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Valorization Of Food Waste For Bioethanol Production: Towards A Circular Bioeconomy

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Abstract: *The global increase in energy demand and the environmental concerns associated with fossil fuels have intensified the need for renewable, sustainable energy sources. As a result, different approaches must be discovered. Bioethanol, a prominent biofuel, has emerged as a viable alternative due to its renewable nature and reduced environmental impact. This study, comprehensively utilizes the food waste collected from institute campus, several hotels and restaurants for bioethanol production, exploring its potential as a renewable energy source. The food waste was exposed to different pretreatment strategies for breaking the complex structure of cellulose and 4% H_2SO_4 was found to be effective revealing significant change in the composition of food waste with 58.9% cellulose, 32.4% hemicellulose and 10.3% lignin. Cellulase enzyme from Aspergillus niger was further utilized to hydrolyze the food waste and the effect of hydrolysis was confirmed using SEM analysis of the treated and untreated food wastes. A variety of factors influence the production of ethanol, and one such factor is the effect of ethanol on the growth of yeast. The isolated Saccharomyces cerevisiae was able to utilize food waste and yielded bioethanol production of 74.7g/L. The presence of obtained ethanol was confirmed using chromic acid method. These findings would be useful to promote the shift towards sustainable bioethanol production from food waste, leading also in a step forward towards enhancing advances and sustainability practices on economically feasible exploitation of second-generation feedstocks for more effective environmental-friendly strategies.*

Keywords: *Food Waste, Bioethanol, Hydrolysis, Semandsaccharomyces Cerevisiae.*

1. INTRODUCTION

The need for renewable energy has grown exponentially over recent decades as fossil fuel reserves decline and environmental concerns escalate (Rezania*et al.,* 2020). Conventional energy sources such as coal, oil, and natural gas have fueled global development for centuries, but their detrimental effects on the environment—ranging from greenhouse gas emissions to ecosystem destruction—have pushed society to seek sustainable alternatives. Bioethanol, a type of biofuel derived from biomass, has emerged as a promising renewable energy source capable of offsetting some reliance on fossil fuels (Singh *et al.,* 2022). The production of bioethanol leverages biological feedstocks, like lignocellulosic biomass, which includes agricultural residues, forestry byproducts, and dedicated energy crops (Saini *et al.,* 2015). Unlike traditional fossil fuels, bioethanol offers a lower carbon footprint, making it an environmentally favorable option in the global shift toward green energy.With all these different feedstocks available for bioethanol, it has huge prospects of being a replacement for petroleum-based fuels and is also a biofuel generated through sugar fermentation. The possibilities of saline farming for both reducing greenhouse gas emissions and generating rural economies are huge, but production efficiencies still need to be prioritized (Ajorloo*et al.,* 2022).

Global energy consumption trends continue to highlight a heavy dependency on fossil fuels, a scenario that has heightened the urgency for renewable solutions (Lamichhane *et al.,* 2021). Countries worldwide have increasingly adopted renewable energy policies, with bioethanol production expanding in regions like the United States, Brazil, and parts of Europe (Raj *et al.,* 2022). India, too, has joined the movement, setting ambitious targets for bioethanol production to reduce its fossil fuel imports and improve its energy security. These policy shifts underline bioethanol's critical role in not only lowering carbon emissions but also fostering energy independence (Malik *et al.,* 2022).

The previous three years, global food waste output totaled 1.3 billion tonnes annually, with China producing the most (21.2%), India (12.6%), and Brazil (7.3%) (ICFO, 2023). Fruits, root crops, and vegetables account for 40-50 % of worldwide food waste, according to the FAO. Fruit and vegetable waste

alone account for 37% of overall agricultural waste in Asia. Every year, roughly 35-40% of fruits and vegetables are thrown away as trash. Food waste is created even after eating, storage, and industrial processing, and its management is an issue. The present study aims to explore the efficiency of bioethanol production from food waste by optimizing pretreatment methods and evaluating the potential of large-scale implementation for sustainable biofuel development.

2. MATERIALS AND METHODS

2.1. Collection and processing offood waste

The pre-consumption and post-consumption food waste was collected from different localities of campus, hotels and restaurants since food ingestion differs from person to place. The food waste was processed by cutting into small pieces and drying in a hot air oven at 70℃ for 24hrs. The dried waste was then grounded to powder using laboratory blender. The powdered waste was stored in an air tight container at room temperature until further use.

