EVALUATING THE POTENTIAL OF SEDGE GRASS (*S. TABERNAEMONTANI*) FOR BIOETHANOL PRODUCTION

Freweyni Hailu, Legesse Adane, Zerihun Demrew Yigezu, AND *Tesfakiros Semere**

1. Introduction

Fossil fuels remain the dominant sources of energy for meeting global demand (Bauer et al., 2016). However, their continued use presents several challenges.

*Freweyni Hailu Asfaha is a Lecturer in the Department of Biotechnology at the College of Dryland Agriculture and Natural Resources, Mekelle University, Ethiopia. She earned her B.Sc. in Biotechnology from Mekelle University in 2017 and subsequently served as a Graduate Assistant from July 2017 to June 2018. She completed her M.Sc. in Bioenergy Science and Technology at Hawassa University in November 2021. Her research interests focus on bioenergy and environmental pollution control. The author is thankful to the NORAD EnPe project, Hawassa University, for financial support and also to Mekelle University for giving her the opportunity to attend the Master's program at Hawassa University.

Legesse Adane holds a Ph.D. in Medicinal Chemistry from the National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Punjab, India, and an M.Sc. in Organic Chemistry from Addis Ababa University, Ethiopia. His research interests include natural product chemistry—particularly medicinal plants—computer-aided drug design and discovery, organic synthesis, environmental pollution control, and bioenergy-related studies. He has supervised more than 100 M.Sc. students at Jimma University and Hawassa University, as well as three Ph.D. candidates, and has published over 50 articles in peer-reviewed international journals. He is currently a Professor of Organic Chemistry at Hawassa University, Ethiopia.

Zerihun Demrew Yigezu, Ph.D., is an Associate Professor of Bioenergy and Biotechnology at Hawassa University, Ethiopia. He earned his Ph.D. in Bioenergy Science from the Department of Biochemical Engineering at Anna University, Chennai, India, and his M.Sc. in Biotechnology and Molecular Biology from Haryana Agricultural University, India. Dr. Yigezu's research focuses on bioenergy resources and biomass conversion technologies. He has supervised over 32 M.Sc. and 5 Ph.D. students and has published more than 26 articles in internationally reputable peer-reviewed journals. (continued)

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First, as non-renewable resources, fossil fuel reserves are steadily depleting and may be exhausted in the near future (Birhanu and Ayalew, 2017). Second, global dependence on fossil fuels has generated adverse consequences, including the depletion of crude oil reserves, severe air pollution, rising global temperatures, and increasingly unpredictable weather patterns. This dependency also reinforces geopolitical and economic vulnerabilities, particularly for nations reliant on imports from a limited number of crude oil-exporting countries. Third, fossil fuel markets are characterized by high and often volatile prices, largely controlled by a small group of producing countries (Afolabi et al., 2023; Wood et al., 2022; Miller and Sorrell, 2014). Collectively, these limitations have compelled the search for alternative energy sources that are renewable, environmentally sustainable, and cost-effective (Ahorsu et al., 2018). Among these, biofuels have received significant attention as a potential solution (Aditya et al., 2016).

Biofuels are derived from biomass sources such as wood, agricultural crops and residues, aquatic plants, forestry products, and animal wastes (Usman et al., 2018). They are renewable and, although their combustion releases carbon dioxide (CO_2) similar to fossil fuels, the feedstock plants absorb CO_2 during growth. As a result, the net contribution to atmospheric CO_2 is considered close to zero (Birhanu and Ayalew, 2017; Hailemichael, 2016; Malla et al., 2022; Kapasi et al., 2010). Among the various biofuels, the most widely utilized are bioethanol and biodiesel (Dharma et al., 2016). Bioethanol is produced through microbial fermentation of biomass-derived sugars and typically contains 35% oxygen, while biodiesel is produced from bio-oils (Gebreegziabher et al., 2017). The availability of feedstocks for bioethanol production, however, varies seasonally and geographically (Balat et al., 2008). While first-generation bioethanol technologies are more established, second- and third-generation pathways—based on lignocellulosic and algal biomass—hold significant promise if effectively implemented (Aditya et al., 2016).

Sedge grass (*Schoenoplectus tabernaemontani*), a member of the Cyperaceae family, is a widely distributed aquatic grass with lignocellulosic composition similar to other bioenergy crops. It is abundant in lakes and wetlands worldwide, including those in Ethiopia. Despite its availability, there are no existing studies on its lignocellulosic profile or its potential for ethanol production (Brown and Elliman, 2020; Mekbeb et al., 2022). Therefore, this study was undertaken to evaluate

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Tesfakiros Semere is an Associate Professor of Plant Breeding and Biotechnology at Mekelle University, Ethiopia, where he also serves as Head of College Research and Community at the College of Dryland Agriculture and Natural Resources. He earned his Ph.D. in Plant Breeding and Seed Systems from Mekelle University in 2024 and holds an M.Sc. in Molecular Biology from Katholieke Universiteit Leuven (KU Leuven), Belgium. His research interests include molecular plant breeding, genomics, agrobiodiversity, seed systems, and bioenergy. Dr. Semere has supervised five M.Sc. students and is currently supervising five Ph.D. candidates. He has authored more than 15 peer-reviewed publications, with several additional articles currently under review.

sedge grass as a feedstock for bioethanol production by assessing its chemical composition, reducing sugar yield, and ethanol conversion efficiency.

