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Analysis of Yeast Biomass for Bioethanol Production Using Sugarcane Bagasse

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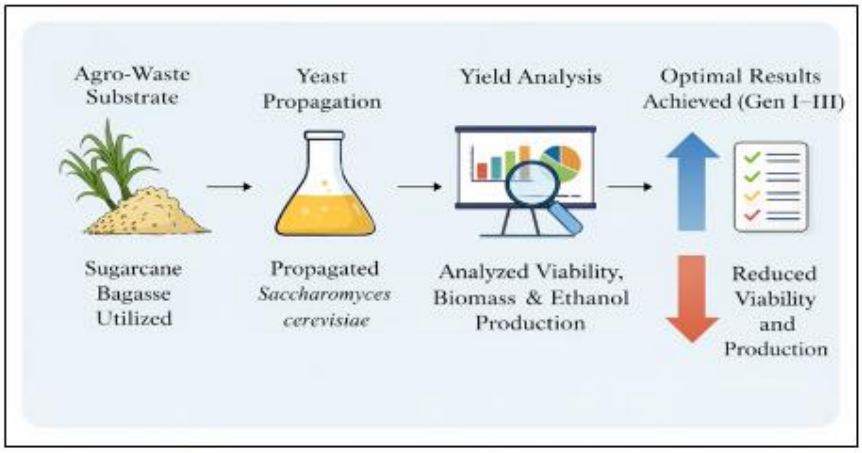
Abstract: *Bioethanol is one of the most extensively used renewable biofuels worldwide due to its capability to reduce dependence on fossil fuels and minimize environmental pollution. Yeast mediated fermentation is the most preferred biological route for bioethanol production because of its efficiency, cost-effectiveness, and scalability. The present investigation focuses on the propagation and analysis of yeast biomass for bioethanol production using a crude extract medium prepared from sugarcane bagasse, an abundantly available agro-industrial waste. Successive generations of Saccharomyces cerevisiae were propagated under controlled sterile conditions with continuous aeration and zinc supplementation. Yeast viability, biomass yield, contamination status, and ethanol production efficiency were evaluated at different propagation stages. The results revealed maximum yeast viability (96.15%), froth formation, and ethanol productivity up to the third generation. A gradual decline in metabolic activity and fermentation efficiency was observed in later generations. The study confirms that sugarcane bagasse-based crude medium can be efficiently utilized for large-scale yeast propagation and sustainable bioethanol production.*

Keywords: Bioethanol, Yeast Propagation, Sugarcane Bagasse, Biomass, Saccharomyces cerevisiae

Graphical Abstract:Analysis of Yeast Biomass for Bioethanol Production Using Sugarcane Bagasse



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I. INTRODUCTION

The continuously increasing global energy demand, coupled with the rapid depletion of fossil fuel reserves, has intensified the need for alternative, renewable, and sustainable energy sources [1]. Bioethanol has emerged as one of the most promising renewable biofuels due to its biodegradability, lower greenhouse gas emissions, and compatibility with existing fuel infrastructure [2]. Microbial fermentation is widely employed for bioethanol production because of its eco-friendly nature, high conversion efficiency, and suitability for large-scale industrial applications [3].

Yeast plays a central role in bioethanol production by converting fermentable sugars into ethanol and carbon dioxide. Among various microorganisms, Saccharomyces cerevisiae is the most extensively utilized yeast species owing to its high ethanol tolerance, rapid growth rate, and ability to ferment a broad range of sugars [4]. Several studies have reported that the efficiency of bioethanol fermentation is strongly influenced by yeast viability, metabolic activity, and biomass quality [5]. Proper yeast propagation ensures higher ethanol yield, improved fermentation kinetics, and consistent industrial performance.

The economic feasibility of bioethanol production is largely dependent on the cost of raw materials and growth media. Consequently, the utilization of low-cost agro-industrial waste materials has received considerable research attention [6]. Sugarcane bagasse, a lignocellulosic by-product of the sugar industry, is generated in large quantities and is rich in carbohydrate content. Previous studies have demonstrated that sugarcane bagasse can serve as a cost-effective substrate for microbial growth and bioethanol production while simultaneously contributing to sustainable waste management [3,7].

Efficient yeast propagation strategies, including controlled aeration, micronutrient supplementation, and strict contamination control, have been shown to significantly enhance fermentation efficiency and ethanol productivity [2,4]. Demirbas and Karslioglu [4] reported that sugar fermentation using properly propagated yeast cultures results in higher ethanol yields compared to direct yeast inoculation methods. However, despite these advancements, limited information is available on generation-wise analysis of yeast biomass propagation using crude sugarcane bagasse extract under industrial conditions.

