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Bioethanol Production from Jowar Husk (*Sorghum bicolor*) Using Indigenous Cellulase-Producing Yeast *Geotrichum candidum.*

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KEYWORDS

Bioethanol production, Jowar husk (Sorghum bicolor), Geotrichum candidum, Lignocellulosic biomass, Cellulase enzyme.

ABSTRACT:

Growing interest in sustainable biofuels has encouraged the exploration of agricultural residues as alternative energy resources. In this study, indigenous yeast strains were isolated from agro-organic substrates and evaluated for their ability to convert lignocellulosic biomass into bioethanol using jowar husk (Sorghum bicolor) as the feedstock. Soil, compost, fruit waste, and sugarcane bagasse samples were collected from Raipur, Chhattisgarh, and screened for yeast abundance and cellulolytic potential. Marked variation in yeast populations was observed, with fruit waste and compost supporting comparatively higher counts. Among the isolates obtained, a cellulase-producing strain identified as Geotrichum candidum demonstrated superior enzymatic performance based on morphological, microscopic, and molecular analyses. Alkali-pretreated jowar husk was subjected to enzymatic hydrolysis using crude cellulase derived from this isolate, resulting in the release of 45–70 g L^{-1} fermentable sugars. Subsequent fermentation of the hydrolysate produced 18–22 g L^{-1} ethanol after 72 hours, corresponding to a conversion efficiency of approximately 0.40–0.42 g g^{-1} of fermentable sugars. Product confirmation was carried out using FTIR spectroscopy, which showed characteristic absorption bands associated with alcohol functional groups, while HPLC analysis revealed a prominent ethanol peak, indicating successful fermentation. The results support the potential of agro-waste-derived yeasts and jowar husk as a viable combination for bioethanol production and highlight the relevance of regionally available biomass in sustainable bioenergy strategies.

Introduction

The continued reliance on fossil fuels has raised serious concerns regarding resource depletion and environmental sustainability. As a result, renewable biofuels have gained considerable attention, with bioethanol emerging as one of the most practical alternatives due to its compatibility with existing fuel systems and comparatively lower greenhouse gas emissions (Moreira et al., 2001). While first-generation bioethanol relies on food-based feedstocks, increasing emphasis is now placed on lignocellulosic biomass, which is abundant, inexpensive, and does not compete directly with food supplies. Agricultural residues represent a particularly attractive resource in this context, offering dual benefits of energy recovery and improved waste management (Chengappa et al., 1984).

In India, large quantities of crop residues are generated annually, and a significant portion is either underutilized or disposed of through open-field burning. Jowar (*Sorghum bicolor*), widely cultivated across semi-arid regions, produces substantial amounts of husk as a by-product (Fung et al., 1990). Despite its high cellulose and hemicellulose content, jowar husk is frequently discarded or used inefficiently as low-grade feed. Converting this lignocellulosic residue into bioethanol presents an opportunity to enhance resource efficiency while supporting sustainable energy initiatives (Diosma et al., 2014).

The bioconversion of lignocellulosic biomass, however, is inherently complex. The rigid structure of plant cell walls, primarily due to lignin crosslinking, restricts enzyme accessibility and limits saccharification efficiency. Effective pretreatment is therefore essential to improve enzymatic hydrolysis (Emam et al., 2023). Alkali pretreatment has been widely applied to agricultural residues because it facilitates partial lignin removal under relatively mild conditions, thereby enhancing cellulose availability. Following pretreatment, cellulolytic enzymes catalyze the breakdown of structural polysaccharides into fermentable sugars.

A major constraint in this process remains the cost of commercial enzyme preparations (Oyeleke & Jibrin,

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2009). This limitation has stimulated interest in exploring indigenous microorganisms capable of producing cellulases efficiently and economically. Yeasts isolated from carbohydrate-rich environments are of particular relevance due to their adaptability, extracellular enzyme secretion, and resilience under variable operational conditions. Compost, fruit waste, and agro-residues provide ecological niches where metabolically versatile yeasts naturally thrive (Lamichhane et al., 2023).

