International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; 9(1): 105-109
www.biochemjournal.com
Received: 15-10-2024
Accepted: 21-11-2024

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Comparative analysis of bioethanol production from agricultural wastes using *Saccharomyces cerevisiae*, *Aspergillus niger*, and their co-culture

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DOI: https://doi.org/10.33545/26174693.2025.v9.i1b.3461

Abstract
The present study aimed to analyze the proximate composition of waste potatoes, wheat straw, and sugarcane bagasse collected from various locations and to optimize fermentation variables for maximum bioethanol yield using *Saccharomyces cerevisiae*, *Aspergillus niger*, and their co-culture.
Initially, the chemical composition of the substrates was analyzed, revealing that waste potatoes, wheat straw, and sugarcane bagasse contain significant levels of total soluble solids (TSS), making them suitable for bioethanol production. Subsequently, experiments were conducted to maximize ethanol yield by varying fermentation parameters. The substrates were tested at different TSS levels (18°Brix, 20°Brix, and 22°Brix), pH levels (3.5, 4.0, and 4.5), and incubation temperatures (27 °C, 30 °C, and 33 °C) using the three microbial cultures individually and in co-culture.
Experimental findings demonstrated that the co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger* efficiently produced ethanol across various substrates under optimal conditions of 20°Brix TSS, pH 4.0, and an incubation temperature of 30 °C. Among the tested substrates, sugarcane bagasse yielded the highest ethanol concentration at 6.1%, followed by waste potatoes at 5.7%, and wheat straw at 4.6%. These results highlight the superior efficiency of sugarcane bagasse for ethanol production, with the same co-culture and process parameters proving consistently effective across all substrates.
This study underscores the potential of utilizing agricultural waste for sustainable bioethanol production and provides valuable insights into optimizing fermentation processes for maximum yield.

Keywords: Proximate composition, waste potatoes, wheat straw, sugarcane bagasse

Introduction
The growing demand for sustainable energy solutions has driven significant research into bioethanol production, a promising alternative to fossil fuels (Patel and Singh, 2024; Novia *et al*. 2023; Vasić *et al*. 2021; Huang *et al*. 2020) [14, 13, 11, 12]. Agricultural waste, a readily available and low-cost resource, offers immense potential as a substrate for bioethanol production due to its rich carbohydrate content (Sikiru *et al*. 2024) [15]. Among the numerous feedstocks studied, waste potatoes, wheat straw, and sugarcane bagasse stand out for their high availability and substantial levels of fermentable sugars and structural polysaccharides. These characteristics make them attractive candidates for conversion into bioethanol (Falowo *et al*. 2023) [16].
Microbial fermentation is central to bioethanol production, with *Saccharomyces cerevisiae* being widely recognized for its robust fermentative capabilities (Parapouli *et al*. 2020; Walker, and Stewart, 2016) [17, 18]. Similarly, *Aspergillus niger*, known for its enzymatic hydrolysis efficiency, complements the fermentation process by breaking down complex carbohydrates (Wang *et al*. 2015; Bansal *et al*. 2011) [20, 19]. The combination of these two microorganisms in co-culture has shown the potential to enhance ethanol yields through synergistic action, where enzymatic hydrolysis and fermentation occur simultaneously (Izmirlioglu and Demirci, 2016; Itelima *et al*. 2013, Ado *et al*. 2009) [23, 21, 22]. However, optimizing fermentation parameters such as substrate concentration, pH, and temperature is critical to maximizing bioethanol production (Manikandan and Viruthagiri, 2010) [24].
This study aims to assess the feasibility of producing bioethanol from waste potatoes, wheat straw, and sugarcane bagasse, evaluating their chemical composition and fermentation potential. By comparing the performance of *Saccharomyces cerevisiae*, *Aspergillus niger*,

and their co-culture under varying fermentation conditions, the research seeks to identify the optimal parameters for maximum ethanol yield. The findings provide valuable insights into the sustainable utilization of agricultural waste for bioethanol production and contribute to advancing biofuel technologies.

Materials and Methods

This study was conducted in the Environmental Biotechnology and Microbiology Laboratory at S.D.M.V.M. College of Agricultural Biotechnology, Aurangabad (M.H.). The substrates used for bioethanol production included waste potatoes collected from various storage facilities in Aurangabad city, wheat straw sourced from local farmer's fields, and sugarcane bagasse obtained from sugar and jaggery factories. These substrates were selected based on their availability and suitability for bioethanol production.