2.2. Pretreatment of Food Waste

2.2.1 Physical Pretreatment(Saifuddin *Et Al.,* 2016)

The food wastes were subjected to microwave irradiation using a general-purpose laboratory scale microwave oven (Godrej appliances, model: GMX 20GA1 MIZ) with an input power of 600W. About 10mL of distilled water was added to 1g of each type of wastes and were irradiated with microwave oven for different time duration (5, 10, 15 & 20 mins). After pretreatment the samples were then filtered to separate the insoluble solid fiber and cooled at room temperature. After drying, 3,5 Di-nitro salicylic acid (DNSA) test were done for all samples.

2.2.2 Physio-Chemical Pretreatment (Ranjan and Moholkar, 2013)

In 500ml conical flask, 15g of food wastes were added to 150ml of double distilled water and autoclaved at 121℃ at 15lbs for different time duration (15, 30, 45 & 60 mins) and allowed to cool at room temperature. Later the suspension was filtered using a sterile cotton cloth and the pH of the suspension was maintained at 6.0. After drying, DNSA test were done for all samples.

2.2.3 Chemical Pretreatment (Zheng *et al.,* 2018)

For chemical pretreatment, the food waste was treated with sulphuric acid (H_2SO_4), sodium hydroxide(NaOH) and hydrogen peroxide (H_2O_2). acid pretreatment, 5g of dried food wastes were added to a beaker containing different concentration (1, 2, 3, 4 & 5%) of H_2SO_4, NaOHand (20,40,60,80 & 100%) of H_2O_2, and covered with thick aluminum foil for 60mins. The pretreated wastes were then neutralized by rinsing with distilled water until it reaches neutral pH and then dried completely at room temperature. After drying, DNSA test was done for all samples.

2.3. Chemical Composition Analysis Of The Waste (Palupi*et al.,* 2022)

The Chesson technique was used to analyze the chemical composition of treated an untreated food waste. 1g of dry food waste (a) was refluxed in 150 mL of water at 100°C for 60 minutes, then filtered and washed with 300 mL of hot water. The dried residue was weighed (b) and further refluxed in 150 mL of 1N H_2SO_4 at 100°C for 60 minutes. The filtrate was neutralized (300 mL) and dried (c). The residue was then soaked in 10 mL of 72% H_2SO_4 for four hours, followed by the addition of 150 mL of 1N solution. After filtration, neutralization (400 mL), and drying at 105°C, the final residue was weighed (d). The cellulose, hemicellulose and lignin were calculated using the following formula:

Lignin content = [(d-c)/a] x 100%

Cellulose content = [(c-d)/a] x 100%

Hemicellulose content = [(b-c)/a] x 100%

2.3 Isolation And Identification Of Cellulase Producingfungi(Nhan *Et Al.,* 2021)

For the isolation of cellulase producingfungi, 1g of soil was added into the test tube containing sterile distilled water of 9ml each and serially diluted up to a dilution of 10^{-5}. 0.1ml of each dilution was inoculated on PDA plates and incubated at 28℃ for 5 days. After 5 days, the isolates were grown on CMC agar plates containing 2% carboxymethylcellulose (CMC), 0.05g yeast extract and 1.5% agar agar and incubated at 28℃ for 3 days. the plates were flooded by 1% congo red for 20 mins and then the Congo red solution was drained out. The plates were then destained thrice by immersing in 1M NaCl (Sodium Chloride) for 15 mins. The isolates showing clear zone of decolorization around the colony were regarded

as positive for cellulase production.The isolate showing maximum zone of clearance was examined under microscope using lactophenol cotton blue and was identified at species level using 18S rRNA sequencing.

2.5 Enzymatic Hydrolysis of Food Waste (Martin *Et Al.,* 2012)

In a 250 ml flask containing 50 ml of growth medium, a specific inoculum size of *Aspergillus* sp. was transferred from a stock culture. In the rotatory shaker the flask was incubated at 150 rpm for 72 hours at 28°C. The fungal mass was separated by centrifugation at 4500 rpm for 10 minutes. The cell free clear supernatant was collected and used as cellulase enzyme. For hydrolysis, the best pre-treated food waste was autoclaved and added with crude cellulase enzyme and incubated at 30℃ for 6 days.