2. Materials and Methods

2.1. Description of the Study Area: Samples of sedge grass (*Schoenoplectus tabernaemontani*) (Figure 1) were collected at the maturity stage in November 2019 from Lake Hawassa, located 273 km south of Addis Ababa in the Sidama Region of Ethiopia. The lake lies at an altitude of 1,750 m above sea level (7°03′ N latitude and 38°28′ E longitude). The area receives a mean annual rainfall of approximately 971.9 mm and has an average temperature of 20.85 °C (National Meteorology Agency, 2019). Experimental analyses were conducted at the School of Chemical Engineering, Institute of Technology, Hawassa University, and at the College of Veterinary Medicine, Mekelle University, Ethiopia.

2.2. Plant Material Collection and Preparation: A total of 2,000 g of sedge grass biomass was collected from Lake Hawassa, Sidama Region, Ethiopia. The stem portions were cut into small pieces and thoroughly washed with tap water. The material was then oven-dried (Model 100-800) at 60 °C for 48 hours. After drying,

Figure 1
THE SEDGE GRASS FROM LAKE HAWASSA, ETHIOPIA

Source: Photo by H. Freweyni, November 2019.

the biomass was ground into powder with a particle size of approximately 2 mm using a Sortmks-3332 grinder (PFEUFFR, Germany). This process increased the surface area for enhanced interaction with extraction solvents, while also reducing cellulose crystallinity to facilitate subsequent hydrolysis.

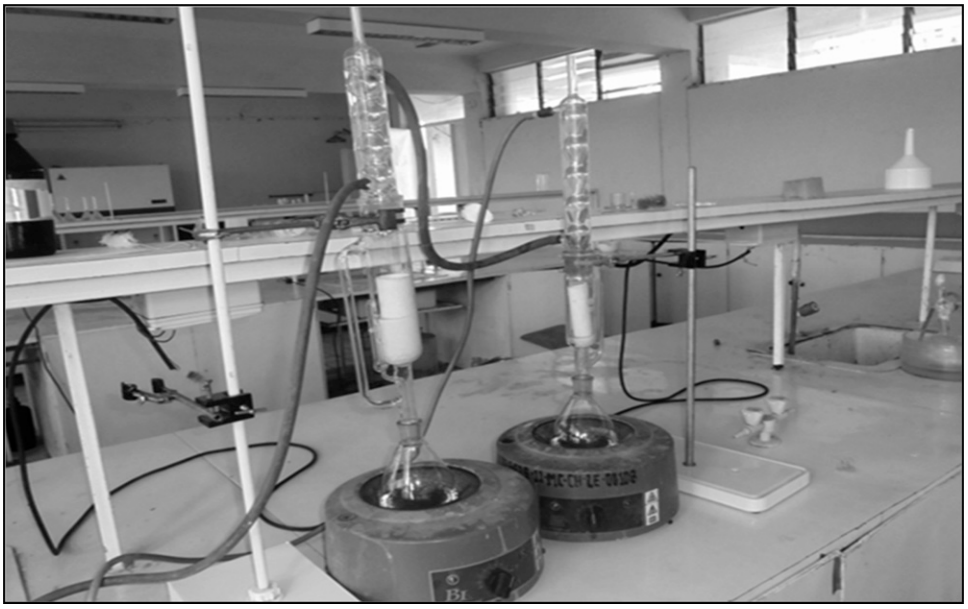
2.3.1. Determination of Chemical Composition:

Determination of Extractives: Approximately 3 g of powdered plant material was subjected to Soxhlet extraction at 60 °C for 72 hours using 150 mL of acetone as the solvent (Figure 2). The boiling and rising stages were maintained at 70 °C and 25 minutes, respectively. Following extraction, the solid residue was air-dried at room temperature and subsequently oven-dried at 105 °C for 1 hour until a constant weight was obtained. The percentage of extractives (w/w) was determined as the weight difference between the raw biomass and the extractive-free sample, following the NREL protocol (2005). Acetone was selected as the extraction solvent due to its low boiling point and ease of evaporation. The extractive content was calculated using Equation (1).

$$Extractive\ \% = \frac{W1 - W2}{W1} \times 100 \tag{1}$$

where $W1$ = oven dried sample and $W2$ = extracted residue.

