Evaluating the Valorization Potential of Food Waste for Bioethanol Production via Sequential Acid Pretreatment and Enzymatic Hydrolysis

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Abstract

Interest in bioethanol as a potential alternative to fossil fuels has increased because of its environmental and economic benefits. Food waste is an abundant and underutilized resource with significant potential for bioethanol production, offering a sustainable solution to global energy demands. The study investigates the feasibility of converting food waste into bioethanol in Pakistan. Bioethanol was obtained through a biochemical process from food waste collected from different households and cafeterias. The food waste was dried and grinded to 1-3 mm size. Dilute acid Pretreatment and Enzymatic hydrolysis were used to break down food waste into reducing sugars. The best conversion rate was achieved with 5% acid concentration pretreatment and hydrolysis at 50°C for 36 hours. The hydrolysate sample was then fermented using Saccharomyces cerevisiae at 35°C for 7 days, producing an experimental bioethanol titer of 6.68 g/L. The overall fermentation process showed approximately 68.6% fermentation efficiency, demonstrating the viability of food waste for bioethanol production. Selecting food waste as a feedstock over traditional starch- and sugar-based materials offers dual benefits: reducing food waste and meeting energy needs. Integrating waste into renewable energy production can help Pakistan achieve its sustainable energy goals and targets.

Keywords: Bioethanol, Food Waste, Acid Pretreatment, Enzymatic Hydrolysis, Saccharomyces cerevisiae.

Introduction

Advances in science have offered new options for exploiting resources to improve human life [3]. As a sustainable substitute for fossil fuels, biofuels are made from organic resources and can address energy and environmental problems. Because they can lower greenhouse gas emissions, they aim to play a major role in the global transition to sustainable energy. Among biofuels, bioethanol is a commonly recommended green biofuel because it is renewable, low-polluting and can replace gasoline in the automotive industry. Bioethanol production is one of the most mature and well-established biofuel production processes. In 2016, global production exceeded 100 billion liters [4]. Bioethanol can be produced from feedstock containing sugar, starch or lignocellulosic materials [5], [6]. Bioethanol production is based on some common feedstocks, such as edible materials like sugarcane and corn. However, this limits bioethanol's potential because competition for agricultural land between energy crops and food crops increases costs [7].

Globally, about 1.3 billion tons of food is wasted annually (about one-third of global food production). This waste contributes to an estimated 3.3 billion tons of Carbon Dioxide (CO2eq) Greenhouse Gases (GHGs), greatly polluting the environment. In addition, fossil fuels account for over 75 percent of global greenhouse gas emissions and nearly 90 percent of all carbon emissions. Given food waste's contribution to environmental damage, it is necessary to explore methods and technologies to convert food waste into alternative fuels that can replace fossil fuels, such as biofuels, while aligning with circular bioeconomy principles that emphasize reduction, reuse, and recycling [8]. Moreover, using food waste to synthesize biofuels aligns with the 2015 United Nations (UN) 2030 Agenda for Sustainable Development [9].

Food waste is the organic, biodegradable portion of Municipal Solid Waste (MSW). Food waste comprises lignocellulosic biomass and has great potential to produce high-calorific-value biofuels, such as bioethanol [10], [11]. Although the composition of food waste varies across seasons, regions and populations' dietary habits, it remains an attractive raw material for biofuel manufacturers because of its richness in carbohydrates, lipids, minerals and protein [12], [13], [14]. Mixed food waste is generally preferred for ethanol production because it contains numerous biomaterials such as starch, proteins, polysaccharides, carbohydrates, cellulose, amino acids, lipids, and vitamins. These biomaterials present an intriguing opportunity for bioethanol production [15]. Mahmoodi, Karimi et al. (2018) examined bioethanol production from municipal solid waste. Chemical pretreatment with 0.5 and 1% (w/w) Sulfuric Acid (H_2SO_4) at 130 and 160 °C for 0, 30, and 60 minutes was used in this study. A hydrolysate containing 23.4 g/L glucose was produced from enzymatic hydrolysis of pretreated solids. Without detoxification, 1% acid treatment for 60 min at 130 °C yielded 44.6 and 44.4 g ethanol per 100 g glucose produced from the hydrolysate. Generally, 194.0 g of ethanol was produced from dry food waste through back-to-back cycles [16]. Bioethanol from

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waste bread is assessed using *Aspergillus Niger* for enzymatic hydrolysis and finally the production of ethanol from glucose by *Saccharomyces cerevisiae*. Waste bread at 55 °C and 300 rpm was hydrolyzed using glucoamylase and protease enzyme extracts. The results show an ethanol yield from glucose (YP/S) of 0.37, indicating a conversion efficiency of 72%, and support developing food waste into a useful commodity like ethanol, thereby supporting the sustainable chemical sector [17].

