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FERMENTATIVE PRODUCTION OF XYLITOL FROM AGRO-INDUSTRIAL BIOMASS USING NOVEL INDIGENOUS BACTERIAL ISOLATES

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ABSTRACT

This study focuses on the microbial production of xylitol, a low-calorie sugar alcohol widely used in food and pharmaceutical applications, using bacterial strains isolated from agricultural soils. Unlike conventional chemical methods that rely on high temperature, pressure, and costly catalysts, this work explores a more sustainable and economical alternative based on microbial fermentation of agricultural waste substrates. A total of twenty microbial isolates were obtained and screened for their ability to utilize xylose and produce xylitol, out of which four bacterial strains—*Escherichia coli*, *Pseudomonas* sp., *Citrobacter* sp., and *Enterobacter* sp.—were selected for further evaluation. Process optimization was carried out using Response surface methodology (RSM) with a central composite design to study the combined effects of pH, inoculum concentration, incubation time, D-xylose, and yeast extract. Among the tested strains, *Citrobacter* sp. showed the best performance, with a maximum xylitol concentration of 124.2 g/L observed at 102 hours. The model predicted optimal conditions at pH 7, 3 % inoculum, and 120 hours of incubation. Overall, the findings suggest that *Citrobacter* sp. has good potential for converting low-cost agricultural residues into xylitol, supporting the development of more sustainable and economically viable bioprocesses.
Additional Keywords: Agro-industrial residues, *Citrobacter sp.*, indigenous bacteria, sustainable bioeconomy, xylitol

RESUMEN

Producción fermentativa de xilitol a partir de biomasa agroindustrial utilizando aislados bacterianos autóctonos

Este estudio se centra en la producción microbiana de xilitol, un alcohol de azúcar de bajo contenido calórico ampliamente utilizado en aplicaciones alimentarias y farmacéuticas, empleando cepas bacterianas aisladas de suelos agrícolas. A diferencia de los métodos químicos convencionales, que dependen de altas temperaturas, presiones y catalizadores costosos, se explora una alternativa más sostenible y económica basada en la fermentación microbiana de sustratos derivados de residuos agrícolas. Se obtuvieron y evaluaron veinte aislados microbianos por su capacidad para utilizar xilosa y producir xilitol; de ellos, se seleccionaron cuatro cepas bacterianas (*Escherichia coli*, *Pseudomonas* sp., *Citrobacter* sp. y *Enterobacter* sp.) para un análisis más exhaustivo. La optimización del proceso se llevó a cabo mediante la metodología de superficie de respuesta (RSM) con un diseño compuesto central, con el fin de estudiar los efectos combinados del pH, la concentración del inóculo, el tiempo de incubación, la D-xilosa y el extracto de levadura. Entre las cepas evaluadas, *Citrobacter* sp. mostró el mejor rendimiento, alcanzando una concentración máxima de xilitol de 124,2 $g \cdot L^{-1}$ a las 102 horas. El modelo predijo condiciones óptimas a un pH de 7, con un 3 % de inóculo y 120 horas de incubación. En conjunto, los resultados sugieren que *Citrobacter* sp. posee un gran potencial para transformar residuos agrícolas de bajo costo en xilitol, lo que respalda el desarrollo de bioprocesos más sostenibles y económicamente viables.
Palabras clave adicionales: Bacterias autóctonas, bioeconomía sostenible, *Citrobacter* sp., residuos agroindustriales, xilitol

INTRODUCTION

Xylitol, chemically known as (2R, 4S)-pentane-1,2,3,4,5-pentol, is a sugar alcohol that has gained remarkable importance as a natural low-calorie sweetener (Wal *et al.*, 2019). Possessing sweetness comparable to sucrose but with about 40 % fewer calories, it provides approximately 2.4 kcal per gram compared to 3.87 kcal per gram of table sugar. Naturally present in small amounts in fruits, vegetables, oats, and mushrooms, xylitol is typically produced from fibrous materials such as corn cobs and sugarcane bagasse (Espinoza, 2020).

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These nutritional and functional benefits have expanded xylitol's applications in food, confectionery, and pharmaceuticals, including use as a sweetener in medicines, chewing gums, and oral care formulations (Gasmi *et al.,* 2020).