Saccharomyces cerevisiae and *Aspergillus niger* were chosen as the microbial strains due to their high ethanol-yielding potential and absence of undesirable by-products. The cultures were grown and maintained on Yeast Extract Peptone Dextrose (YEPD) media under standard laboratory conditions to ensure their activity and viability throughout the experiments.

The fermentation process was carried out following the protocol described by Sharma *et al*. (2006) [1]. This method was used for bioethanol production from waste potatoes, wheat straw, and sugarcane bagasse. The same process was also employed to optimize key fermentation variables, including total soluble solids (TSS), pH, and incubation temperature, to achieve maximum ethanol yield. The experiments involved varying these parameters systematically to determine the conditions that produced the best results.

Analyses of the substrates and bioethanol were performed using established techniques. Total soluble solids (TSS) in both the substrates and bioethanol were measured with an Erma Hand Refractometer. The pH of the samples was determined using a calibrated pH meter, while the acidity of the bioethanol was assessed using the A.O.A.C. (1990) [2] method. Sugar content, including total sugar, reduced sugar, and non-reducing sugar, was quantified using the method outlined by Ranganna (1997) [3]. The ethanol yield from each substrate was determined by employing the distillation and dehydration process described by O'Leary (2000) [4]. These standardized analytical methods ensured accurate and reproducible results, providing a robust framework for evaluating the efficiency of bioethanol production from the selected substrates.

Results and Discussion

This study involved a detailed examination of the chemical composition of waste potatoes, wheat straw, and sugarcane bagasse to assess their suitability as substrates for bioethanol production. The analysis showed that waste potatoes contained 86.40% moisture, 13.60% dry matter, and 73.0% starch. (Table 1.) These results align with previous reports, though minor variations in moisture and dry matter were noted, possibly due to genetic differences and the purity of materials used in earlier studies (Gopalan *et al*., 1984; Kita, 2002) [5, 6]. Wheat straw was found to consist of 39% cellulose, 23% hemicellulose, and 17% lignin, (Table 2.) which are within the range reported in the literature. For instance, Smuga-Kogut *et al*. (2015) [7] documented wheat straw as containing 35–50% cellulose, 15–30% hemicellulose, and 20–30% lignin, alongside smaller amounts of ash and other compounds. Sugarcane bagasse was determined to have 47% cellulose, 26% hemicellulose, and 23% lignin, (Table 3.) which is comparable to earlier findings, such as those by Anita Singh (2012) [8], who reported cellulose, hemicellulose, and lignin levels of 43%, 24%, and 20%, respectively. Slight deviations in the observed values for the substrates could be attributed to environmental conditions, genetic variability, or other factors affecting the composition of these materials.

The bioethanol yield was evaluated under varying conditions of pH and TSS at an incubation temperature of 30 °C using the co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger*. The results indicated that sugarcane bagasse, under optimal conditions of 20°Brix TSS, pH 4.0, and 30 °C, produced the highest ethanol yield of 6.1%. (Fig 3.). These findings are consistent with those of Hernawan *et al*. (2017) [9], who reported ethanol yields of 5.87±0.78% and 6.83±0.07% using simultaneous saccharification and fermentation (SSF) with cellulase enzymes and cellulase enzymes combined with β-glucosidase, respectively. Waste potatoes produced a maximin ethanol yield of 5.7% under the same conditions, (Fig 1.) which aligns with previous studies emphasizing the high starch content of potatoes as a promising feedstock for bioethanol production (Promon *et al*., 2018) [10]. Similarly, wheat straw yielded 4.6% ethanol using the same co-culture and fermentation parameters. (Fig 2.)

The slight differences in ethanol yields between the substrates can be attributed to their distinct chemical compositions. The combination of *S. cerevisiae* and *A. niger* proved effective in breaking down complex carbohydrates, enabling efficient ethanol production. These results underscore the potential of utilizing agricultural residues such as sugarcane bagasse, waste potatoes, and wheat straw for sustainable bioethanol production, consistent with observations by earlier researchers.