Figure 2
THE SOXHLET EXTRACTOR SETUP USED FOR OIL EXTRACTIONS



Determination of Hemicellulose: The hemicellulose content was determined following the procedure described by NREL (2005). Briefly, 1.0 g of extractive-free powdered sample was placed in a 250 mL conical flask, to which 150 mL of 0.5 N NaOH solution was added. The mixture was heated on a hot plate at 60 °C for 3.5 hours. After boiling, the solution was cooled to room temperature and filtered using vacuum filtration. The solid residue was washed thoroughly with distilled water until a neutral pH was achieved, then dried in a conventional oven at 105 °C for 4 hours. The hemicellulose content (% w/w) was calculated as the difference in sample weight before and after treatment (Equation 2).

$$Hemicellulose\ \% = \frac{W1 - W2}{W1} \times 100 \tag{2}$$

where $W1$ = oven dried sample and $W2$ = oven dried extracted residue.

Determination of Lignin: According to the National Renewable Energy Laboratory (NREL) protocol, lignin in biomass is classified into two fractions: acid-insoluble lignin (AIL) and acid-soluble lignin (ASL) (NREL, 2005). Both fractions were quantified to determine the total lignin content of the biomass. For AIL determination, 0.5 g of extractive-free sample was placed in a flask and hydrolyzed with 3 mL of 72% H_2SO_4 at room temperature for 2 hours, with occasional shaking at 30-minute intervals. The mixture was then diluted with 84 mL of distilled water, and a second hydrolysis step was carried out in an autoclave (LaMCS204) at 121 °C for 1 hour. After cooling, the hydrolysate was filtered under vacuum. The solid residue was dried in an oven at 105 °C for 3 hours, followed by incineration in a muffle furnace at 575 °C for 2 hours to account for ash content. The acid-insoluble lignin was calculated according to Equation (3). For ASL determination, the filtrate was neutralized with NaOH solution, and the absorbance was measured at 205 nm using UV spectrophotometry. The acid-soluble lignin content was calculated using Equations (4) and (5). The total lignin content of the biomass was expressed as the sum of AIL and ASL (Equation 6).

$$Acid-insoluble\ lignin = \frac{weight\ of\ AIR - weight\ of\ ash}{weight\ of\ ODS} \times 100 \tag{3}$$

where AIR = acid-insoluble residue and ODS = oven dried sample.

$$Acid\ soluble\ lignin\ \% = \frac{d \times v \times Aa}{a \times w \times L} \times 100 \tag{4}$$

where d = the dilution (dimensionless) and was calculated by Equation (5):

$$d = \frac{volume\ of\ sample + volume\ of\ diluting\ solvent}{volume\ of\ sample} \tag{5}$$

where V = the filtration volume (L), Aa = the average absorbance of the sample (dimensionless), a = absorptivity of the lignin (L/g.cm), W = the oven-dry mass of the sample (g), L = the path length of UV-Vis cell (cm); and the value of "a" at 205 nm = 110. Therefore, we have Equation (6):

$$Total\ lignin\ \% = Acid\ insoluble\ lignin + Acid\ soluble\ lignin \tag{6}$$

Determination of Cellulose: The cellulose content (% w/w) was determined indirectly, as described in the literature (Danmaliki et al., 2016). It was calculated by subtracting the sum of the other major biomass components—hemicellulose, extractives, and lignin—from 100% of the sample. The calculation was performed using the following Equations (7) and (8):

$$WC + WH + WE + WL = 100 \tag{7}$$

$$WC = 100 - (WH + WE + WL) \tag{8}$$

where WC = cellulose content, WH = hemicellulose content, WE = extractive content, and WL = lignin content.

2.3.2. Bioethanol Production Process:

Acid Pretreatment: The pretreatment process was carried out to remove lignin, reduce cellulose crystallinity, and increase material porosity, thereby enhancing sugar release while minimizing carbohydrate loss and inhibitor formation (Kumar et al., 2009). In this study, sedge grass biomass was pretreated with 1.5% (v/v) dilute H_2SO_4 at a solid-to-liquid ratio of 1:10 (w/v). The mixture was placed in a 500 mL flask and autoclaved at 121 °C for 60 minutes. After cooling, the slurry was filtered under vacuum, and the residue was washed four times with distilled water until a neutral pH of 6.78 was achieved. The neutralized residue was oven-dried at 60 °C for 24 hours in preparation for hydrolysis (Kumar et al., 2009).

Dilute Acid Hydrolysis: Hydrolysis was performed using 1.5%, 2.5%, and 3.5% (v/v) H_2SO_4 solutions, following previously reported protocols (Warrand and Janssen, 2007; Gladyshko, 2011). The reactions were carried out at three different temperatures (115, 125, and 135 °C) and three hydrolysis times (40, 60, and 80 minutes). After hydrolysis, the solid fraction (mainly non-fermentable lignin) was separated from the liquid hydrolysate by vacuum filtration. The resulting liquid fraction was retained for fermentation experiments.

pH Adjustment: Prior to fermentation, the pH of the hydrolysate was adjusted to 4–5 to maintain favorable conditions for microbial activity and to prevent microbial death under highly acidic or basic environments (Wondale, 2012). The pH was adjusted using 2N NaOH added dropwise while monitoring with a digital pH meter until the desired range was achieved.