Commercially, xylitol has been produced by catalytic hydrogenation of xylose using nickel catalysts under high temperature and pressure (Gomez *et al.,* 2019). However, the chemical synthesis route is energy-intensive, costly, and environmentally taxing due to its dependence on high-purity xylose and complex downstream processing. As an alternative, biotechnological production using microorganisms offers a sustainable and economically viable approach. Several yeasts, such as *Candida guilliermondii*, *Pichia stipitis*, *Candida tropicalis*, and *Pachysolen tannophilus*, can bioconvert xylose to xylitol via the reducing action of xylose reductase in the presence of NADPH (Saravanan *et al.,* 2023). This bioconversion pathway is largely influenced by the strains used and the composition of the fermentation medium. Although yeast-based production is well studied, the xylitol-producing potential of bacterial species remains underexplored despite their potential for faster growth rates and simpler cultivation requirements.

Given the growing industrial demand and high import dependency, particularly in countries such as India, it is crucial to explore cost-effective methods to produce xylitol using locally available lignocellulosic resources (Lugani *et al.,* 2020). Agricultural wastes, including sugarcane bagasse and corn cobs, are rich in hemicellulose, which can be hydrolyzed to release xylose and other fermentable sugars. Utilizing such agro-industrial residues not only reduces production costs but also provides an eco-friendly alternative to traditional methods (Espinoza, 2020).

This approach emphasizes the need to move away from conventional chemical hydrogenation toward microbial fermentation, which can provide a sustainable and scalable process that supports the circular bioeconomy. By designing an indigenous laboratory-scale fermenter system with a working volume of 15 litres, the study explores the potential to overcome mass transfer limitations and refine the fermentation conditions for optimal xylitol yield. Ultimately, this research contributes to developing cost-effective strategies for large-scale xylitol production from renewable agricultural residues, thereby reducing sugar dependency while addressing both economic and environmental sustainability. Also, this study represents the first systematic effort to isolate xylose reductase (XR) producing microbial strains specifically from agricultural waste soil, a novel approach not previously reported in the literature. While earlier research has primarily focused on isolating such strains from diverse ecological niches such as decomposed herbivore dung, oil palm waste, or sugars-rich industrial residues, agricultural waste soil a plentiful and renewable resource remains largely unexplored as a source for xylose reductase (XR) producers.

Therefore, the present research aims to isolate and characterize xylitol-producing bacterial strains such as *Escherichia coli*, *Pseudomonas sp*., *Citrobacter sp*., and *Enterobacter sp*., from agricultural soils and employ them for fermentative xylitol production using hemi cellulosic hydrolysates derived from agricultural waste. Optimization of key process parameters, including pH, temperature, aeration, and nutrient composition, is essential to enhance enzyme activity particularly xylose reductase and improve xylitol yield.

MATERIALS AND METHODS

The materials used in this experiment included 3,5-dinitrosalicylic acid (analytical grade DNSA), potassium sodium tartrate tetrahydrate, phloroglucinol, glacial acetic acid, hydrochloric acid (HCl), and sodium hydroxide (NaOH). The 3,5-dinitrosalicylic acid reagent (IUPAC name: 2-hydroxy-3,5-dinitrobenzoic acid) is an aromatic compound that reacts with reducing sugars to form 3-amino-5-nitrosalicylic acid, which exhibits a strong absorbance at 540 nm. This property makes it a valuable reagent for quantifying reducing sugars in biochemical assays.

Collection of agro-industrial wastes: soil samples. Different soil samples were collected from various locations, including agricultural fields and wastelands, to isolate xylose reductase (XR)-producing microbial cultures. The samples were aseptically collected using pre-sterilized polythene bags and sterile spatulas, then stored at 4 °C until further use.