Therefore, the present study investigates yeast biomass propagation using a sugarcane bagasse-based crude extract medium and evaluates its suitability for bioethanol production at an industrial level. The study emphasizes yeast viability, biomass yield, contamination control, and ethanol productivity across successive generations of yeast propagation, thereby addressing an important research gap in sustainable biofuel production.

II. MATERIALS AND METHODS (HIGHLY DETAILED-RESEARCH PAPER STANDARD)

2.1 Study Location and Experimental Facility:

The present investigation was carried out in collaboration with United Breweries, Taloja MIDC, Maharashtra, India. Laboratory-scale yeast propagation experiments were performed using Carlsberg flasks, while large-scale yeast propagation and ethanol estimation were conducted under industrial conditions using propagating and pitching vessels. All experimental procedures were executed under strict aseptic and hygienic conditions to prevent microbial contamination and ensure reproducibility of results [8].

2.2 Raw Materials and Chemicals:

Sugarcane bagasse was used as an agro-industrial waste substrate for the preparation of crude extract medium. Commercially available yeast granules of Saccharomyces cerevisiae were used for propagation**(Figure.1)**. Peptone was used as a nitrogen source, and distilled water was used for all preparations. All glassware, vessels, and media were sterilized prior to use [9].

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Figure 1: Raw materials used for yeast propagation - (a) Sugarcane bagasse and (b) commercially available yeast granules

2.3 Preparation of Crude Sugarcane Bagasse Extract Medium:

One kilogram of sugarcane bagasse was added to 10 liters of distilled water and boiled continuously for two hours to extract soluble nutrients. The mixture was allowed to cool to room temperature and then filtered using muslin cloth to remove solid residues. From the obtained crude extract, one liter was taken and supplemented with 10 g of peptone to enhance yeast growth. The pH of the medium was adjusted to 5.0, which is optimal for yeast fermentation. The prepared medium was sterilized by autoclaving at 121°C for 15 minutes and allowed to cool before inoculation [10].

2.4 Yeast Inoculum Preparation:

Commercial yeast granules were rehydrated in sterile distilled water. Approximately 10% (v/v) yeast inoculum was aseptically transferred into the sterile crude sugarcane bagasse medium. Initially, a single yeast cell was selected and propagated to obtain a pure yeast culture, ensuring uniformity and reproducibility throughout the experimental study [11].

2.5 Yeast Propagation and Generation-wise Transfer:

Yeast propagation was carried out through successive generations under sterile conditions using laboratory-scale and industrial-scale propagation and pitching vessels **(Figure 2)**. For each generation, 800 mL of yeast culture was transferred into 800 liters of sterile crude sugarcane bagasse medium. The propagation process was continued from Generation I to Generation VII, with each generation incubated for 48 hours. Continuous aeration was provided using sterile compressed air passed through a 0.2 µm membrane filter to promote yeast multiplication and metabolic activity. Zinc supplementation was maintained at a concentration of 1 ppm during each propagation stage to support enzymatic reactions and cellular growth [12]. Temperature was maintained between 10-12°C, and pH was controlled within the range of 5.0-5.5 throughout the propagation process.

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Figure 2: Yeast propagation and pitching vessels used during successive generations of yeast propagation

2.6 Yeast Biomass Recovery and Processing:

After completion of incubation, yeast biomass was allowed to settle and subsequently recovered by centrifugation at 15,000 rpm for 10 minutes, resulting in clear separation of the supernatant and yeast biomass **(Figure 3)**. The upper supernatant layer was aseptically removed, and the middle yeast-rich layer was carefully skimmed. The recovered yeast biomass was subjected to mild sterilization at 50°C for 30 minutes and then cooled to pitching temperature before further use in fermentation processes [13].

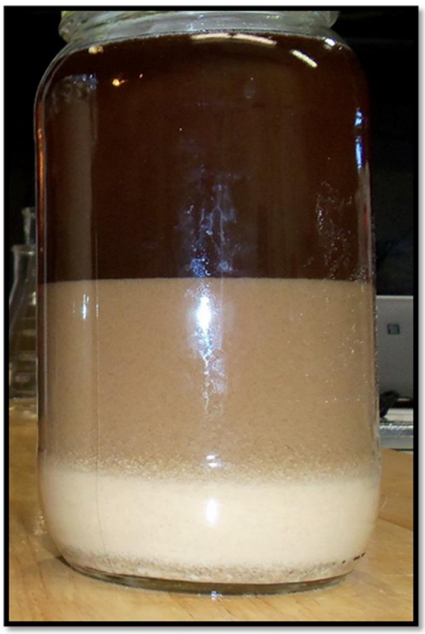

Figure 3: Yeast precipitation after centrifugation

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2.7 Yeast Viability Analysis:

Yeast viability was assessed using hemocytometry combined with viable staining techniques. A known volume of yeast suspension from Generation I was loaded onto a hemocytometer, and viable (unstained/colorless) and non-viable (stained) cells were counted under a light microscope. The percentage of viable yeast cells observed during Generation I is presented in **Table 1**.

