicating successful crystal formation. The FTIR spectrum of standard xylitol showed characteristic absorption lines, indicating the

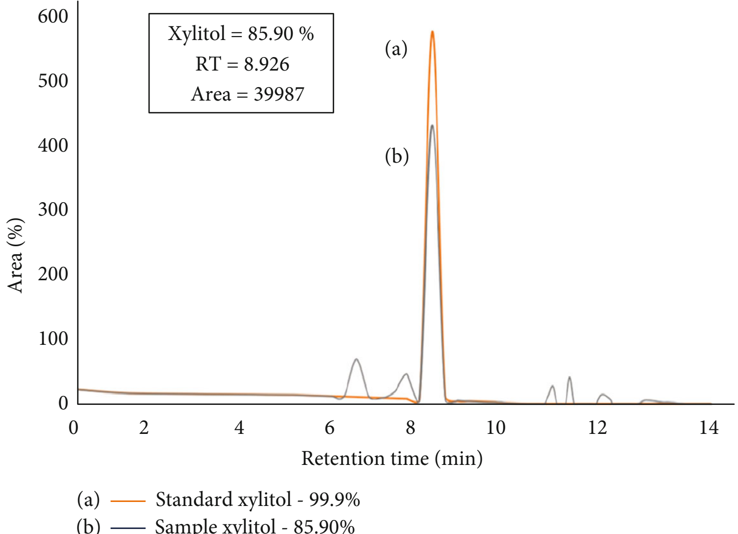


Figure 15: HPLC chromatogram of (a) standard xylitol with (b) purified xylitol crystals.

presence of hydroxyl and other xylitol functional groups. The FTIR spectra of xylitol crystals from BSG fermented broth showed major peaks at ~3350 cm^{-1} (O-H stretching), ~2930 cm^{-1} (C-H stretching), and~1050 cm^{-1} (C-O stretching), showing successful retention of xylitol's functional groups. However, further minor peaks at ~1650 cm^{-1} and ~1400 cm^{-1} suggested the presence of residual impurities, likely from protein-derived amides or lignin-related chemicals in BSG [42].

Finally, the HPLC analysis of the final downstreamed product of xylitol revealed an 85.90% purity, highlighting the need for further purification to achieve higher purity levels (Figure 15).

Overall, HPLC analysis of the final downstream xylitol product demonstrated a purity of 85.90%, as evidenced by a prominent peak at a retention time of 8.926 min, aligning with the xylitol standard (Figure 15). While this initial purity is encouraging, it falls short of the pharmaceutical-grade purity standard of typically > 99% [43]. The presence of approximately 10% unidentified impurities underscores the necessity for rigorous purification procedures. Common contaminants encountered in xylitol production include residual sugars, organic acids, and salts [44]. These impurities can adversely affect the quality and efficacy of xylitol, necessitating their removal to achieve the desired purity levels. To attain the desired purity levels, the integration of chromatographic techniques, such as ion exchange chromatography or adsorption chromatography, and membrane filtration could further reduce impurities to achieve > 99% purity [19]. Moreover, membrane-based separation processes have demonstrated potential in the purification of biobased products [45]. Additional avenues for enhancing product purity include the meticulous optimization of downstream processing parameters, such as temperature, pH, and residence time. Electrodialysis is an energy-efficient technology for eliminating salts and impurities, simplifying the process while obtaining higher purity. Food-grade adsorbents such as bentonite or kaolin, eco-friendly solvent crystallization with isopropanol, enzymatic treatments to break down residual polysaccharides or proteins, and mineral impurity removal via selective precipitation with calcium or phosphate salts are all cost-effective alternatives [46, 47].

4. Conclusion

This study pioneers the bioconversion of brewer's waste grain (BSG), a byproduct of the brewing industry, into high-value xylitol using *Pichia fermentans*, providing a unique and cost-effective alternative to standard xylose-rich substrates. The optimized fermentation process produced a maximum xylitol concentration of 34.57 g/L, and downstream processing successfully recovered xylitol crystals with 85.90% purity. Unlike existing techniques that rely on refined substrates, this work shows BSG as a sustainable, low-cost feedstock, transforming a plentiful industrial waste stream into a commercially valuable product. While these results show that BSG-derived xylitol synthesis is feasible, further crystallization and purification stages must be optimized to obtain pharmaceutical-grade purity. Future research should concentrate on scaling up from laboratory bioreactors to industrial pilot plants, performing technoeconomic evaluations, and assessing the environmental impact. This study establishes the groundwork for an innovative, circular bioeconomy approach to xylitol production that converts waste into a sustainable, in-demand sweetener.

Data Availability Statement

All raw data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Ethics Statement

This study did not involve human or animal subjects, and no ethical approval was required.

Disclosure

This manuscript has not been published and is not under consideration for publication elsewhere.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization, S.M., D1.K., and V.K; validation, D1.K., V.K., and D6.K.; formal analysis, D1.K., D6.K., V.K., and R. V, data curation, S.M.; writing-original draft preparation, S.M; writing—review and editing, S.M.; visualization, S.M.; supervision, D1.K., V.K., D6.K., R.V., and H.L. All authors have read and agreed to the published version of the manuscript. All authors meet the ICMJE criteria.

Funding

No funding was received for this research.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)* (1) Raw HPLC chromatograms for sugar profiles. This file contains the four raw chromatograms obtained from HPLC analysis, showing xylitol profile present in the samples analyzed compared with standard graphs. (2) Detailed purification protocol. This file provides a step-by-step protocol detailing the purification process used for xylitol separation and crystallization.

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Mathur S, Kumar D, Kumar V, et al., (2025) Xylitol production from brewer’s spent grain via Pichia fermentans fermentation: optimization, scaling, and isolation. Journal of Food Processing and Preservation, Volume 2025, Article number 9721088

https://doi.org/10.1155/jfpp/9721088

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