Enrichment and isolation of microbial strains. For the enrichment and isolation of

microbial strains, each soil sample (1 % w/v) was separately inoculated into pre-sterilized enrichment media (pH 7.0-5.0) contained in individual flasks with xylose (10.0 $g \cdot L^{-1}$) and peptone (3.0 $g \cdot L^{-1}$) to promote the growth of XR-producing microorganisms. The flasks were incubated, at 37 and 30 °C for 24-48 hours, to facilitate microbial proliferation. Xylose was included in the enrichment media to support the growth of xylose-utilizing microorganisms, since XR is required for xylose metabolism. Following incubation, aliquots from the enrichment culture were streaked onto agar plates containing xylose (10.0 $g \cdot L^{-1}$), peptone (3.0 $g \cdot L^{-1}$), and agar (20.0 $g \cdot L^{-1}$). The plates were incubated at 37 °C, and pure cultures were obtained by repeated streaking (3-4 times). The purified bacterial and yeast isolates were maintained on nutrient agar slants comprising peptone (5.0 $g \cdot L^{-1}$), NaCl (5.0 $g \cdot L^{-1}$), beef extract (1.5 $g \cdot L^{-1}$), yeast extract (1.5 $g \cdot L^{-1}$), and agar (20.0 $g \cdot L^{-1}$) at pH 7.0. Mold isolates were maintained on potato dextrose agar (PDA) slants containing potato extract (200.0 $g \cdot L^{-1}$), dextrose (20.0 $g \cdot L^{-1}$), and agar (20.0 $g \cdot L^{-1}$) at pH 5.0. A total of 20 microbial strains (5 bacterial, 5 fungal and 10 yeasts) were stored at 4 ± 1 °C and sub-cultured aseptically every two weeks for maintenance (Kusumawati *et al*., 2023).

Screening of xylose reductase producing cultures. For the screening of xylose reductase-producing cultures, starter cultures of the isolates were prepared using media containing peptone (3.0 $g \cdot L^{-1}$) and dextrose (5.0 $g \cdot L^{-1}$) at pH 7.0 for bacteria, and pH 5.0 for yeast and mold cultures. Bacterial starter cultures were incubated at 37 °C for 12-24 hours at 150 rpm, while yeast and mold cultures were incubated at 30 °C for 24-48 hours. Sterilized fermentation media containing xylose (5.0 $g \cdot L^{-1}$) and peptone (3.0 $g \cdot L^{-1}$) were prepared separately for bacterial and fungal (yeast and mold) cultures, adjusted to pH 7.0 and 5.0, respectively. Each medium was inoculated with 10 % (v/v) of the respective starter culture. Bacterial and yeast flasks were incubated at 37 and 30 °C, respectively, for 48-96 hours at 150 rpm, whereas mold cultures were incubated under stationary conditions at 30 °C. The cell biomass was harvested by centrifugation at 5000 × g for 10 minutes at 4 °C (Eppendorf 5840R). Intracellular and extracellular XR activities were analysed in the cell pellet and supernatant, respectively. Prior to disruption by sonication (20 kHz, VCX 500 Sonics), the cells were pretreated with EDTA and β-mercaptoethanol for enhanced intracellular enzyme extraction (Kaur *et al*., 2023).

Estimation of xylanase activity. Xylose-degrading enzymes catalyse the hydrolysis of the linear polysaccharide xylan into xylose. The 3,5-dinitrosalicylic acid (DNSA) method was used to quantify xylose released during the reaction. In this assay, birchwood xylan in acetate buffer served as the substrate. Approximately 600 µL of 2 % xylan and the crude enzyme (culture supernatant) were combined, followed by the addition of 800 µL of distilled water to make up a total volume of 2000 µL. The mixture was incubated at 50 °C for 30 minutes in a water bath. Subsequently, 2 mL of DNSA reagent was added, and the mixture was boiled for 10 minutes to develop colour. A control without enzyme was maintained under identical conditions. The absorbance was measured at 540 nm. One unit of xylanase activity ($U \cdot mL^{-1}$) was defined as the amount of enzyme that liberates 1 µmol of xylose equivalent per minute under the specified assay conditions (Dhaver *et al*., 2022).

Xylose estimation. For xylose estimation, 0.5 g of phloroglucinol was dissolved in 100 mL of glacial acetic acid and mixed with 10 mL of concentrated HCl to prepare the colour reagent. A 200 µL sample aliquot was added to 5 mL of this reagent, heated at 100 °C for 4 minutes, and then cooled to room temperature. The absorbance of the generated colour complex was recorded at 540 nm using a UV-visible spectrophotometer (Hermida *et al*., 2020).