Among such microorganisms, *Geotrichum candidum* has attracted attention for its enzymatic diversity, including cellulase and hemicellulase production. Although extensively studied in food fermentation and certain biotechnological applications, its application in lignocellulosic bioethanol systems remains comparatively less explored. Utilizing cellulase-producing strains derived from local environments may provide a sustainable alternative to commercially sourced enzymes (Ratanawimarnwong et al., 2022; Mgeni et al., 2025).

In light of these considerations, the present study focused on isolating and screening yeast strains from agro-organic substrates for cellulolytic activity and assessing their application in bioethanol production from jowar husk. By integrating microbial screening, enzymatic hydrolysis, and fermentation processes, this work seeks to evaluate the feasibility of developing a cost-effective and environmentally sustainable approach to agricultural residue valorization (Sakakihara et al., 2024).

Materials and Methods:

Sample Collection

Environmental samples, including soil, compost, fruit waste, and sugarcane bagasse, were obtained from different locations across Raipur, Chhattisgarh, India. Care was taken to collect the materials aseptically using sterile containers to prevent external contamination. Each sample was clearly labeled with details of the collection site along with the date and time of sampling before being transported to the laboratory under ambient conditions. To preserve microbial viability and minimize changes in population dynamics, processing was initiated within 24 hours of collection (Saulawa et al., 2021).

Enrichment and Isolation of Yeasts

To promote the growth of cellulolytic and xylanolytic yeasts, portions of each environmental sample were introduced into liquid enrichment media containing cellulose and xylan as the primary carbon sources. The cultures were maintained at 30°C under static conditions, creating an environment that supported yeast proliferation while limiting the dominance of rapidly growing bacteria. After the enrichment phase, aliquots were streaked onto the corresponding solid media and incubated at 30°C for 48–72 hours. Distinct colonies displaying typical yeast characteristics—smooth texture, creamy appearance, and circular margins—were selected for further purification. Repeated subculturing was performed until morphologically uniform isolates were obtained (Rabah et al., 2011).

Enumeration of Yeast Population

The yeast load present in each environmental sample was determined using the serial dilution and plate count technique. Briefly, samples were diluted stepwise in sterile diluent, and appropriate dilutions were spread in triplicate onto suitable agar media. Plates were incubated at 30°C, after which visible colonies were enumerated. Yeast density was calculated as colony-forming units per gram (CFU g^{-1}) based on the average colony counts obtained from replicate plates (Lee et al., 2012).

Morphological and Microscopic Characterization

Purified yeast isolates were initially examined for colony morphology on Yeast Extract Peptone Dextrose (YPD) agar following 24–48 hours of incubation. Observable characteristics such as colony color, surface texture, margin, elevation, and overall growth pattern were documented for comparative analysis. Microscopic evaluation was carried out using cultures grown on Potato Dextrose Agar (PDA). Gram staining was performed to assess cellular morphology, including cell shape, budding characteristics, and arrangement, under a light microscope (Saleh et al., 2013).

Molecular Identification of the Potent Yeast Isolate

The isolate exhibiting the highest enzyme-producing capability was subjected to molecular identification. Genomic DNA was extracted using standard protocols, followed by amplification of ribosomal DNA regions through PCR. The amplified products were sequenced,

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and the resulting nucleotide sequence was analyzed for similarity against reference sequences available in public databases using BLAST. Species-level identification was assigned based on sequence homology and alignment results (Visioli et al., 2014).

Raw Material Collection and Preparation for Bioethanol Production

Millet husk (jowar husk; Sorghum bicolor) was procured from local millet processing units and agricultural fields in the Raipur district of Chhattisgarh, India. The collected biomass was first washed thoroughly with distilled water to remove adhering soil particles and residual debris, followed by air-drying at room temperature. Subsequently, the material was oven-dried at 60°C for 24 hours until a constant weight was attained. The dried husk was mechanically milled and sieved to achieve a uniform particle size ranging between 250 and 500 µm, ensuring consistency during pretreatment and enzymatic hydrolysis (Izah & Ohimain, 2015).

Pretreatment of Jowar Husk

To enhance the accessibility of structural carbohydrates, the milled jowar husk was subjected to alkali pretreatment. The biomass was treated with 2% (w/v) sodium hydroxide at a solid-to-liquid ratio of 1:10 (w/v) and autoclaved at 121°C for 30 minutes. After thermal treatment, the slurry was cooled to room temperature and repeatedly washed with distilled water to remove residual alkali until a neutral pH was attained. The pretreated material was subsequently oven-dried at 60°C, then stored in airtight containers for subsequent hydrolysis experiments (Yemets et al., 2020).