Roukas & Kotzekidou (2022) thoroughly reviewed the process of turning food-sector waste into second-generation bioethanol. It describes the three primary processes that are involved: pretreatment, enzymatic hydrolysis, and fermentation. The review covers a range of pretreatment techniques, such as chemical, physical (milling, pyrolysis, microwave irradiation), physicochemical (steam explosion, hydrothermal processes, ammonia fiber explosion), and biological (fungi, bacteria) treatments. The review also covers enzymatic hydrolysis, which uses enzymes such as cellulase, β-glucosidase, and pectinase to convert complex carbohydrates into fermentable sugars. The review concludes by discussing the fermentation stage, in which microbes turn these sugars into bioethanol. The review also covers challenges across all steps, including pretreatment optimization and biomass management [18]. In the context of a circular bioeconomy, Bender et al. (2024) explored using food waste to manufacture bioethanol. To increase bioethanol output, the study investigates various enzyme combinations for hydrolyzing food waste and assesses how well Spirulina platensis supplements the process. In keeping with the circular bioeconomy's zero-waste tenet, the study also examines the possibility of turning residual waste from bioethanol production into animal feed. The results suggest this strategy is feasible for producing both biofuel and useful byproducts [19].

In this study, mixed food waste was used as a feedstock to produce bioethanol through biochemical processes. The main goal was to produce bioethanol from mixed food waste via yeast fermentation. In addition, the study identified optimum pretreatment and hydrolysis concentrations to maximize fermentation yield. The food waste was collected from different sources, including households and cafeterias. Dilute acid pretreatment using H_2SO_4 and enzymatic hydrolysis using *Aspergillus Niger* are employed to break the feedstock into reducing sugars. After hydrolysis, the sample was fermented using Saccharomyces cerevisiae at 35°C for 7 days. The conversion of reducing sugars to the product was analyzed every 24 hours at 45°C.

Materials and Methods

Feedstock Preparation

The food waste used as a feedstock was collected from various sources, including households and cafeterias around the University of Engineering and Technology, Taxila. The collected food waste was sun-dried for two weeks. After drying, the sample was ground using a high-speed laboratory-grade knife mill. The sample was then passed through a series of standardized mechanical mesh sieves to achieve a particle size of 1-3 mm. The sample was then stored in a zip-lock plastic bag at 4 degrees Celsius for further use.

Characterization of Feedstock Sample

The feedstock sample was characterized chemically by estimating pH, moisture content, total solids, volatile solids, ash content, Total Kjeldahl Nitrogen (TKN), cellulose, hemicellulose, and lignin content. Each parameter was measured using standardized protocols.

- A pH meter was used to determine the pH of a mixture, which was prepared by mixing 20g of the sample with distilled water [20].
- Moisture content was determined by measuring the weight difference before and after drying at 103-105°C for 24 hours [21].
- Total solids were also calculated by measuring the weight difference before and after drying at 103-105°C for 24 hours, while volatile solids were measured after further combustion in a muffle furnace at 550°C.
- Ash content was measured by burning the dried sample at 550°C for 1 hour [22].
- Total Kjeldahl nitrogen (TKN) was measured using the Kjeldahl method. Protein content was determined by multiplying TKN by 6.25 (factor) [18].
- Cellulose and hemicellulose contents were determined by chemical treatments with sodium hydroxide and sulfuric acid, respectively, with lignin content measured after sulfuric acid treatment [23], [24].

Dilute Acid Pretreatment

The food waste was pretreated with 1%, 3%, and 5% H_2SO_4 for 1–3 days using a 1:3 sample-to-acid ratio (5g sample in 15 mL acid). Using the dinitrosalicylic acid (DNS) method (after washing and filtering the pretreated sample with distilled water), we identified reducing sugars. The remaining pretreated sample was dried at 50°C for 2 days and stored in airtight containers for subsequent hydrolysis [25] .

Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted using enzymes produced from *Aspergillus niger* strain. The strain, sourced from the Department of Microbiology, Quaid-I-Azam University (QAU), was cultivated on Potato Dextrose Agar (PDA) medium at 30°C for 5 days. Spore suspensions were prepared by washing the plate surface with a 50% glycerol solution. Submerged fermentation was used for enzyme production in a basal growth medium (67 g/L wheat bran and other nutrients) with 15 g urea to maintain a carbon-to-nitrogen (C/N) ratio of 10. The enzyme cocktails were extracted from the culture broth and stored for hydrolysis experiments [26].

The pretreated sample was hydrolyzed using 25 mL of centrifuged enzyme solution for 24, 36, and 48 hours at 45°C, 50°C, and 55°C. Based on standardized metrics for this specific *Aspergillus niger* cultivation, the crude enzymatic cocktail possessed an estimated cellulolytic activity profile of 3.5-5.2 FPU/mL, delivering an approximate localized enzyme loading of 17.5-26 FPU/g of dry substrate. We measured reducing sugars every 24 hours

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using the DNS method. After hydrolysis, the sample was washed and filtered, and the extract was used to estimate sugars. The solid residue was dried at 50°C for two days and stored in airtight containers for fermentation [25], [26].

Estimation of Reducing Sugars

To measure reducing sugars using the DNS method, we prepared a standard calibration curve with known glucose concentrations. Absorbance values were measured with a spectrophotometer, and a regression curve was constructed using the values shown in Table 1. With an R^2 value of 0.9792, the regression equation was derived from the curve, showing a satisfactory match [27].

To perform the DNS method, 1 mL of sample extract was mixed with 2 mL DNS reagent, heated at 90°C for 15 minutes, and diluted with 4 mL distilled water. Absorbance at 540 nm was measured against glucose standards (0.1 g/L to 1 g/L) prepared from a 1 g/L stock solution (Figure 1) [25].

Table 1:Standard Curve Values for Reducing Sugar Estimation

Concentrations (g/L)	Absorbance
0.1	0.045
0.2	0.216
0.3	0.381
0.4	0.46
0.5	0.634
0.6	0.779
0.7	0.938
0.8	1.122
0.9	1.437
1.0	1.666

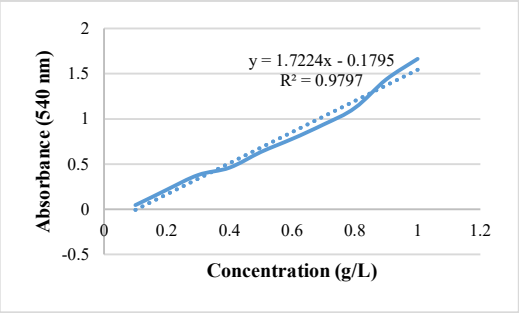


Figure 1: Calibration Curve for Reducing Sugars Estimation

Fermentation

Saccharomyces cerevisiae was used for fermentation at 35°C for 7 days. The hydrolysate was centrifuged, neutralized to pH 5.0, and sterilized. Fermentation was carried out at 35°C for seven days in 300 mL of medium. Reducing sugars were measured every 24 hours using the DNS method. Fermentation results were analyzed to determine the optimum time for maximum ethanol production[1], [25].

Fractional Distillation

Fractional distillation was performed to separate ethanol from the fermentation broth. The setup was constructed by placing a 500 mL single-neck round-bottom flask within a controlled heating mantle. The neutralized, centrifuged fermentation effluent was poured into the flask. A glass distillation adapter holding a laboratory thermometer was connected directly to the flask, allowing continuous monitoring of the emerging vapor temperature at the exit throat. Gradual heating was applied to keep maintaining a steady vapor temperature profile matching the volatility window of the escaping bioethanol. The vapor was then cooled using a water-cooled Liebig condenser. The condensed bioethanol was collected into a graduated receiving cylinder until the boiling temperature rose toward the boiling point of water, signaling completion of volatile-phase recovery [28].

Results and Discussions

Chemical Characterization

The chemical composition of the food waste sample is summarized in Table 2. The high cellulose and lignin contents (28.53% and 36.89%, respectively) suggest that an effective pretreatment strategy is needed to break down the biomass and release fermentable sugars. Overall, these values suggest that the feedstock contains significant fermentable components, which enhance the potential for enzymatic hydrolysis.