Xylitol production under optimal conditions. Optimization of xylitol production was carried out using Response Surface Methodology (RSM) to evaluate the combined influence of multiple process variables. A Central Composite Design (CCD) was applied, with two variables, considering five important factors-pH, inoculum concentration, incubation time, D-xylose concentration, and yeast extract concentration-each examined at five levels and three replicates at the central point, was employed to fit a second-order response surface model. The experimental layout included factorial points, axial (star) points (2k, where k is the number of variables), and replicated centre points. These

centre point replicates were particularly useful for estimating pure experimental error and assessing the reproducibility of the system, although it is recognized that they alone are not sufficient to judge model adequacy; therefore, the suitability and significance of the model were further confirmed through appropriate statistical analysis. Rotatability of the design was maintained by selecting a suitable axial distance (α), ensuring consistent prediction variance across the experimental space. The optimal conditions for xylitol production were identified by examining both the individual and interaction effects of the variables using the developed model. In parallel, preliminary experiments were conducted to study the effect of pH (3, 7, and 9) on bacterial growth, where optical density measurements indicated that pH 7 supported the highest growth; however, xylitol production itself was evaluated separately under the optimized conditions obtained through the RSM approach (Singh *et al*., 2024).

RESULTS AND DISCUSSION

For commercial usage, researchers were concentrated on finding effective microbial sources with high XR output at cheap cost (Lugani *et al.,* 2021). Thus, the goal of the current investigation has been to isolate an effective xylose reductase-producing bacterium that can quickly and efficiently create a large amount of xylitol.

The isolates from the soil sample collection were screened for their potential for Xylose Reductase production based on extracellular and intracellular activities along with their NADPH and NADH specificity. After screening for xylose reductase production, the four isolates which showed positive were subjected to biochemical tests and tentatively identified as *Escherichia coli, Pseudomonas sp., Citrobacter sp., and Enterobacter sp.,* The OD values obtained for each bacterium in the xylose activity phase are obtained at 540 nm. Among the four bacteria, *Citrobacter* has the highest activity illustrated by the high OD value of 1.05 compared to the other three bacterial strains.

Table 1 lists the xylitol production in terms of OD values. This method was used as a qualitative method to differentiate the better xylitol producer from the isolated set of organisms. From the estimated values for xylitol production show that the *Citrobacter* produces the highest amount of xylitol (124.2 $g \cdot L^{-1}$). In similar conditions, the other three bacterial strains consume more time but produces less amount of xylitol. Thus, it can be concluded that the selected bacteria *Citrobacter sp.* can be used in the production of xylitol which emerged as a top candidate. This pioneering exploration not only fills a critical knowledge gap but also opens new avenues for utilizing agricultural residues in enzyme production and value-added bioproducts, thereby enhancing the eco-friendly biotechnological exploitation of lignocellulosic biomass. The novelty and significance of this study are underscored by the distinct source of isolation and its potential impact on microbial xylitol production applications.

Table 1. Xylose estimation**.**

Bacteria	OD values	Xylitol ($g \cdot L^{-1}$)
Pseudomonas	0.05	82.17
E.coli	0.03	54.23
Enterobacter	0.02	35.45
Citrobacter	0.07	124.2

There is very little information on bacterial XR, and most of the prior research on XR-producing microbes has been identified with yeasts. A few numbers of bacteria were discovered to make XR, including *Mycobacterium smegmatis*, *Serratia marcescens*, *Cellulomonas cellulans*, *Enterobacter liquefacien*s, and *Corynebacterium sp*. (Domingues *et al.,* 2021) but there is no literature available on the fermentative production of NADPH dependent XR from *Citrobacter sp.* Hence, the selected isolate i.e. *Citrobacter sp*. has been proved as an efficient culture with maximum NADPH dependent XR activity amongst bacterial cultures.

The Response Surface Methodology (RSM) used to optimize the variables aligns with the approach in modern biotechnological studies to enhance xylitol yields efficiently. 26 conical flasks were used. Each of the conical flasks had been supplied with 500µl of bacteria sample culture. Each conical flask was maintained at room temperature with different pH and different inoculum concentration. Additionally, incubation time, D-Xylose and yeast extract were also varied from 1 flask to another. Then, the cultures are transferred to 26 test tubes for testing**.** Finally, the readings were taken using at 540nm using a UV-visible spectrophotometer. These readings were used to determine the optimal conditions for xylitol production by the bacteria. Table 2 presents the results.