Sterilization: All reactors and fermentation equipment were sterilized by autoclaving at 121 °C for 15 minutes to ensure aseptic conditions (Hossain et al., 2015).

2.3.3. Absorbance Reading for Standard Glucose: The total reducing sugar content produced during acid hydrolysis was determined using Benedict's solution method, as described by Danmaliki et al. (2016). In this procedure, the hydrolyzed sample was mixed with Benedict's reagent and heated in a water bath at 90 °C for 5 minutes. The reaction induced a characteristic color change, which provided a semi-quantitative estimate of reducing sugar concentration: blue (no sugar), green (0.5%), yellow (1%), orange (1.5%), red (2%), and brown (highest concentration). This colorimetric response results from the reduction of blue copper (II) ions to brick-red copper (I) oxide in hot alkaline conditions. The absorbance of the reaction mixtures was measured at 540 nm using a UV–visible spectrophotometer and reducing sugar concentrations were calculated against a glucose calibration curve.

Preparation of Standard Glucose Solutions: Standard glucose solutions were prepared by dissolving 0, 0.2, 0.4, 0.6, 0.8, and 1.0 g of glucose in 5 mL of distilled water in six separate test tubes. Each standard solution (1 mL) was then added to a test tube containing 5 mL of Benedict's reagent. The mixtures were heated in a water bath at 90 °C for 5 minutes, rapidly cooled, and filtered. The absorbance of each filtrate was recorded at 540 nm. A calibration curve was constructed by plotting absorbance (Y) against glucose concentration (x), and the slope (m) and intercept (b) of the line were obtained according to Equation (9):

$$Y = mx + b \tag{9}$$

where Y = absorbance, x = glucose concentration, m = the slope, and b = the intercept.

2.3.4. Determination of Sample Total Reducing Sugar Concentration: The concentrations of unknown sugar samples were determined using a glucose standard calibration curve ($Y = 0.024X - 0.002$; $R^2 = 0.998$). The absorbance of each sample was first measured at 540 nm. A total of 27 test tubes were prepared (in triplicate), each containing 5 mL of Benedict's solution. From each hydrolyzed sample, 1 mL of reducing sugar solution was pipetted into the respective tubes. The mixtures were heated in a water bath at 90 °C for 5 minutes, rapidly cooled, and filtered. The absorbance of each filtrate was recorded at 540 nm using a UV–visible spectrophotometer. Reducing sugar concentrations and yields were calculated according to Equations (10) and (12), respectively.

$$conc.\ of\ unknown\ sample\ (TRSC) = \frac{aborbance\ of\ unknown\ sample\ (y) - y\ intercept\ (b)}{slope(m)} \tag{10}$$

After the concentration of total reducing sugar (TRS) is calculated, the total reducing sugar in grams was calculated using the following Equation (11).

$$TRS\ produced\ in\ gram\ -\frac{TRSC}{milliliter\ of\ solution} \tag{11}$$

$$TRSY(\%) = \frac{TRS\ produced\ in\ gram}{raw\ material\ used} \times 100 \tag{12}$$

where $TRSY$ = Total Reducing Sugar Yield and $TRSC$ = Total Reducing Sugar Concentration.

$$Raw\ material\ used = \frac{gram\ of\ sample\ used}{milliliter\ of\ soution} \tag{13}$$

2.3.5. Fermentation: The experiment aimed to evaluate ethanol production from hydrolyzed sedge biomass using two fermenting microorganisms, *Saccharomyces cerevisiae* and *Fusarium oxysporum*, across different fermentation periods (Wang et al., 2016). The microbial strains were donated from the Ethiopian Biodiversity Institute (EBI), Addis Ababa. The hydrolysate yielding the highest reducing sugar content (49.83%) was selected for fermentation. Fermentation media were prepared at a 1:10 ratio (media to sample) and transferred into flasks covered with aluminum foil to maintain anaerobic conditions. Separate fermentations were conducted with *S. cerevisiae* and *F. oxysporum* at 30 °C and pH 5.45 under continuous shaking. During the course of fermentation, data were taken after 3 days, 5 days and 7 days. Before conducting fermentation processes, media for yeast *S. cerevisiae* and *F. oxysporum* were prepared under optimum condition for growth as described below.

(i) Media for yeast *Saccharomyces cerevisiae*: Yeast growth medium was prepared in a 500 mL conical flask containing 4 g dextrose, 0.5 g yeast extract (agar), 4 g peptone, 2.5 g urea, 2.5 g $MgSO_4{\cdot}7H_2O$, and 250 mL distilled water. The pH was adjusted to 5.0, and the medium was sterilized by autoclaving at 121 °C for 15 minutes. After cooling, 1.5 mL of *S. cerevisiae* inoculum was transferred into a 350 mL conical flask containing the medium, which was sealed with aluminum foil. The culture was incubated at 30 °C under shaking conditions (200 rpm) for 24 hours to promote yeast growth.