2.6 Surface Characterization of Hydrolyzed Food Waste

The surface morphology of best hydrolyzed and unhydrolyzedfood waste was analyzed by Scanning Electron Microscope (SEM). Scanning Electron Microscopy was used to study the topography of the substrates before and after the pretreatment method. The specimens were analyzed at a very high magnification from 3000X to 30,000X.

2.7 Isolation and Identification of Yeast (Wuczkowski*et Al.,* 2003)

Fresh curd and batter were collected and fermented for 3 days at room temperature and were then serially diluted on PDA (Potato Dextrose Agar) medium and incubated at room temperature for 48-72 hours. The isolated yeast strains were identified by studying specific colony morphology such as colony color, shape and texture and examining under light microscope. The isolate showing characteristics similar to*Saccharomyces* sp. was selected and identified at molecular level.

2.8 Production of Bioethanol

The production of bioethanol was done using 250ml of food waste hydrolysate in 500ml S-type airlock capped Erlenmeyer flask, inoculated with identified *Saccharomyces* strain. The production was carried out at 30℃ for 120 hrs with mild agitation (100rpm). After incubation, the sample was retrieved to determine the concentration of ethanol produced using chromic acid method. Ethanol was recovered by distillation at 78℃ for 4-6 hrs and was taken for further confirmation by specialized analytical technique.

3. RESULTS AND DISCUSSION

The growing interest in environmentally sustainability and its economic implications has prompted scientific investigations into the potential of biomass as a novel alternative source (Martini and Afroze, 2021).The food waste used in the study was initially screened for any soil and grit materials using mesh screens. Subsequently, the waste was washed under running water and finally rinsed with distilled water to remove excess dust particles followed by sun drying under ambient conditions so that the samples were not over-exposed to sunlight and were cut into small pieces of 1cm size using scissors and grounded to power as shown in figure 1.

A B

Figure 1. Collection of Food Waste (A. Raw Waste B. Powdered Waste)

Food wastes are difficult to degrade because of their complex composition and physiochemical structure. To overcome this barrier, the substrates are put through pretreatment processes (Awogbemi and Kallon, 2022). Pretreatment is the primary and crucial step in accessing cellulose for enzymatic hydrolysis and sugar fermentation (Balat*et al.,* 2008; Karimi and Tehrazadeh, 2008). Hence, the influence of various

pretreatment methods on structure and composition of substrates mainly, percentage of release of reducing sugar was studied. Among all the methods, pretreatment using 4% sulphuric acid was found to be most effective in high release of reducing sugars of 584 µg/ml as displayed in figure 2. Therefore, 4% sulphuric acid was used in the subsequent experiments.