Table 2 presents the experimental results obtained under different combinations of process variables. Although the highest observed xylitol concentration (121 $g \cdot L^{-1}$) was recorded in the 18th run, corresponding to pH 7, inoculum concentration of 3 %, incubation time of 120 hours, D-xylose concentration of 10 %, and yeast extract concentration of 0.6 %, this value alone should not be considered as the definitive optimum. The optimal conditions were determined using Response Surface Methodology (RSM) based on a Central Composite Design (CCD), which evaluates both the individual and interactive effects of the variables through a fitted second-order model. The three-dimensional response surface plots (Figure 1) illustrate these interactions and help identify the region of maximum xylitol production more reliably than individual experimental runs.

Furthermore, Figure 2 shows the time-course profile of xylitol production from agricultural wastes by different bacterial strains. It can be observed that xylitol production increases up to a certain incubation period and then gradually declines. Among the strains studied, Citrobacter sp. exhibited the highest production, reaching 124.2 $g \cdot L^{-1}$ at 102 hours before decreasing thereafter. In comparison, the other bacterial strains produced relatively lower amounts of xylitol under similar conditions.

Under the optimized conditions predicted by RSM, the *Citrobacter sp*. demonstrated a maximum xylitol production of 121 $g \cdot L^{-1}$ at pH 7, inoculum concentration of 3 %, incubation time of 120 hours, D-xylose concentration of 10 %, and yeast extract concentration of 0.6 %. In addition, time-course analysis revealed that the strain achieved a peak production of 124.2 $g \cdot L^{-1}$ at 102 hours, after which a decline in xylitol concentration was observed, indicating possible product re-utilization or metabolic shift. These values clearly indicate that Citrobacter sp. exhibits high volumetric productivity under the tested conditions.

In comparison to commonly reported yeast producers such as Candida and Wickerhamomyces, which typically yield high xylitol concentrations under controlled conditions, the present bacterial isolate demonstrates comparable productivity levels. Moreover, the Citrobacter strain offers practical advantages, including faster growth rate, shorter generation time, and the ability to produce xylitol under relatively simple and flexible conditions. This suggests that the strain could serve as a promising alternative for industrial applications involving xylose reductase-mediated bioconversion.

The findings are consistent with earlier reports that both bacteria and yeasts are capable of efficient xylitol production, and that Citrobacter species have shown potential for high-yield production under optimized conditions. Additionally, yeast strains such as Candida and Wickerhamomyces are known to exhibit optimal xylitol production at mildly acidic pH (5-7) and temperatures ranging from 30 to 37 °C (Zahoor *et al.*, 2021). While the present study was conducted at room temperature, these observations suggest that further optimization of temperature could enhance production even further.

The optimal conditions identified for xylitol production by *Citrobacter* sp. (pH 7, incubation time of 120 hours, and inoculum concentration of 3 %) agreed with previous reports indicating that bacterial xylitol production is generally favoured near neutral pH and longer incubation periods. In the present analysis, although the model evaluated five independent variables using RSM, the effects of D-xylose concentration (factor D) and yeast extract concentration (factor E) were not highlighted in this specific comparison because they were maintained at their central levels (10 % and 0.6 %, respectively). This approach allows clearer interpretation of the primary variables while minimizing additional variability.

The selection of these central values is justified, as they represent balanced conditions within the experimental range and are commonly used in Central composite design to evaluate the effects of other factors more effectively. Furthermore, like observations in yeast-based systems, oxygen availability is known to influence xylitol production, where reduced aeration often enhances yield. This suggests that, in addition to the variables studied, factors such as aeration may also play a significant role in optimizing production depending on the microbial system.