(ii) Media for *Fusarium oxysporum*: For the growth of *F. oxysporum* wild-type strain f3, potato dextrose agar (PDA) medium was used following Christakopoulos et al. (1996). PDA was prepared by dissolving 39 g of PDA powder in 1 L of distilled water. The mixture was heated, sterilized by autoclaving at 121 °C for 15 minutes, and poured aseptically into three sterile Petri dishes (90 mm diameter), with 25 mL of medium per plate. After cooling to room temperature under laminar

airflow, the fungal inoculum was dispensed onto the agar plates. The cultures were incubated at 28 °C for 3 days to allow sufficient fungal growth.

Distillation: Bioethanol was separated from the fermentation broth by simple distillation, following the procedure described by Tekalign (2018). Distillation was conducted at 78 °C, and ethanol was collected over a period of 3 hours.

Estimation of Ethanol Content Using Potassium Dichromate Solution:

Ethanol Standard Curve Preparation: A potassium dichromate ($K_2Cr_2O_7$) solution was prepared by dissolving 33.8 g of $K_2Cr_2O_7$ and adding 325 mL of 96% H_2SO_4 per liter of distilled water. Standard ethanol solutions (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0% v/v) were prepared in six test tubes by diluting 1 mL of each ethanol concentration with 9 mL of distilled water (1:10 ratio). From each standard solution, 1 mL was transferred into a separate tube, and 2 mL of potassium dichromate solution was added. The mixtures were heated in a water bath at 60 °C for 20 minutes, rapidly cooled, and their absorbance was measured at 600 nm (Mussatto and Roberto, 2004).

Estimation of Ethanol Content: To estimate the ethanol concentration, each sample was diluted by mixing 1 mL of ethanol solution with 9 mL of distilled water. Triplicate preparations were made for accuracy. From each diluted sample, 1 mL was transferred into a test tube, followed by the addition of 2 mL of $K_2Cr_2O_7$ solution. The mixtures were heated in a water bath at 60 °C for 20 minutes, cooled, and the absorbance was measured at 600 nm. Ethanol concentration was determined using the standard calibration curve prepared from ethanol solutions. The ethanol yield (%) was then calculated using Equation (14).

$$\textit{Percentage of sample ethanol yield } (\%) = \frac{(Cs)}{Cu}\frac{(Au)}{As} \times 100 \tag{14}$$

where Cs = Concentration of standard ethanol, Cu = Concentration of sample ethanol, Au = Absorbance of standard ethanol, and As = Absorbance of sample ethanol.

Fourier Transform Infrared (FT-IR) Analysis: Following recovery from the distillation column, the condensed distillate was analyzed using a PerkinElmer Spectrum 65 Fourier Transform Infrared (FT-IR) spectrometer. Functional groups present in the ethanol were identified by recording percent transmittance across the wavenumber range of 4000–400 cm^{-1}, in accordance with Rubio-Arroyo et al. (2011). The analysis was carried out at the Department of Chemistry, Addis Ababa University, Ethiopia. The resulting spectra were plotted as percent transmittance versus wavenumber to confirm ethanol functional group characteristics.

Experimental Design: The determination of the chemical composition of sedge grass biomass was conducted using a Completely Randomized Design (CRD) with three replications. Hydrolysis experiments were arranged in a factorial CRD with three replications, incorporating three factors: temperature (115, 125, and 135 °C),

hydrolysis time (40, 60, and 80 minutes), and H_2SO_4 concentration (1.5%, 2.5%, and 3.5%). For the fermentation stage, *Saccharomyces cerevisiae* and *Fusarium oxysporum* were employed as microbial inoculants, and ethanol yield was measured at three fermentation periods (3, 5, and 7 days).

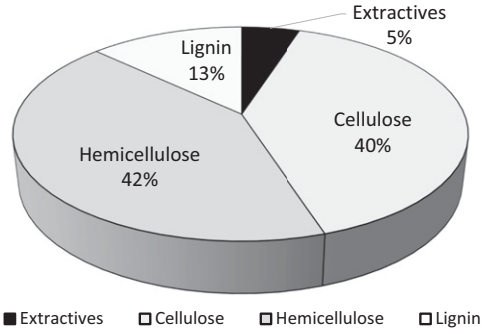
Statistical Analysis: Data were analyzed using analysis of variance (ANOVA) with the PROC MIXED procedure in SAS software version 9.0 (SAS Institute, 2009) to evaluate treatment effects on the dependent variables. Mean comparisons were performed using the Least Significant Difference (LSD) test at a 1% level of significance.