Yeast viability was calculated using the following equation:

$$Viability\ (\%) = \frac{\text{Number of viable cells}}{Total\ number\ of\ cells} \times 100$$

Observed data (Generation I):

Viable cells = 25

Total cells = 26

$$Viability\ (\%) = \frac{25}{26} \times 100 = 96.15\%$$

Table 1: Yeast Viability Analysis (Generation I)

Parameter	Observation
Generation	I
Viable yeast cells	25
Total yeast cells	26
Yeast viability (%)	96.15

2.8 Yeast Biomass Estimation by Solid Weight Method:

Yeast biomass was estimated using the solid-by-weight method. The empty weight of a sterile centrifuge tube was recorded as X, and the final weight after biomass collection was recorded as Y.

The percentage of yeast solid content was calculated using the following equation:

$$Yeast\ Solid\ by\ weight\ (\%) = \frac{X}{Y} \times 100$$

Where: X = 42.1358 g (empty tube weight)

Y = 62.2373 g (tube weight with yeast biomass)

Based on this calculation, the yeast solid by weight was found to be 67.70%, indicating effective biomass recovery during the propagation process. The detailed calculation is presented in **Table 2.**

Table 2: Yeast biomass solid-by-weight calculation

Parameter	Value
Empty centrifuge tube weight (X)	42.1358 g
Final tube weight with yeast biomass (Y)	62.2373 g
Yeast solid by weight (%)	67.70

2.9 Contamination Analysis:

Microbial contamination during yeast propagation was assessed using Nutrient Agar and Rakaray Agar media, which are commonly employed for the detection of bacterial and wild yeast contaminants in fermentation processes. Samples from each generation were streaked onto agar plates and incubated under appropriate conditions. Minimal contamination was observed throughout the propagation process, which can be attributed to strict aseptic handling, effective sterilization of media and equipment, and controlled processing conditions [14,15].

2.10 Quantitative Estimation of Ethanol:

Ethanol concentration was quantitatively estimated using an Anton PaarAlcolyzer at United Breweries, Taloja MIDC. Prior to sample analysis, the instrument was calibrated using distilled water and 100% ethanol according to the

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manufacturer's guidelines. Ethanol production was recorded for each yeast generation to evaluate fermentation efficiency and alcohol yield [16].

III. RESULTS AND DISCUSSION

3.1 Generation-wise Observation of Yeast Propagation:

Successive generations of Saccharomyces cerevisiae propagated in crude sugarcane bagasse extract medium showed clear variations in growth behavior and metabolic activity. Generation I exhibited rapid initiation of growth, confirming the suitability of the crude medium for yeast adaptation. Generation II showed enhanced froth formation and increased biomass accumulation, indicating active aerobic metabolism supported by continuous aeration and zinc supplementation.

Generation III demonstrated maximum froth formation, compact yeast precipitation, and intense metabolic activity, representing the most efficient propagation stage. This generation correlated with the highest ethanol productivity observed during industrial evaluation. In contrast, Generations IV to VII exhibited progressively reduced froth formation and weaker fermentation vigor, suggesting a gradual decline in yeast metabolic efficiency due to repeated recycling stress [4,6,12]. These qualitative propagation characteristics are illustrated in **Figure 4.**

Figure 4. Illustrates the frothy yeast biomass observed during Generation III.

3.2 Yeast Viability Analysis:

Yeast viability plays a crucial role in fermentation efficiency and ethanol yield[5,6]. Hemocytometric analysis combined with viable staining revealed **96.15% viable yeast cells in Generation I**, indicating excellent cellular health under optimized propagation conditions. High viability during the initial stage ensured efficient sugar utilization and stable fermentation performance.A gradual reduction in yeast viability was observed in subsequent generations, which correlated with decreased fermentation vigor. This decline can be attributed to nutrient depletion, accumulation of metabolic by-products, and physiological stress caused by repeated generation-wise transfers[6,12].Quantitative yeast viability data are presented in **Table 1**.

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3.3 Yeast Biomass Yield and Solid Content:

Yeast biomass estimation using the solid-by-weight method confirmed effective biomass recovery from the sugarcane bagasse-based crude medium. The yeast solid content was calculated as **67.70%**, indicating efficient biomass accumulation during early propagation stages.Although apparent biomass weight increased in later generations, microscopic and physical observations revealed that this increase was largely due to water retention rather than active cell multiplication. Dense and compact yeast pellets were observed up to Generation III, whereas loosely packed biomass was obtained in later generations, reflecting reduced cellular integrity[6,13].The biomass recovery calculation is presented in **Table 2**.