Table 2. Optimum conditions for xylitol production**.**

RUN	A: pH	B: Inoculum concentration (%)	C: Incubation time (hours)	D: D-Xylose (%)	E: Yeast extract (%)	Response: Xylanase activity ($U \cdot mL^{-1}$)	Xylitol ($g \cdot L^{-1}$)
1	7	3	120	10	0.6	0.09	76
2	3	4.5	48	15	0.9	0.07	65
3	7	3	120	10	0.6	0.08	71
4	3	4.5	192	15	0.3	0.09	75
5	9	4.5	48	15	0.3	0.11	98
6	3	1.5	48	5	0.3	0.12	101
7	9	3	120	10	0.6	0.06	58
8	9	4.5	48	5	0.9	0.05	49
9	7	3	192	10	0.6	0.08	77
10	7	3	120	10	0.6	0.11	103
11	7	3	120	5	0.6	0.10	93
12	7	3	120	10	0.6	0.07	63
13	9	1.5	48	15	0.9	0.06	52
14	9	1.5	192	15	0.3	0.07	61
15	3	1.5	192	15	0.9	0.09	79
16	9	4.5	192	5	0.3	0.13	110
17	7	1.5	120	10	0.6	0.12	104
18	7	3	120	10	0.6	0.155	121
19	7	3	120	10	0.3	0.05	45
20	7	3	120	15	0.6	0.09	78
21	3	3	120	10	0.6	0.06	51
22	7	4.5	120	10	0.6	0.08	72
23	7	3	120	10	0.9	0.11	102
24	3	4.5	192	5	0.9	0.06	54
25	9	1.5	192	5	0.9	0.09	82
26	7	3	48	10	0.6	0.07	63

The emphasis on NADPH and NADH specificity in the isolated bacteria reflects the key understanding that XR's coenzyme dependency affects xylitol yield and efficiency. This is supported by detailed biochemical studies showing variable XR activity impacts. The reported highest optical density (OD) for *Citrobacter sp.* in xylose activity suggests strong enzymatic conversion capability, which is compatible with the role of xylose reductase as a rate-limiting enzyme in many microbial xylitol-producing pathways (Ranieri *et al*., 2024).