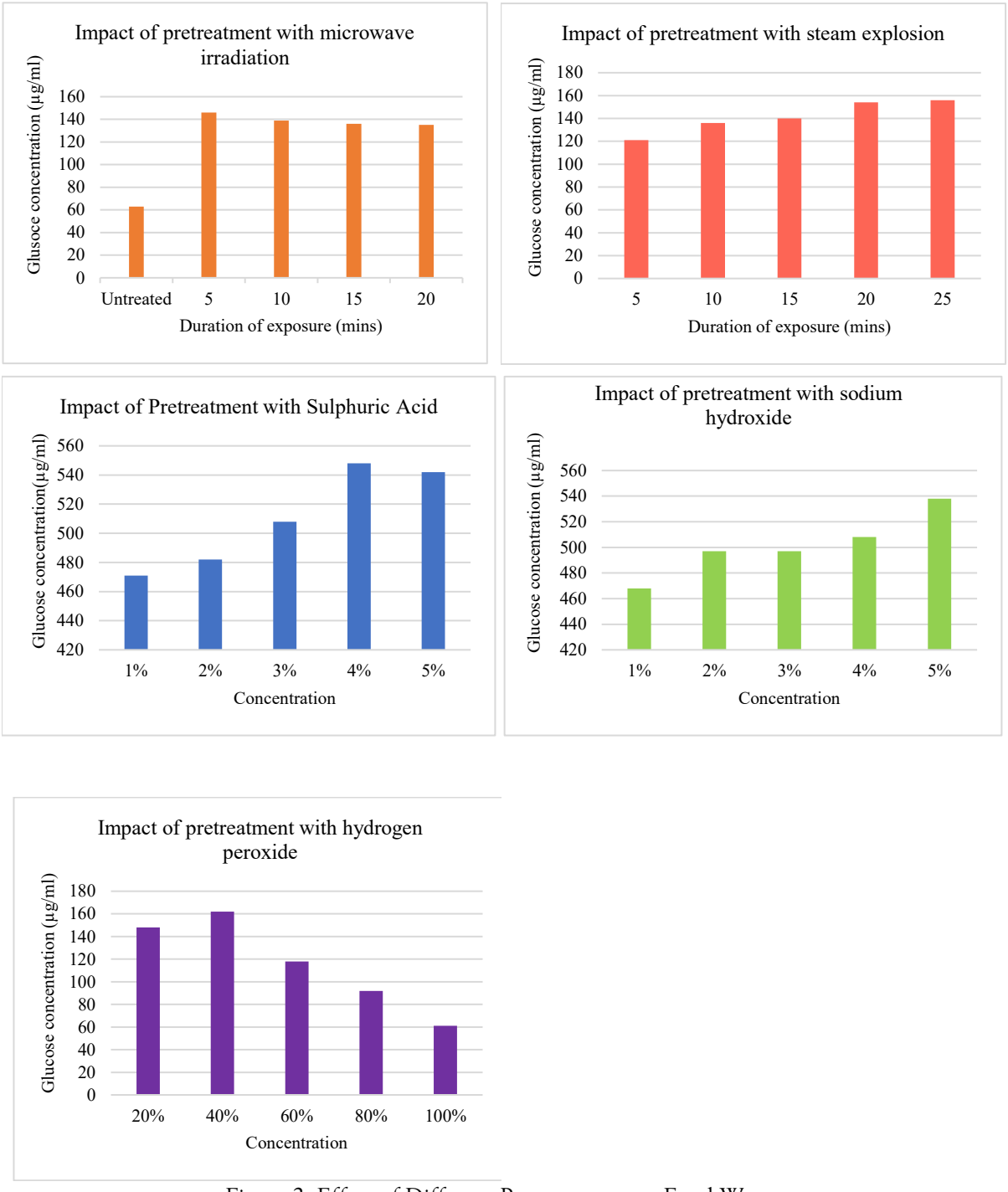


Figure 2. Effect of Different Pretreatment on Food Wastes

In any biomass, cellulose, hemicellulose, and lignin are the main components that account for 34-50%, 19-34% and 11-30% respectively (Chiranjeevi *et al.*, 2012). The characteristics of the food waste such as moisture, cellulose, hemicellulose and lignin were estimated to be %, which are summarized in figure 3.

Similarly, Chowdhury *et al.*, (2018) presented the composition of food waste in their study, showing a notable resemblance to that of the obtained results of the present study.

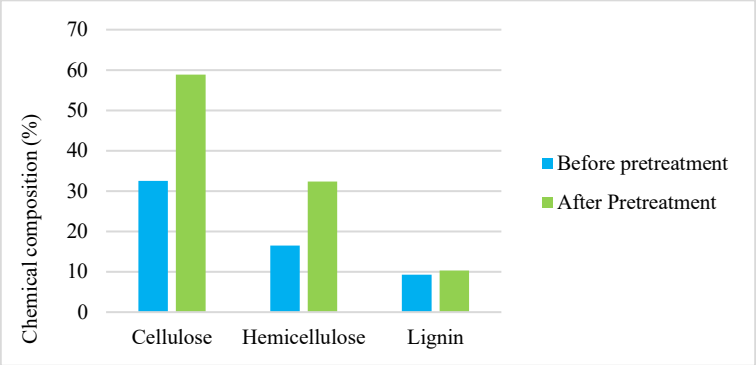


Figure 3. Composition Analysis of Food Waste before and After Pretreatment

Cellulase enzyme produced from microorganisms holds significant economic worth because of their extensive use in textile industry, pharmaceutical manufacturing and biofuel generation (Kuhad*et al.*, 2011; Eiaz*et al.*, 2021). The most crucial stage in enzymatic hydrolysis is the isolation of potential cellulolytic microorganisms that can produce effective cellulase enzyme necessary for the hydrolysis of biomass.A total of 11 distinct fungal strains were isolated from soil sample. The growth of selected fungal strain (VN8) which displayed the ability to produce a clear halo zone on CMC agar after staining with Congo red is shown in figure 4.