3.4 Contamination Status During Yeast Propagation:

Contamination analysis using Nutrient Agar and Rakaray Agar media showed **negligible microbial contamination throughout the propagation process**. Strict aseptic handling, effective sterilization of media and equipment, and controlled aeration using filtered air played a key role in maintaining culture purity.The absence of contaminating microorganisms ensured stable yeast performance and prevented competition for nutrients during fermentation [5,14,15].

3.5 Ethanol Production Efficiency:

Quantitative ethanol estimation using an Anton PaarAlcolyzer demonstrated **maximum ethanol production during Generation III,** which strongly correlated with high yeast viability, optimal biomass quality, and vigorous fermentative activity(**Figure 5**). Reduced ethanol production observed in later generations highlighted the limitations of excessive yeast recycling under industrial conditions.These findings emphasize the importance of controlled yeast propagation and limited reuse cycles for achieving reliable bioethanol production[4,7,16].

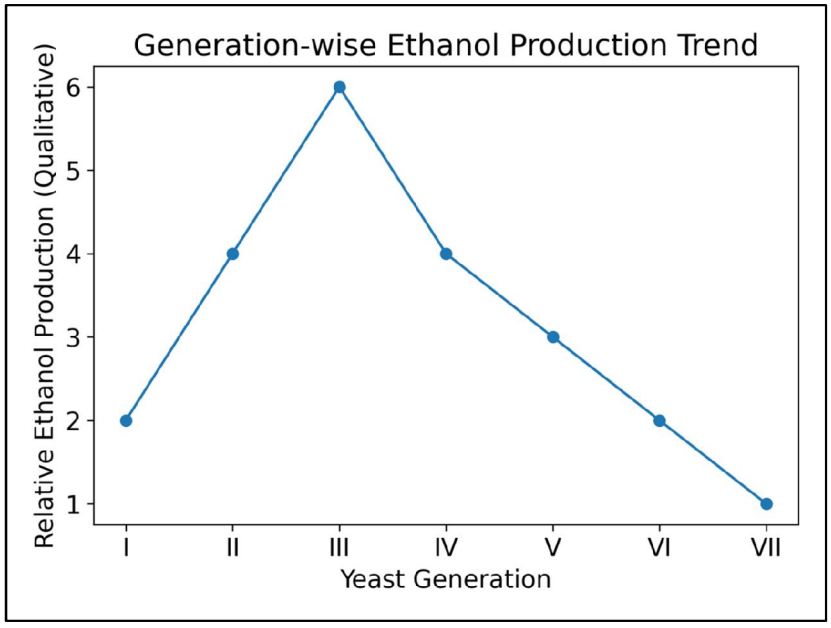

Figure 5.Generation-wise qualitative trend of ethanol production highlighting optimum performance at Generation III.

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IV. CONCLUSION

The present study demonstrates that crude sugarcane bagasse extract can be effectively utilized as a low-cost and sustainable medium for yeast biomass propagation and bioethanol production. Saccharomyces cerevisiae propagated under controlled conditions with continuous aeration and zinc supplementation exhibited high viability, efficient biomass recovery, minimal contamination, and reliable fermentation performance during early propagation stages.Maximum yeast activity, froth formation, and ethanol production were observed up to the third generation, indicating that limited yeast recycling enhances fermentation efficiency. Beyond this stage, a gradual decline in metabolic activity and ethanol productivity was observed, highlighting the negative impact of repeated yeast reuse under industrial conditions.

Overall, the findings confirm that optimized yeast propagation using agro-industrial waste substrates such as sugarcane bagasse offers an economically viable and environmentally sustainable approach for industrial bioethanol production. The study provides practical insights into generation-wise yeast management for improving fermentation efficiency and process consistency in large-scale bioethanol industries.

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Credit Authorship Contributions Statement

Ms. Aparna A. Dhumal[1]: Analytical experiments, data validation, investigation, manuscript preparation.

Ms. Kajal R. Gaikwad[2]: Conceptualization, microbiological analysis, result interpretation, data curation, writing - original draft preparation.

Dr. Vishal Naik[3]: Supervision, methodology development, resources, writing-review and editing.

Dr. Kirti J. Mhatre[4]: Formal analysis, validation, critical revision of the manuscript, technical guidance.

Ms.Neha S. Mehta[5]: Experimental support, data collection, laboratory assistance

Declaration of Competing Interest

The author declares no conflict of interest, financial or personal, that could have influenced the work reported in this paper.

Consent to Publish Declaration

Not applicable

Ethics and Consent to Participate Declarations

Not applicable.

Data Availability:

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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