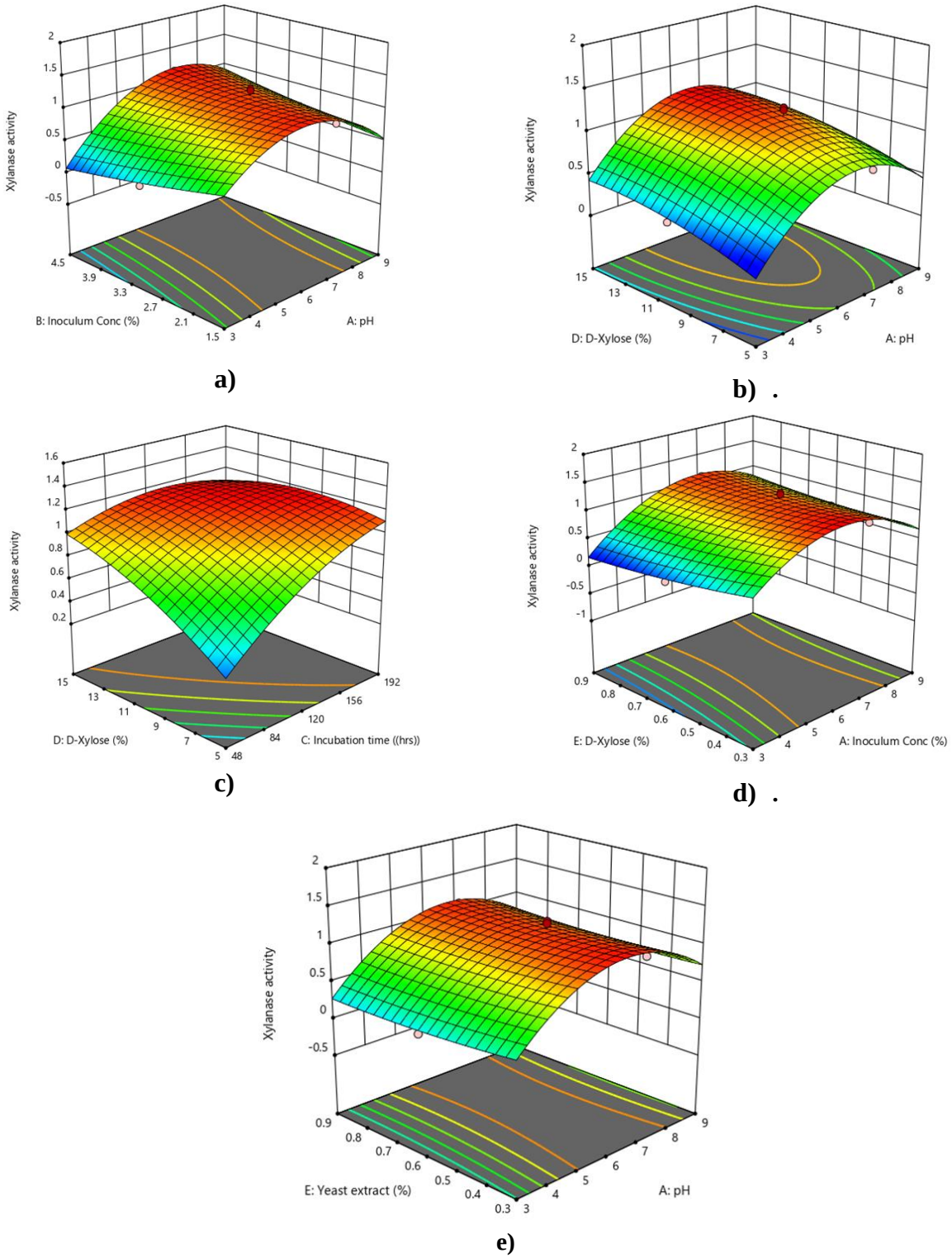

Figure 1. Interaction effects on xylanase activity. a: pH and Inoculum concentration on xylanase activity. b: pH and D-Xylose on xylanase activity. c: D-Xylose and Incubation time on xylanase activity. d: D-Xylose and Inoculum concentration on xylanase activity. e: pH and yeast extract on xylanase activity.

The xylitol yield of 124.2 g·L^{-1}obtained from *Citrobacter* sp. in the present study appears notably high when compared with previously reported bacterial systems. For example, *Pseudomonas putida* has been reported to produce approximately 35.2 g·L^{-1} xylitol under batch fermentation conditions (Lugani and Sooch, 2020), while *Pseudomonas gessardii* achieved yields in the range of 48-72 g·L^{-1} from lignocellulosic hydrolysates (Ravindran *et al*., 2022). In contrast, metabolically engineered strains such as *Corynebacterium glutamicum* have demonstrated higher production levels, reaching up to 166 g·L^{-1}under optimized fed-batch conditions, although such systems rely on genetic modification and tightly controlled fermentation parameters (Becker *et al*., 2011). Yeast-based systems, including *Candida* and *Wickerhamomyces* species, typically produce between 80 and 110 g·L^{-1} xylitol under optimized conditions (Zahoor *et al*., 2021), placing the present findings within the upper range of reported microbial production. These comparisons suggest that the *Citrobacter* isolate possesses promising potential for xylitol production. Furthermore, the use of agricultural waste as a substrate supports the growing emphasis on sustainable bioprocessing and aligns with current trends in the circular bioeconomy, where low-cost lignocellulosic biomass is valorised into high-value products (Kumar *et al*., 2022).