3. Results and Discussion

3.1. Chemical Composition Analysis of Sedge Grass: The estimation of chemical components in lignocellulosic biomass is essential for assessing its conversion efficiency into bioenergy and other value-added products (Timung et al., 2016). Analysis of the stem portion of sedge grass revealed a composition of 39.87% cellulose, 42% hemicellulose, 13.07% lignin, and 5.06% extractives (Figure 3). These values fall within the ranges reported for other grass biomasses: 25–40% cellulose, 35–50% hemicellulose, 10–30% lignin, and 1–5% extractives (Alamgir, 2017; Sun and Cheng, 2002). Minor deviations from literature values may be attributed to differences in biomass type, plant part analyzed, environmental and habitat conditions, harvesting time, and pretreatment or experimental procedures (Seidl and Goulart, 2016; Tye et al., 2017).

3.2. Effects of Hydrolysis Factors on Total Reducing Sugar Yield: The yield of total reducing sugars (TRS) hydrolyzed from lignocellulosic biomass is influenced by several factors, including solid-to-liquid ratio, particle size, plant part, hydrolysis time, temperature, and acid concentration (Sindhu et al., 2014). In this study, powdered sedge grass was subjected to dilute acid hydrolysis to evaluate the

Figure 3
THE PERCENT OF CHEMICAL COMPOSITION OF SEDGE GRASS BIOMASS



effects of H_2SO_4 concentration, hydrolysis time, and temperature on TRS production. Typically, TRS yield increases to an optimum level before declining due to sugar reversion and degradation. Analysis revealed that TRS yield was significantly affected by the main effects of these parameters, as well as by two-way and three-way interactions, at the 1% probability level ($p < 0.01$). Quantification of TRS was performed using a calibration curve prepared from standard glucose solutions, with Benedict's reagent as the indicator (Singh and Singh, 2015). Absorbance was measured at 540 nm, and the calibration curve of glucose concentration versus absorbance is presented in Figure 4.

3.2.1. Main Effects of Hydrolysis Factors on Total Reducing Sugar Yield: Previous studies have shown that dilute acid hydrolysis is a simple and effective method for converting lignocellulosic materials into fermentable sugars, with optimal conditions reported at temperatures of 80–200 °C, H_2SO_4 concentrations of 0.25–8 wt%, and reaction times of 10–2000 minutes (Warrand and Janssen, 2007; Gladyshko, 2011). In the present study, biomass was hydrolyzed under varying conditions of acid concentration, temperature, and time (Table 1). At a hydrolysis time of 60 minutes and 1.5% H_2SO_4, the TRS yield increased from 42.8% to 45.02% as the temperature rose from 115 °C to 125 °C, with the maximum yield obtained at 125 °C (Table 1). Beyond this temperature, TRS yield declined slightly. These results align with previous findings that hydrolysis temperature plays a critical role in sugar release from lignocellulosic biomass (Taherzadeh and Karimi, 2007; Chandel et al., 2012). The reduced TRS yield at lower temperatures can be attributed to insufficient thermal energy to disrupt the glucosidic and hydrogen bonds of lignocellulose, thereby limiting the conversion of cellulose and hemicellulose into glucose and xylose (Balat and Balat, 2009). Conversely, the decline in TRS yield at higher temperatures is likely due to sugar degradation and the formation of other by-products instead of glucose.

Figure 4
THE CALIBRATION CURVE OF STANDARD GLUCOSE SOLUTION

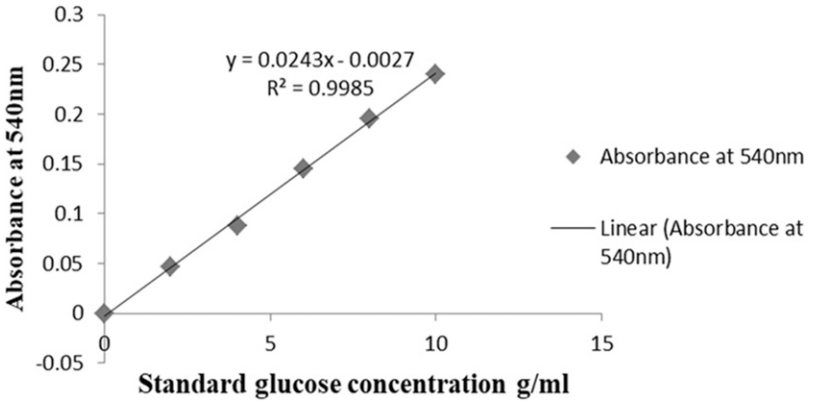


Table 1
THE EFFECT OF TEMPERATURE, HYDROLYSIS TIME, AND H_2SO_4 CONCENTRATION ON TOTAL REDUCING SUGAR YIELD (TRSY) IN %

Treatments	TRSY (%)	SE
Temperature	***	
115 °C	42.8 c	± 0.014
125 °C	45.02 a	
135 °C	43.5 b	
LSD	0.04	
Hydrolysis Time	***	
40 min	41.89 c	± 0.014
60 min	45.5 a	
80 min	43.42 b	
LSD	0.04	
H_2SO_4 conce.	***	
1.5%	44.51 a	± 0.014
2.5%	44.18 b	
3.5%	42.63 c	
LSD	0.04	
CV	0.168	

Table Note: TRSY (%) = Total Reducing Sugar Yield in percent, SE = Standard Error, LSD = Least Significant Difference, CV= Coefficient of Variation, Values are expressed as mean and means in different letters are statically significant according to LSD test and (***) indicates very highly significance (significant at $P < 0.01$).