Enzymatic Hydrolysis of Pretreated Jowar Husk

Saccharification of the alkali-pretreated jowar husk was performed using crude cellulase produced by *Geotrichum candidum*. Hydrolysis reactions were conducted in 250 mL Erlenmeyer flasks containing 5% (w/v) pretreated biomass suspended in 50 mM sodium citrate buffer (pH 5.0). The crude enzyme extract was added to the reaction mixture, which was then incubated at 50°C with continuous agitation at 150 rpm for 48–72 hours under optimized conditions. At defined intervals, samples were withdrawn and centrifuged at 10,000 × g for 10 minutes to separate residual solids. The resulting supernatant was analyzed for reducing sugar concentration using the DNS method (Miller, 1959). Although the initial enrichment process employed both cellulose and xylan to select for lignocellulose-degrading yeasts, cellulase was prioritized during saccharification because jowar husk contains a comparatively higher proportion of cellulose (Kiran & Prasanna, 2023).

Fermentation for Bioethanol Production

The enzymatic hydrolysate obtained after saccharification served as the substrate for ethanol fermentation. Prior to inoculation, the hydrolysate was sterilized either by membrane filtration or by autoclaving at 121°C for 15 minutes to eliminate potential contaminants. Fermentation was carried out using *Saccharomyces cerevisiae* as the ethanologenic strain. The process was initiated with an inoculum size of 5% (v/v), and the initial sugar concentration of the medium was adjusted to 80–100 g L^{-1}. Incubation was performed at 30°C with the pH maintained between 4.5 and 5.0 under static conditions for 72 hours. Samples were withdrawn at 24-hour intervals to evaluate residual sugar levels and ethanol production (Nanda et al., 2024; Mtashobya et al., 2025).

FTIR Analysis of Bioethanol

Fourier Transform Infrared (FTIR) spectroscopy was performed to verify the presence of ethanol in the fermented jowar husk hydrolysate. Prior to analysis, samples were centrifuged at 10,000 × g for 10 minutes to remove yeast cells and residual particulate matter. The resulting supernatant was carefully collected and analyzed using an ATR-FTIR spectrophotometer. Spectral data were recorded within the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. Commercial ethanol served as the reference standard for comparison. Ethanol formation was confirmed by identifying characteristic absorption bands associated with O–H stretching, C–H stretching, and C–O stretching vibrations (Favaro et al., 2017).

HPLC Quantification of Ethanol

Ethanol concentration in the fermented samples was quantified using High Performance Liquid Chromatography (HPLC). Prior to analysis, fermentation broths were filtered through a 0.22 µm membrane filter to remove residual cells and suspended particles. Chromatographic separation was achieved using an Aminex HPX-87H column (300 × 7.8 mm) with 5 mM H_2SO_4 as the mobile phase delivered at a flow rate of 0.6

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mL min^{-1}. The column temperature was maintained at 60°C, and ethanol detection was carried out using a refractive index detector (RID) (Jeevan Kumar et al., 2020; Ramesh et al., 2025). An injection volume of 20 µL was employed for each run. Quantification was based on comparison of retention time and peak area with those of ethanol standards prepared within the concentration range of 0.5–10 g L^{-1}. A calibration curve generated from standard solutions was used to calculate ethanol concentration in the samples.

Results:

3.1 Yeast Population Density in Agro-Organic Samples

Yeast populations varied considerably among the different agro-organic substrates collected from Raipur, Chhattisgarh (Table 1). The total yeast count ranged from **0.038 × 10^{-5} to 820 × 10^{-5} CFU g^{-1}**, indicating strong substrate-dependent microbial abundance.

Fruit waste samples supported the highest yeast populations, with **sample S11** (fruit juice shop waste) recording the maximum yeast density (**820 × 10^{-5} CFU g^{-1}**), followed by **S9 (210 × 10^{-5} CFU g^{-1})**. Compost samples also showed substantial yeast abundance, particularly **S6 (109.33 × 10^{-5} CFU g^{-1})** and **S8 (72.67 × 10^{-5} CFU g^{-1})**. In contrast, soil and sugarcane bagasse samples exhibited comparatively lower yeast counts.