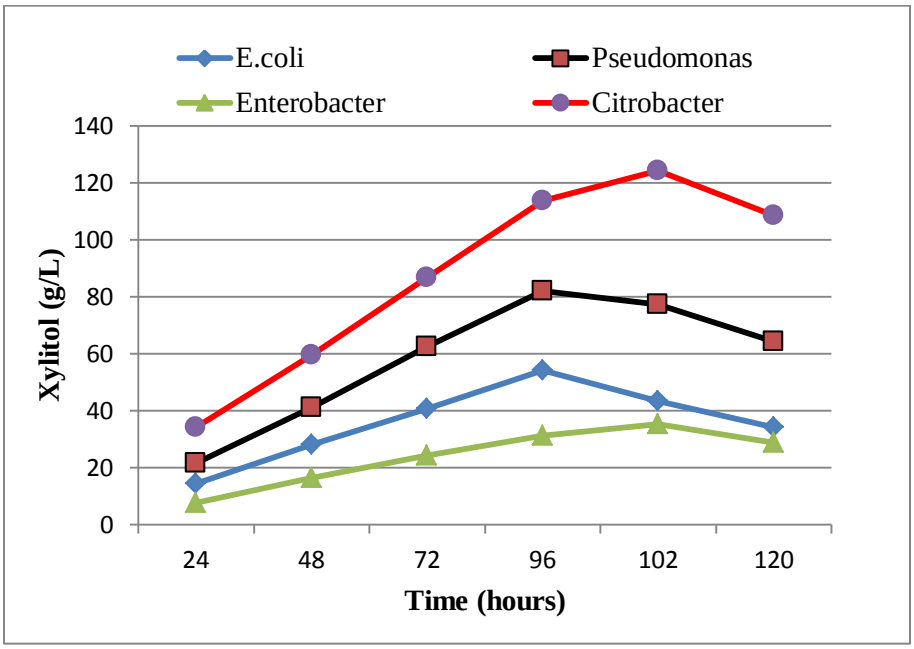

Figure 2. Xylitol Production from agricultural wastes.

The xylitol concentration of 124.2 g·L^{-1} achieved by *Citrobacter* sp. in this study is relatively high when viewed alongside values reported for other bacterial systems. For example, *Pseudomonas putida* has been shown to produce about 35.2 g·L^{-1} under batch fermentation conditions (Lugani and Sooch, 2020), whereas *Pseudomonas gessardii* typically yields between 48 and 72 g·L^{-1}when grown under optimized conditions (Ravindran *et al*., 2022). In this context, the performance of the present isolate suggests that it could be a promising candidate for efficient xylitol production. Another important aspect of this work is the use of agricultural residues as substrates, which supports a more sustainable approach to bioprocessing. Converting low-cost lignocellulosic materials into value-added products such as xylitol is increasingly being explored within industrial biotechnology, particularly in line with circular bioeconomy

principles that aim to reduce waste while improving resource utilization (Kumar *et al*., 2022).

The successful isolation and optimization of xylitol-producing bacteria from agricultural soil contribute valuable strains to the biotechnological toolkit. The predominance of *Citrobacter* in producing the highest xylitol concentration, combined with optimal pH and nutrient conditions, suggests it could be a superior candidate for scale-up fermentation processes compared to more commonly studied yeasts and bacteria. The screening and bioprocess optimization methods adopted are in line with current best practices in microbial biotechnology research, ensuring reproducibility and industrial relevance.

Moreover, the results underscore the importance of understanding enzyme cofactor specificity and activity in maximizing bioconversion efficiency. Future work might explore genetic or metabolic engineering of these strains to further enhance production, as well as investigate the effects of aeration, temperature adjustments, and substrate heterogeneity on product yields.

CONCLUSIONS

From a total of 20 isolated microbial strains, four bacterial species candidates *Escherichia coli*, *Pseudomonas sp.*, *Citrobacter sp*, and *Enterobacter sp*. were selected based on their ability to utilize xylose efficiently. *Citrobacter sp*, demonstrated the highest xylitol yield, confirming its superior xylose reductase activity as validated through xylose activity and estimation assays using colorimetric analysis.

Optimization of the process parameters using Response Surface Methodology identified pH 7.0, inoculum concentration 3 %, D-xylose 10 %, yeast extract 0.6 %, and an incubation period of 120 hours as the model-predicted optimum conditions for xylitol production. However, based on the experimental data, the highest xylitol concentration observed was 124.2 $g \cdot L^{-1}$ at 102 hours. This distinction is important, as the statistical model predicts an optimum within the design space, whereas the experimentally measured maximum reflects the actual system performance under specific conditions. The corresponding volumetric at 102 hours was approximately 1.22 g/L/h. These results indicate that *Citrobacter* sp. is capable of efficient xylose conversion, although slight differences between predicted and observed values are expected due to biological variability and model limitations.

Furthermore, the use of agro-industrial waste as a substrate emphasizes the sustainability of the process and supports its relevance in current biotechnological applications focused on biomass valorisation. Overall, while the model provides a useful framework for optimization, the experimentally observed maximum response is considered more representative of the system's performance in this study.

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