(a) (b)

Figure 4. Isolation of Cellulolytic Fungi (A) Pure Culture of Selected Cellulolytic Fungi (B) Selected Isolate Showing Clear Zone on CMC Plate

Based on the colony characteristics and sporulation features evident from the microscopic observation, the fungal isolate 'VN8' was identified belonging to the genus *Aspergillus* (figure 5). The identification of the isolate was further done by molecular characterization. The nucleotides of the isolate were deposited in NCBI database with Accession number: **PQ7376104** and the results confirms that the sequenced isolate VN8 is highly similar to *Aspergillus niger*(figure 6).

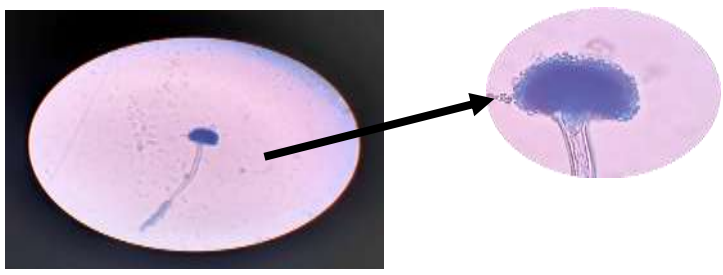


Figure 5. Microscopic Observation of the Isolate

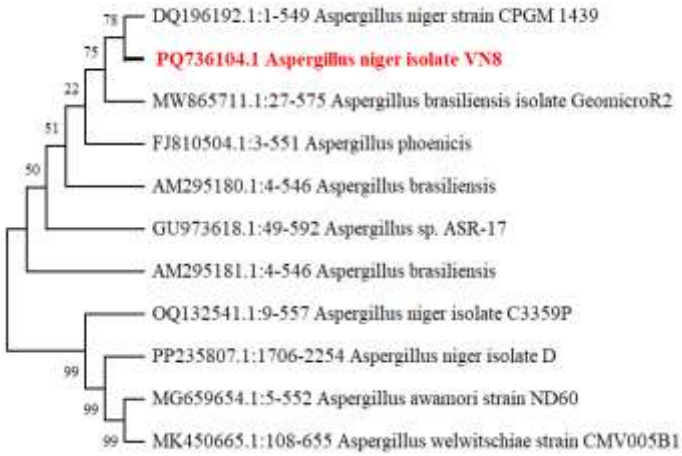
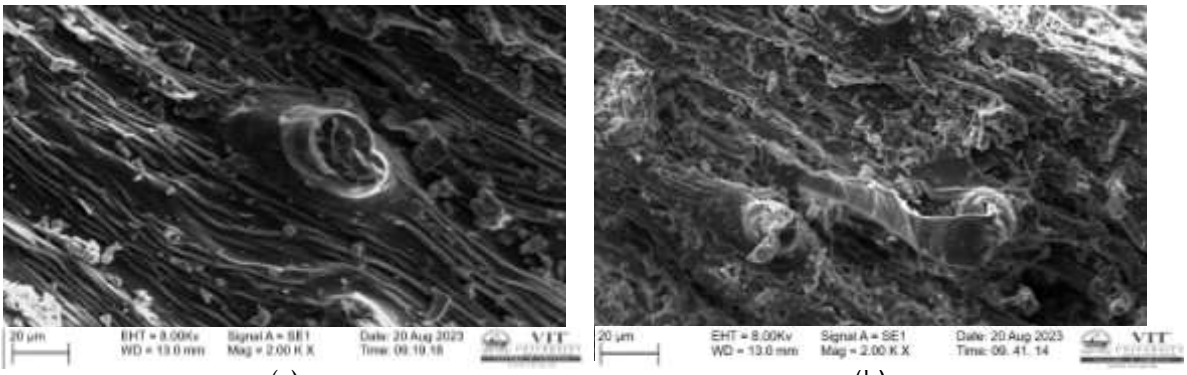


Figure 6. Phylogenetic Analysis of Cellulolytic Isolate (VN8)

After appropriate pretreatment, the food waste enriched in cellulose was hydrolyzed to yield fermentable sugars for their subsequent fermentation to ethanol. The hydrolysis of the pretreated food waste is shown in figure 7. Scanning electron microscopy was used to study the surface morphology of hydrolyzed and unhydrolyzed waste. The effect of acid pretreatment and cellulase hydrolysis on the waste is clearly shown in figure 8. The untreated food waste displayed smooth, intact and organized compact structure (figure 8 (a)). Whereas, the structure of the treated food waste turned rough, brittle and disrupted (figure 8 (b)). The morphology of pretreated substrates clearly shows the swollen and cracked fiber and increased porosity which leads to improvement of exposed areas of cellulosic substrates. Similar structural changes using SEM were observed in sulfuric acid pretreated corn substrate, where more rough and disordered fibers were found in comparison to raw material (Arelli*et al.,* 2020). Whereas, Carvalho *et al.,* (2016) reported rough cell wall surface, fiber swelling using alkaline pretreatment.