These results suggest that nutrient-rich substrates such as fruit waste and compost provide favorable conditions for yeast proliferation.

Table 1. Yeast sample collection from different area and their CFU Count with mean value.

S.No.	Sample ID	Sample type	R1	R2 ×10^{-5}	R3 ×10^{-5}	Mean ×10^{-5}
1	S1	Soil	0.23×10^{-5}	0.25×10^{-5}	0.21×10^{-5}	0.23×10^{-5}
2	S2	Soil	0.18×10^{-5}	0.20×10^{-5}	0.19×10^{-5}	0.19×10^{-5}
3	S3	Soil	0.035×10^{-5}	0.041×10^{-5}	0.038×10^{-5}	0.038×10^{-5}
4	S4	Soil	0.62×10^{-5}	0.59×10^{-5}	0.65×10^{-5}	0.62×10^{-5}
5	S5	Compost	32.0×10^{-5}	29.0×10^{-5}	35.0×10^{-5}	32.0×10^{-5}
6	S6	Compost	110.0×10^{-5}	98.0×10^{-5}	120.0×10^{-5}	109.33×10^{-5}
7	S7	Compost	4.50×10^{-5}	5.00×10^{-5}	4.80×10^{-5}	4.77×10^{-5}
8	S8	Compost	73.0×10^{-5}	69.0×10^{-5}	76.0×10^{-5}	72.67×10^{-5}
9	S9	Fruit waste	210.0×10^{-5}	190.0×10^{-5}	230.0×10^{-5}	210.0×10^{-5}
10	S10	Fruit waste	55.0×10^{-5}	61.0×10^{-5}	58.0×10^{-5}	58.0×10^{-5}
11	S11	Fruit waste	820.0×10^{-5}	790.0×10^{-5}	850.0×10^{-5}	820.0×10^{-5}
12	S12	Fruit waste	14.0×10^{-5}	16.0×10^{-5}	15.0×10^{-5}	15.0×10^{-5}
13	S13	Sugarcane bagasse	0.92×10^{-5}	1.10×10^{-5}	1.00×10^{-5}	1.01×10^{-5}
14	S14	Sugarcane bagasse	4.50×10^{-5}	3.90×10^{-5}	4.20×10^{-5}	4.20×10^{-5}
15	S15	Sugarcane bagasse	0.068×10^{-5}	0.075×10^{-5}	0.062×10^{-5}	0.068×10^{-5}

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3.2 Morphological, Microscopic, and Molecular Identification of the Potent Yeast Isolate

Based on enzyme screening, the most efficient isolate was selected for detailed characterization. Colonies were white to cream-colored, dry, chalky, and powdery with irregular margins. Microscopic examination revealed hyaline, septate hyphae fragmenting into rectangular arthroconidia. Molecular identification using ribosomal DNA sequencing confirmed the isolate as *Geotrichum candidum*.