Table 1: Chemical Composition of Waste Potato Pulp and Its Suitability for Bioethanol Production

Sr. No.	Constituents	Amount (In %)
1.	Moisture	86.40
2.	Dry matter	13.60
3.	Starch	73.00

Table 2: Chemical Composition of Wheat Straw and Its Suitability for Bioethanol Production

Sr. No.	Constituents	Amount (In %)
1.	Cellulose	39.00
2.	Hemicellulose	23.00
3.	Lignin	17.00

Table 3: Chemical Composition of Sugarcane Bagasse and Its Suitability for Bioethanol Production

Sr. No.	Constituents	Amount (In %)
1.	Cellulose	47.00
2.	Hemicellulose	26.00
3.	Lignin	23.00

Table 4: Effect of Different pH and TSS Levels on Bioethanol Yield at an Incubation Temperature of 30 °C Using Waste Potato and Co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger*

Sr. No.	TSS (%)	Ethanol yield (%)		
		Ph		
		3.5	4.0	4.5
1.	18	3.8	4.0	3.6
2.	20	5.0	5.7	5.1
3.	22	4.0	4.2	4.1

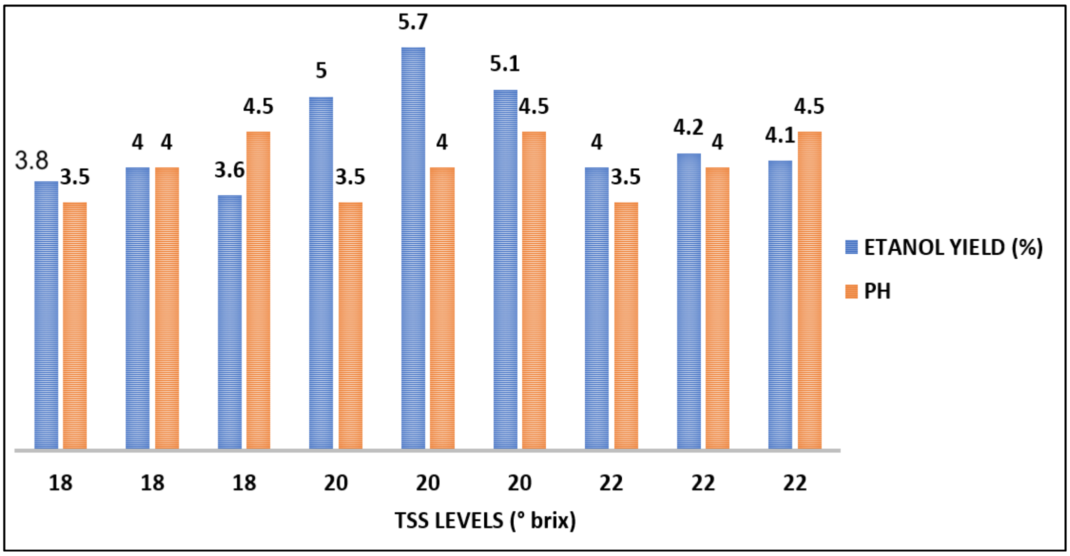

Fig 1: Effect of Different pH and TSS Levels on Bioethanol Yield at an Incubation Temperature of 30 °C Using Waste Potato and Co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger*

Table 5: Effect of different pH and TSS levels on bioethanol yield at an incubation temperature of 30 °C. With Wheat straw using a co-culture of *Saccharomyces cerevisiae* and *Aspergillus Niger*

Sr. No.	TSS (%)	Ethanol yield (%)		
		Ph		
		3.5	4.0	4.5
1.	18	3.5	4.2	3.7
2.	20	4.2	4.6	4.3
3.	22	3.4	4.0	3.8