The influence of hydrolysis time on TRS yield was evaluated at a constant temperature of 125 °C and 1.5% H_2SO_4 concentration. Results showed that TRS yield increased from 41.89% to 44.51% as hydrolysis time was extended from 40 to 60 minutes, with the maximum yield achieved at 60 minutes. Beyond this optimum time, TRS yield declined, suggesting that cellulose and branched hemicellulose structures are most efficiently hydrolyzed at intermediate reaction times, while prolonged hydrolysis promotes sugar degradation. The effect of acid concentration was also examined under constant conditions (125 °C, 60 minutes). Maximum TRS yield (44.51%) was obtained at 1.5% H_2SO_4, followed by 44.18% and 42.63% at 2.5% and 3.5% concentrations, respectively (Table 1). These results indicate that lower acid concentrations are more effective in liberating fermentable sugars compared to higher concentrations. This observation is consistent with earlier reports on wet coffee pulp and highland bamboo hydrolysis, where sugar yields decreased as acid concentration increased from 1.5% to 3.5% (Kefale et al., 2012; Tsegaye et al., 2019). The decline in TRS yield at elevated acid concentrations

may be attributed to the degradation of monomeric sugars (glucose and xylose) into inhibitory by-products such as furfural and hydroxymethylfurfural (HMF), or to further conversion of glucose into levulinic and formic acids under strong acidic conditions (Kefale et al., 2012; Loow et al., 2016).

3.3. Interaction Effects of Hydrolysis Factors on Total Reducing Sugar Yield: Previous studies have shown that multiple factors influence the hydrolysis efficiency of lignocellulosic biomass and, consequently, the yield of total reducing sugars (TRS) (Sun and Cheng, 2002). In addition to the individual effects of temperature, acid concentration, and hydrolysis time, their interactions also play a significant role. Two-way interactions—specifically between temperature and acid concentration, temperature and hydrolysis time, and hydrolysis time and acid concentration—were found to significantly influence TRS yield ($p < 0.01$). Similarly, three-way interactions among temperature, acid concentration, and hydrolysis time also showed significant effects at the 1% probability level ($p < 0.01$). These results highlight the complex interplay among hydrolysis conditions in determining optimal sugar release from lignocellulosic biomass.

3.3.1. Effect of Temperature and Time on Total Reducing Sugar Yield: The interaction between hydrolysis temperature and time on TRS yield was evaluated at a constant acid concentration of 1.5%. Three temperature levels (115, 125, and 135 °C) and three hydrolysis times (40, 60, and 80 minutes) were tested (Figures 5 and 6). The highest TRS yield (47.23%) was obtained at 125 °C and 60 minutes, while the lowest yield (39.91%) was recorded at 115 °C and 40 minutes. Thus, the