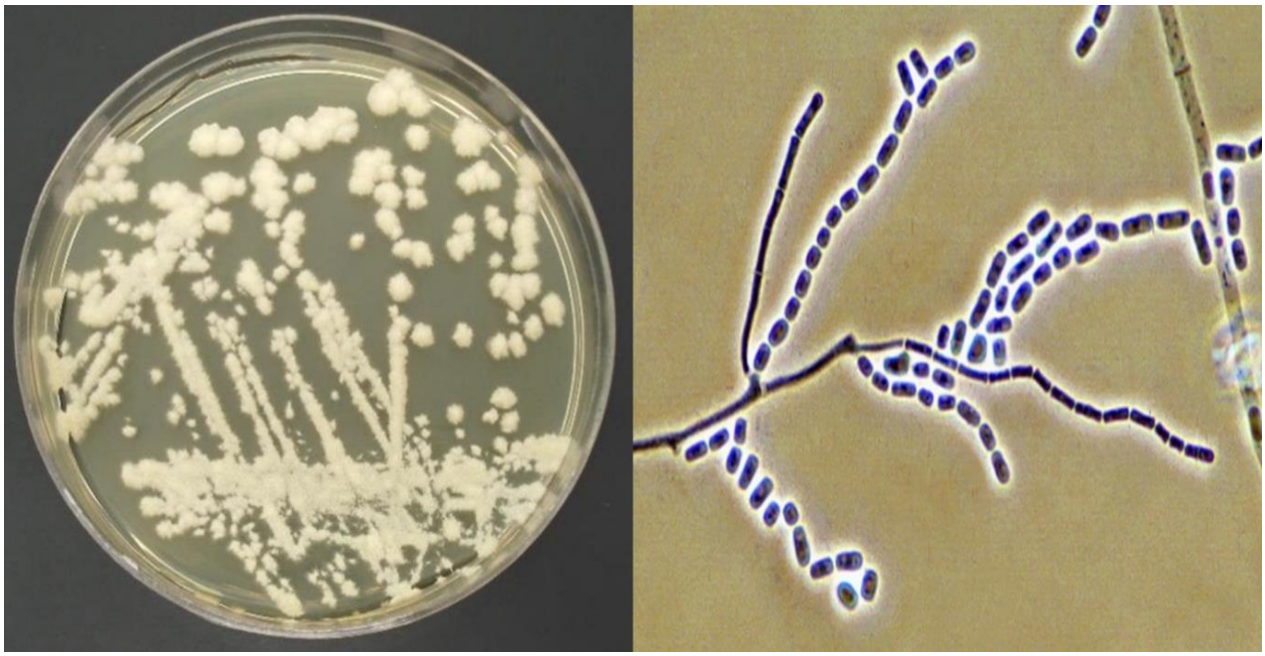

Figure 1. Showing the morphological colony and microscopic view of the yeast Geotrichum candidum.

3.3 Molecular Analysis:

TCCGTAGGTGAACCTGCGGAGGGCTACGGTATT
CGTTCTGAGCATGTCATTCGTGCATGTAACACAA
CAGAGTCGGGTCGGTATACTCGGTCTCTCTTCC
GGCTATGTTGTGGGATATGAGTTCCTGGTCGTGA
TGCGTGCTATAACGGTACTAGCTCGACAGTCTTA
CAATCATGGTTCAACCTGCGGCTTTGCTAGCGC
CTTAGCCAGGTCTCCTGAAGCTGCGTTTGGCAA
CTGAATCTAAACGGTATGCGACCAGGATTGCCA
TAGAGATCGAGCAAGTACTACCGTTCTTTTGTAT
ATATGAGGAGTAGGTGGTTGTCTGTATCGCGGCC
TCCGAAACCTAGTTAGATCGAAAGAGTAGCCAA
GATTTCTGAGCAGAAGGACATCCGATGAGGGAA
GGAATAACACGCACGCCAAGATGTGAAGGCAC
CGTCGTAACTGGTCAAAACGACACAGAGTAGG
AGCCGCACACGAACCGATGAGCGGATTCCGTAA
CGTGGGGACGACTACACGTATCGTGAAACACGC
CCCTTGTGTGGTCGATGTTGGAGGATTTATACTG
GGGACTGATCCTATGTGAAAGAGACTTGACACA
TGTGGCGGACATGCTGCGTTCTTCATCGATGCC
ACGGGCAGTTATGAGCAGT

3.4 Qualitative Screening of Yeast Isolates for Cellulase Activity: A total of 30 yeast isolates were qualitatively screened for cellulase activity using the CMC agar plate assay. Clear zone formation following Congo red staining indicated cellulose degradation (Table 1). Clear zone diameters ranged from **8.0 to 60.0 mm**, demonstrating wide variation in cellulolytic potential. The highest cellulase activity was observed in isolate **YFW10 (60.0 mm)**, followed by **YFW4 (52.67 mm)** and **YC11 (49.0 mm)**. Several isolates including **YC2, YFW3, YFW9, and YSB1** exhibited strong cellulolytic activity, whereas isolates such as **YFW2 and YFW13** showed weak activity.

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Table 2. Comparative yeast population density in different sample types.