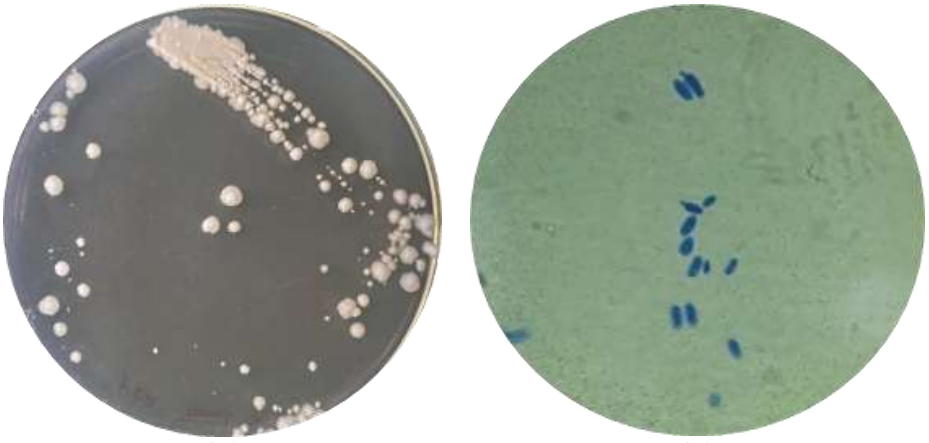
Figure 7. Hydrolysis of Acid Treated Food Waste Using Cellulase Enzyme



(a) (b)

Figure 8. SEM Analysis of Food Waste (A) Untreated (B) Hydrolyzed

Isolation of yeasts from natural resources is the most successful technique to obtain yeast isolates that have abilities to utilize and ferment various exogenous compounds (Banat *et al.,* 1998). Strains exhibiting morphological characteristics similar to*Saccharomyces* were selected. Several researchers have reported isolation of yeast from different food samples (Thapa *et al.,* 2015 and Yuma, 2020). The selected isolates showed white to cream coloured colonies, circular colony shape, smooth texture an oval in shape (figure 9). These results align with the observation made by Halim *et al.,* (2024) regarding the macroscopic characteristics of *Saccharomyces.*



(A) (B)

Figure 9. Colony Morphology and Microscopic Observation of Isolated Yeast

The Molecular identification is a more reliable method for identifying microorganism than the morphological characterization. Therefore, the selected isolates were subjected to molecular identification using 18srRNA sequencing. The nucleotides of the isolate were deposited in NCBI database with Accession number PQ363679 and matched with already existing sequences to construct phylogenetic tree and the screened yeast isolate was identified as *Saccharomyces cerevisiae* (figure 10).

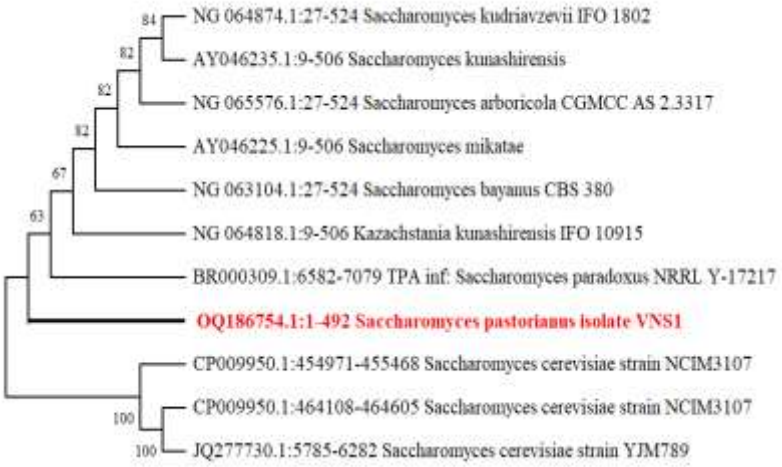


Figure 10. Phylogenetic Analysis of Isolated Yeast Strain

Ethanol production from enzymatic hydrolysate of pretreated food waste conducted in 500ml S-shaped Erlenmeyer's flask is shown in figure 11. The parameters such as residual reducing sugars and ethanol yield, were measured every 24 hours. Maximum ethanol production by *Saccharomyces pastorianus,* was observed at 72 hrs of fermentation, yielding 74.7g/L respectively.Figure 12, illustrates that the total reducing sugars have decreased with the ethanol fermentation.