Table 2: Chemical Composition of Food Waste Sample

Parameter	Content
pH	4.76
Total Solids (%)	32.9
Ash (%)	2.63
Volatile Solids (%)	29.6
Moisture Content (%)	67.02
TKN (%)	0.1456
Cellulose (%)	28.53
Hemicellulose (%)	8.36
Lignin (%)	36.89
Protein (%)	0.91

Effect of Dilute Acid Pretreatment on reducing sugar Yield

Reducing sugars were released from the food waste after pretreatment with 1%, 3%, and 5% H_2SO_4 for 1-3 days. The maximum reducing sugar concentration (14.8 g/L) was obtained with 5% H_2SO_4 on day 3. This result confirms that increasing acid concentration and pretreatment duration enhance reducing-sugar release, but prolonged exposure may reduce sugar concentrations and cause the formation of inhibitory compounds such as furfural and hydroxy-methyl-furfural (HMF) [29]. Figure 2 compares the reducing sugar yields obtained across all tested pretreatment conditions.

It may be noted that the study did not conduct the direct quantification of furfural and HMF generated during the pretreatment; however, downstream fermentation kinetics (Section 3.4) verified that inhibitor concentrations remained well below toxic thresholds. Referencing Liu et al. (2004), *Saccharomyces cerevisiae* exhibits a robust in situ detoxification response, metabolizing toxic furan aldehydes into less harmful alcohol derivatives after a brief

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adaptive phase. [30] The rapid reduction in reducing sugars by S. cerevisiae during the initial 48 hours indicates no severe, lingering metabolic inhibition, confirming that post-pretreatment washing and neutralization preserved hydrolysate compatibility (Table 3).

Table 3: Reducing Sugars produced after pretreatment

Time (Day)	Acid Concentration (%)	Reducing Sugars (g/L)	Avg. Reducing Sugars (g/L)
1	1	7.314	7.179
		7.082	
		7.140	
	3	9.753	9.560
		9.172	
		9.753	
	5	11.553	11.670
		11.670	
		11.786	
2	1	8.359	8.390
		8.301	
		8.534	
	3	10.915	10.857
		10.798	
		10.857	
	5	12.657	12.783
		12.744	
		12.947	
3	1	9.114	8.960
		9.231	
		8.534	
	3	11.786	11.941
		12.076	
		11.960	
	5	14.689	14.786
		14.864	
		14.805	

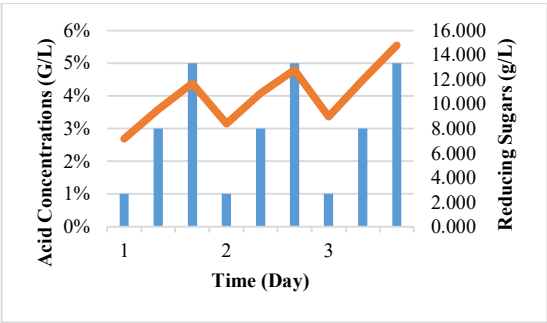


Figure 2: Comparison of Reducing Sugar (RS) after acidic pretreatment

Enzymatic Hydrolysis

The pretreated samples underwent enzymatic hydrolysis at various temperatures and durations. Reducing sugars from the sample hydrolyzed at 50°C for 36 hours yielded the highest concentration (27.6 g/L) compared with other temperature and time conditions. Hydrolysis at 50°C for 36 hours produced the highest reducing sugar concentration (27.6 g/L). This finding suggests that enzyme activity peaks under these conditions, maximizing sugar release for subsequent fermentation. Figure 3 compares the reducing sugar yields obtained under the different hydrolysis conditions.

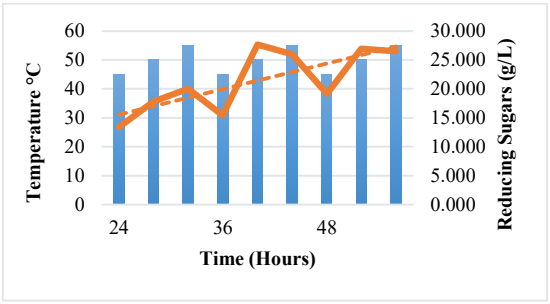


Figure 3: Comparison of reducing sugars (RS) produced after acid hydrolysis at different times and acid concentrations

Fermentation

The hydrolysate was fermented using *Saccharomyces cerevisiae* at 35°C for 7 days. Every 24-hour measurement shows a consistent reduction in reducing sugar concentration, indicating successful product formation. The values are shown in Table 4. Figure 4 illustrates that maximum product formation occurred in the first 4 days of the fermentation, with approximately a 62% drop in reducing sugar concentration. After day 4, the reduction in reducing sugars slowed, reaching a near-constant rate by day 6 and day 7. By day 7, the final reducing sugar concentration dropped to 8.592 g/L, representing about 68.91% substrate utilization efficiency. After fractional distillation, 2.5 mL of ethanol was obtained from 300 mL of fermentation broth.