S.No.	Yeast Isolates	Replicate 1 (mm)	Replicate 2 (mm)	Replicate 3 (mm)	Mean (mm)
1	YS1	15	11	14	13.33
2	YC1	35	34	38	35.67
3	YC2	45	43	46	44.67
4	YC3	18	14	16	16.00
5	YC4	8	11	14	11.00
6	YC5	28	28	30	28.67
7	YC6	34	31	37	34.00
8	YC7	27	30	28	28.33
9	YC8	28	30	28	28.67
10	YC9	13	12	13	12.67
11	YC10	28	24	29	27.00
12	YC11	49	50	48	49.00
13	YFW1	12	12	14	12.67
14	YFW2	9	9	9	9.00
15	YFW3	36	38	38	37.33
16	YFW4	55	52	51	52.67
17	YFW5	25	24	30	26.33
18	YFW6	12	15	15	14.00
19	YFW7	16	20	19	18.33
20	YFW8	13	12	15	13.33
21	YFW9	35	31	34	33.33
22	YFW10	60	62	58	60.00
23	YFW11	27	30	29	28.67
24	YFW12	18	17	23	19.33
25	YFW13	8	11	13	10.67
26	YFW14	27	23	24	24.67
27	YSB1	32	30	37	33.00
28	YSB2	14	14	13	13.67
29	YSB3	21	18	21	20.00
30	YSB4	24	27	27	26.00

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3.5 Quantitative Estimation of Cellulase Activity: Quantitative cellulase activity, determined using the DNS assay, ranged from 0.147 to 2.289 U mL^{-1} among the yeast isolates (Table 2). Based on superior cellulase activity and consistent performance across screening assays, the isolate identified as Geotrichum candidum was selected for enzyme production and downstream bioethanol studies. The highest cellulase activity was recorded in YS9 (2.289 U mL^{-1}), followed by YS3 (2.105 U mL^{-1}) and YS28 (1.993 U mL^{-1}). Moderate enzyme activity was observed in isolates YS6, YS16, YS24, and YS10, while minimal activity was detected in YS27 and YS4.

Table 3. Quantitative cellulase activity of yeast isolates determined by DNS assay.

S.No.	Sample	Replicate 1	Replicate 2	Replicate 3	Mean
1	YS1	1.578	1.436	1.511	1.508
2	YS2	0.629	0.700	0.682	0.670
3	YS3	2.154	2.030	2.131	2.105
4	YS4	0.188	0.104	0.190	0.161
5	YS5	0.181	0.091	0.226	0.166
6	YS6	1.993	1.895	2.070	1.986
7	YS7	0.416	0.487	0.408	0.437
8	YS8	0.299	0.308	0.246	0.284
9	YS9	2.396	2.220	2.250	2.289
10	YS10	1.751	1.648	1.727	1.709
11	YS11	0.843	0.774	0.930	0.849
12	YS12	1.132	1.020	1.169	1.107
13	YS13	0.348	0.240	0.320	0.303
14	YS14	1.452	1.332	1.410	1.398
15	YS15	0.256	0.232	0.240	0.243
16	YS16	1.982	1.845	1.912	1.913
17	YS17	0.565	0.492	0.563	0.540
18	YS18	0.714	0.621	0.748	0.694
19	YS19	1.325	1.220	1.290	1.278
20	YS20	0.942	0.850	0.963	0.918
21	YS21	1.200	1.120	1.241	1.187
22	YS22	0.437	0.366	0.419	0.407
23	YS23	0.992	0.870	0.985	0.949
24	YS24	1.647	1.502	1.610	1.586

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25	YS25	0.334	0.280	0.355	0.323
26	YS26	1.070	0.984	1.061	1.038
27	YS27	0.158	0.120	0.163	0.147
28	YS28	2.012	1.905	2.062	1.993
29	YS29	1.410	1.320	1.422	1.384
30	YS30	0.783	0.702	0.767	0.751

3.6 Bioethanol Production from Jowar Husk

Enzymatic hydrolysis of pretreated jowar husk using cellulase from Geotrichum candidum resulted in efficient release of fermentable sugars, yielding approximately 45–70 g L^{-1} reducing sugars. The hydrolysate supported ethanol fermentation under optimized conditions, producing an ethanol concentration of ~18–22 g L^{-1} after 72 h, corresponding to an ethanol conversion efficiency of approximately 0.40–0.42 g g^{-1} fermentable sugars. Prior to fermentation, the hydrolysate sugar concentration was adjusted to 80–100 g L^{-1} using concentrated hydrolysate or sterile glucose solution to ensure consistent fermentation conditions.