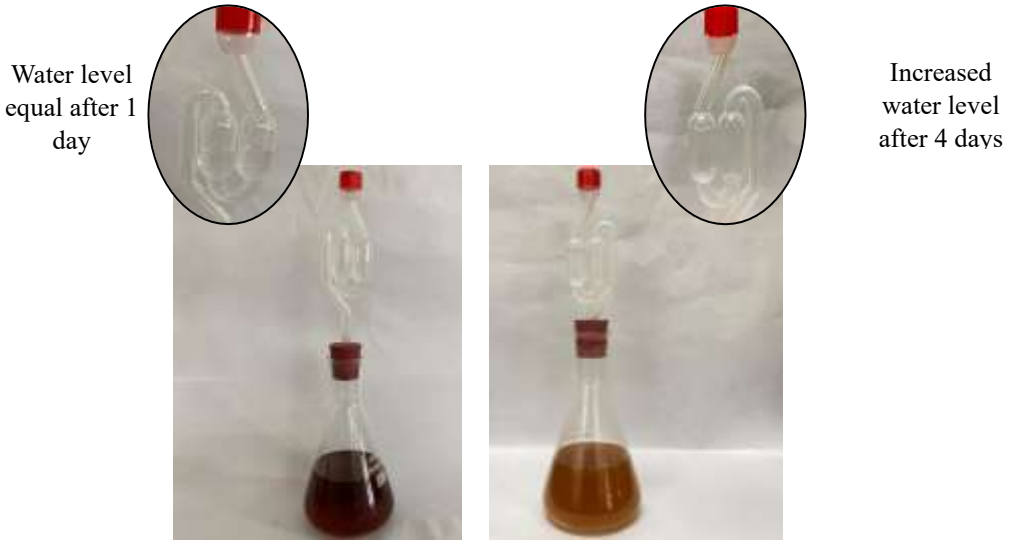


Figure 11. Bioethanol Production In S-Type Airlock-Capped Flasks

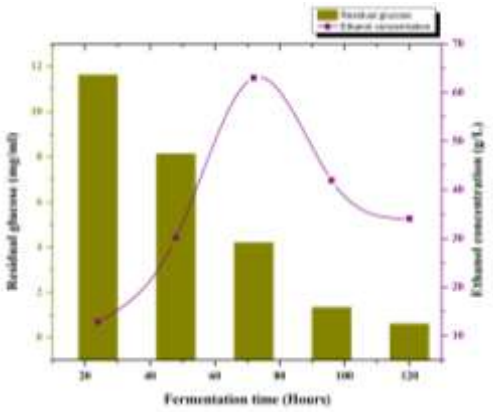


Figure 12. Production of Bioethanol Using Saccharomyces Cerevisiae

4. CONCLUSION

This study highlights an efficient and sustainable approach for converting food waste into bioethanol, through a series of pretreatment, hydrolysis, and fermentation processes, contributing to waste valorization and renewable energy development. Among the tested pretreatment methods, 4% sulfuric acid treatment proved to be the most effective, maximizing the release of reducing sugars (584 µg/mL). The enzymatic hydrolysis using cellulase from *Aspergillus niger* enhanced sugar availability, facilitating efficient fermentation by *Saccharomyces cerevisiae*. The highest ethanol yield of 74.7 g/L was achieved after 72 hours of fermentation. SEM analysis confirmed significant structural modifications in food waste after pretreatment, improving cellulose accessibility. The results provide valuable information for further development of an economically sustainable bioethanol production process using sugarcane bagasse, which appears to be a promising feedstock. Further research is warranted to confirm these results on larger scales and can focus on scaling up the process and optimizing fermentation conditions for commercial applications. Our study provides significant contributions to advancing robust biofuel production approaches and a critical shift towards economic and environmental concerns in the clean energy sector.

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