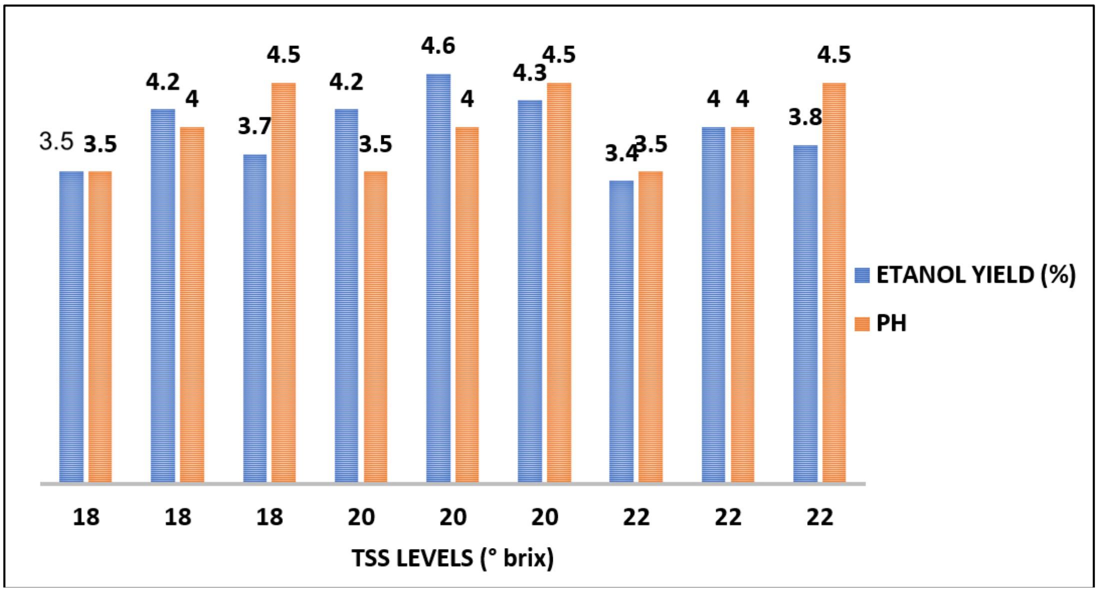

Fig 2: Effect of different pH and TSS levels on bioethanol yield at an incubation temperature of 30 °C. With Wheat straw using a co-culture of *Saccharomyces cerevisiae* and *Aspergillus Niger*

Table 6: Effect of different pH and TSS levels on bioethanol yield at an incubation temperature of 30 °C. With sugarcane bagasse using co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger*

Sr. No.	TSS (%)	Ethanol yield (%)		
		pH		
		3.5	4.0	4.5
1.	18	4.2	4.7	4.6
2.	20	5.1	6.1	4.8
3.	22	4.7	5.2	4.7

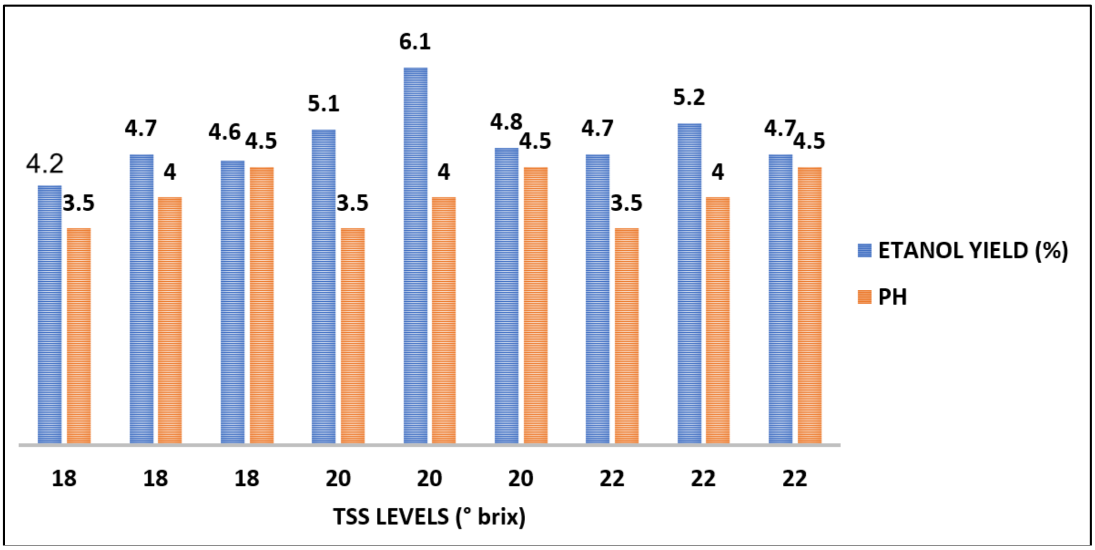

Fig 3: Effect of different pH and TSS levels on bioethanol yield at an incubation temperature of 30 °C. With sugarcane bagasse using co-culture of *Saccharomyces cerevisiae* and *Aspergillus Niger*

Conclusion

This study highlights the potential of waste potatoes, wheat straw, and sugarcane bagasse as viable substrates for bioethanol production due to their distinct chemical compositions. Waste potatoes exhibited high starch content, while wheat straw and sugarcane bagasse contained significant cellulose and hemicellulose levels. The co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger* proved effective in converting these substrates into ethanol, with sugarcane bagasse yielding the highest ethanol production at 6.1%. The findings emphasize the importance of utilizing agricultural residues for sustainable bioethanol production, aligning with previous research on alternative feedstocks for renewable energy.

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