3.7 FTIR Analysis of Bioethanol Produced from Jowar Husk

Fourier Transform Infrared (FTIR) spectroscopy was employed to confirm the presence of ethanol in the fermented jowar husk hydrolysate. The FTIR spectrum of the fermented sample (Figure X) displayed characteristic absorption bands corresponding to alcohol functional groups, confirming successful bioethanol production. A broad and intense absorption band observed in the region of 3300–3400 cm^{-1} was attributed to O–H stretching vibrations, a characteristic feature of alcohols, indicating the presence of hydroxyl groups associated with ethanol. The absorption peaks appearing around 2970–2870 cm^{-1} correspond to C–H stretching vibrations of methyl ($–CH_3$) and methylene ($–CH_2$) groups, which are typical of ethanol molecules. Additionally, a prominent absorption band detected in the region of 1040–1100 cm^{-1} was assigned to C–O stretching vibrations, further confirming the presence of ethanol in the fermented broth. Minor peaks observed in the fingerprint region may be attributed to residual fermentation metabolites; however, the dominant peaks were consistent with those of standard ethanol.

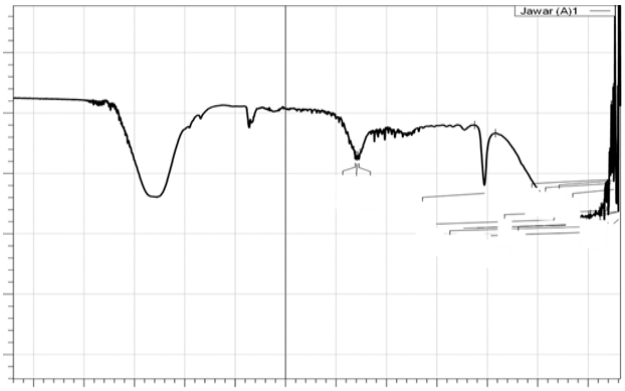

Figure 2. FTIR spectrum of fermented jowar husk hydrolysate showing characteristic functional groups of ethanol.

3.8 HPLC Analysis of Bioethanol Produced from Jowar Husk

High Performance Liquid Chromatography (HPLC) was employed for the quantitative confirmation of ethanol produced during fermentation of enzymatically hydrolyzed jowar husk. The chromatographic profile of the fermented sample (Sample A) revealed multiple peaks, indicating the presence of ethanol along with minor fermentation-derived metabolites. A dominant peak observed at a retention time of approximately 4.52 min accounted for the highest area percentage (65.21%), indicating the presence of a major compound in the fermented broth. This retention time closely corresponded with that of the ethanol standard analyzed under identical chromatographic conditions, confirming ethanol as the principal fermentation product. Several minor peaks were detected at retention times ranging from 1.84 to 3.35 min, representing low-molecular-weight by-products formed during fermentation. These

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peaks collectively contributed a relatively small fraction of the total chromatographic area, suggesting efficient conversion of fermentable sugars toward ethanol formation. Quantification of ethanol was carried out by comparing the peak area of the ethanol-associated peak with a previously generated ethanol calibration curve. Based on this analysis, the ethanol concentration in the fermented jowar husk hydrolysate after 72 h of fermentation was estimated to be approximately 18–22 g L^{-1}, which is consistent with effective lignocellulosic ethanol fermentation under batch conditions.

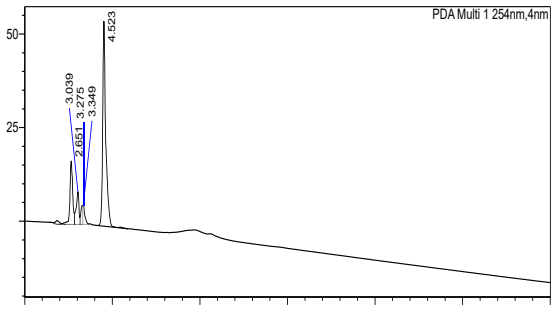

Figure 3. HPLC chromatogram of fermented jowar husk hydrolysate showing a dominant ethanol peak at ~4.52 min.