Table 4: Reducing sugars produced after Fermentation process

Time (Day)	Reducing Sugars (g/L)
0	27.639
1	25.723
2	23.110
3	19.858
4	10.392
5	9.172
6	8.592
7	8.592

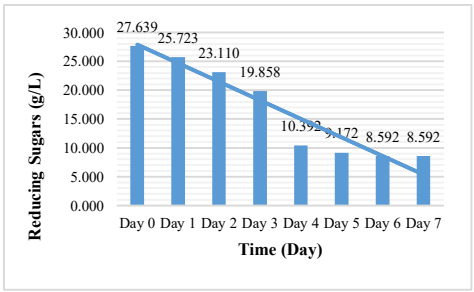


Figure 4: Conversion of Reducing Sugars into biofuel after Fermentation

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Material Balance and Efficiency

The material balance for a 300 mL fermentation broth indicates that the initial reducing sugar concentration was 27.639 g/L, totaling 8.29 g of reducing sugars at the start. After 7 days of fermentation at 35°C, the final reducing sugar concentration dropped to 8.592 g/L, leaving 2.58 g of residual sugars in the medium. Thus, this represents a substrate utilization efficiency of 68.91% by Saccharomyces cerevisiae, consuming a total of 5.71 g of reducing Sugars.

Following fractional distillation, a final volume of 2.54 mL bioethanol was recovered. Considering the density of pure ethanol (0.789 g/mL), this volume equates to approximately 2.0 g of ethanol. This results in an actual experimental ethanol titer of 6.68 g/L and an experimental yield of 0.35 g/g (2.0 g ethanol / 5.71 g consumed reducing sugars). According to the theoretical Gay-Lussac maximum yield (0.511g ethanol/g glucose) [31], the experimental setup can produce approximately 2.917 g of bioethanol. This corresponds to an actual fermentation efficiency of approximately 68.6% relative to the theoretical maximum yield (2.0 g of actual bioethanol yielded / 2.917 g maximum theoretical bioethanol yield).

Compared to other studies, where substrate utilization efficiencies range between 60% and 75% under similar conditions [26], [31], the achieved fermentation profiles align well with established literature. The enzymatic hydrolysis step, performed at 50°C for 36 hours, was particularly effective, yielding a reducing sugar concentration of 27.639 g/L and significantly enhancing ethanol production. Fractional distillation successfully separated ethanol, providing a pure product. While direct analytical quantification (such as Gas Chromatography or HPLC) was not performed to profile secondary metabolites or track minute metabolic side-routing, physical recovery via fractional distillation verifies successful bioethanol production. Overall, the process efficiency and ethanol yield confirm that food waste is a viable feedstock for bioethanol production, offering a sustainable solution for waste valorization and renewable energy generation.

Conclusion

This study demonstrates the feasibility of bioethanol production from food waste through dilute acid pretreatment, enzymatic hydrolysis, and fermentation. The study provides optimized conditions of 3 days of pretreatment with 5% H_2SO_4, enzymatic hydrolysis using *Aspergillus Niger* strain at 50°C for 36 hours, and fermentation at 35°C for 7 days, which yielded the highest bioethanol yield. The production process studied, including pretreatment, hydrolysis, fermentation, and fractional distillation, takes approximately 12 days to complete (excluding 15 days for feedstock preparation). Overall, producing bioethanol from food waste offers a workable solution to the world's waste and energy problems. Moreover, this study provides opportunities for future work focusing on sustainable, low-chemical pretreatment and continuous fermentation systems for scalable bioethanol production. One area that needs improvement is optimizing drying methods for high-moisture food waste.

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Competing Interests

The authors declare no conflict of interest.

Authors' Contribution

Conceptualization and methodology, D.A., and S.M.A.; formal analysis, investigation, resources, data curation, D.A., F.T. and M.F.; visualization, D.A. and S.M.A.; writing—original draft preparation, D.A., S.M.A., L.A and M.U.A.; writing—review and editing, D.A., S.A, and M.U.A. All authors have read and agreed to the published version of the manuscript.

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