Discussion

The results of this study indicate that agro-organic substrates serve as effective reservoirs of lignocellulose-degrading yeasts. Noticeable differences in yeast population density were observed among the collected samples, suggesting that substrate composition plays a decisive role in shaping microbial abundance and enzymatic potential. Fruit waste and compost supported comparatively higher yeast counts than soil and sugarcane bagasse. This trend likely reflects the presence of readily available carbohydrates, organic nutrients, and adequate moisture, all of which create favorable growth conditions for metabolically active yeasts. Similar observations have been reported for fruit-derived and composted materials, which frequently harbor carbohydrate-utilizing microorganisms.

Screening on CMC agar revealed clear differences in cellulolytic capability among the isolates. Variations in halo diameter indicated differing levels of extracellular enzyme secretion and substrate degradation efficiency. Isolates recovered from fruit waste and compost generally exhibited stronger cellulase activity, reinforcing the idea that environmental adaptation influences metabolic traits. The agreement between plate-based assays and DNS quantification further supports the reliability of the screening strategy adopted. Combining qualitative and quantitative evaluation proved essential for identifying the most promising candidate.

Among the isolates examined, *Geotrichum candidum* showed the highest cellulase activity and was selected for detailed investigation. Colony morphology, including the characteristic chalky appearance and formation of arthroconidia, corresponded well with previously documented descriptions. Ribosomal DNA sequencing provided additional confirmation at the species level, ensuring accurate taxonomic identification. The demonstrated ability of *G. candidum* to produce extracellular cellulase under mild conditions strengthens its relevance for lignocellulosic biomass conversion systems.

Pretreatment played a decisive role in enhancing saccharification efficiency. Alkali treatment of jowar husk disrupted lignin structures, thereby improving enzyme accessibility to cellulose fibers. The release of 45–70 g L^{-1} reducing sugars indicates that the combined pretreatment and hydrolysis strategy was effective. Importantly, the use of crude cellulase suggests that costly purification steps may not be necessary for achieving substantial sugar yields, which has implications for overall process economics.

The hydrolysate supported efficient fermentation by *Saccharomyces cerevisiae*, resulting in ethanol concentrations of 18–22 g L^{-1} after 72 hours. The calculated conversion efficiency (approximately 0.40–0.42 g g^{-1} fermentable sugars) approaches theoretical expectations for ethanol production, indicating minimal sugar loss to competing pathways. Considering the structural complexity of lignocellulosic substrates and the possible formation of inhibitory compounds during pretreatment, these values demonstrate satisfactory fermentation performance.

Analytical validation further confirmed ethanol formation. FTIR spectra displayed characteristic absorption bands corresponding to O–H, C–H, and C–O functional groups typical of ethanol. HPLC chromatograms showed a dominant peak with a retention time consistent with ethanol standards, representing the

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majority of the total peak area. Minor peaks observed may correspond to low-molecular-weight by-products commonly generated during batch fermentation.

Taken together, the integration of indigenous enzyme production, alkali pretreatment, and conventional yeast fermentation supports the feasibility of converting jowar husk into bioethanol. Utilizing locally sourced microbial strains and agricultural residues offers a practical approach aligned with circular bioeconomy concepts. Further optimization of enzyme loading, pentose sugar utilization, and process scale-up could enhance overall productivity and improve industrial applicability.

Conclusion

This study demonstrates the feasibility of producing bioethanol from jowar husk (*Sorghum bicolor*) using a locally isolated cellulase-producing yeast, *Geotrichum candidum*. Agro-organic substrates, particularly fruit waste and compost, proved to be valuable sources of metabolically active yeasts with notable cellulolytic capability. Through systematic screening, a potent *G. candidum* isolate was identified and confirmed by both qualitative and quantitative enzyme assays. Alkali pretreatment enhanced the accessibility of lignocellulosic components, enabling effective enzymatic saccharification and the release of significant amounts of fermentable sugars. The crude cellulase preparation derived from *G. candidum* was sufficient for hydrolyzing pretreated biomass, suggesting that enzyme purification may not be essential for productive conversion. Fermentation with *Saccharomyces cerevisiae* resulted in ethanol yields approaching theoretical conversion efficiencies under batch conditions. Analytical validation using FTIR and HPLC confirmed the formation and relative purity of ethanol in the final product. Overall, the integration of indigenous microbial enzymes with an underutilized agricultural residue supports the development of a cost-effective and regionally adaptable bioethanol production strategy. Further refinement of process parameters and scale-up evaluation would help clarify its industrial potential.

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