Figure 5
INTERACTION EFFECT OF TEMPERATURE WITH HYDROLYSIS TIME ON TRS YIELD WITH CONSTANT 1.5% OF H_2SO_4

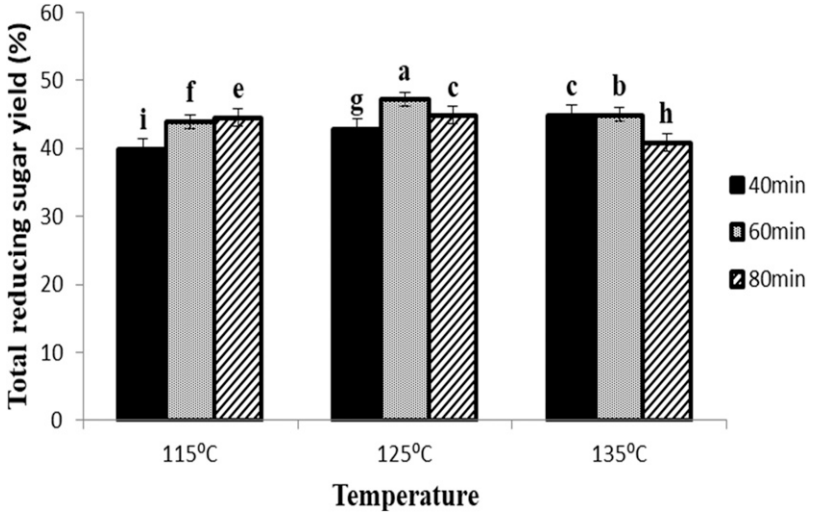
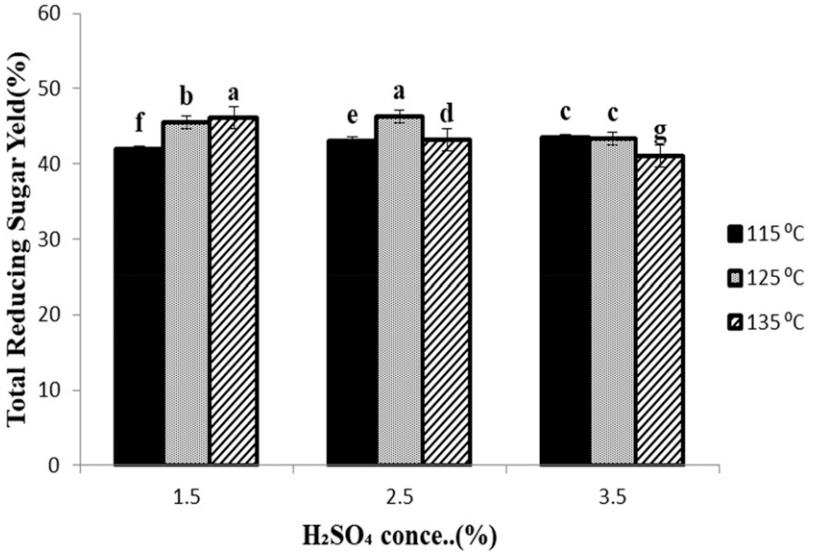


Figure 6
INTERACTION EFFECT OF TEMPERATURE AND H_2SO_4 CONCENTRATION ON TRS YIELD WITH CONSTANT HYDROLYSIS TIME OF 60 MINUTES



optimal conditions for maximum TRS production were identified as 125 °C and 60 min. Beyond these conditions, increasing temperature or prolonging hydrolysis time led to a decline in TRS yield, consistent with previous findings (Timung et al., 2016; Loow et al., 2016).

3.3.2. Effect of Temperature and Acid Concentration on Total Reducing Sugar Yield: The interaction between temperature and acid concentration significantly influenced TRS yield when hydrolysis time was fixed at 60 minutes (Figure 5). Hydrolysis was carried out at three temperatures (115, 125, and 135 °C) combined with three H_2SO_4 concentrations (1.5%, 2.5%, and 3.5%). The highest TRS yield (46.22%) was achieved at 125 °C with 2.5% H_2SO_4, followed closely by 46.17% at 135 °C with 1.5% H_2SO_4. No significant difference ($p \leq 0.01$) was observed between these two treatments. In contrast, the lowest TRS yield (41.09%) occurred at 135 °C with 3.5% H_2SO_4, which was significantly lower ($p \leq 0.01$) than the other combinations (Table 1).

Lower hydrolysis temperatures combined with high acid concentrations enhanced TRS yield, whereas high acid concentrations at elevated temperatures led to reduced yields. Similar findings were reported by Mahelete et al. (2019), who demonstrated in bamboo-to-ethanol studies that simultaneous increases in both temperature and acid concentration decreased TRS yield, while lower acid levels with increasing temperatures improved sugar release. The decline in yield under harsher conditions is attributed to sugar degradation into by-products such as

hydroxymethylfurfural (HMF) and furfural (Sindhu et al., 2014). The interaction effects of hydrolysis temperature and acid concentration on TRS yield are presented in Figure 6.

3.3.3. Effect of Hydrolysis Time and Acid Concentration on TRS Yield: The interaction between hydrolysis time (40, 60, and 80 minutes) and H_2SO_4 concentration (1.5%, 2.5%, and 3.5%) at a constant temperature of 125 °C was evaluated for its effect on TRS yield (Figure 7). The highest TRS yield (46.4%) was obtained with 60 minutes hydrolysis time and 2.5% H_2SO_4, which was significantly greater ($p \leq 0.01$) than other treatments. In contrast, the lowest yield (41.07%) occurred at 80 minutes with 3.5% H_2SO_4 ($p \leq 0.01$). These results are consistent with previous studies, which reported that lower acid concentrations combined with moderate or extended hydrolysis times enhance sugar release, whereas higher acid concentrations with prolonged reaction times promote sugar degradation and reduce TRS yield (Hailemichael, 2016; Biniyam et al., 2016).

3.3.4. Three-Way Interaction Effects on Total Reducing Sugar Yield: The combined effects of acid concentration, temperature, and hydrolysis time on TRS yield demonstrated that yield decreased under conditions of both low temperature with short hydrolysis time and high severity (high acid concentration, elevated temperature, and prolonged hydrolysis). Conversely, TRS yield increased under optimum conditions of temperature, acid concentration, and time (Figure 8). The maximum

Figure 7
INTERACTION EFFECT OF TEMPERATURE AND H_2SO_4 CONCENTRATION ON TRS YIELD WITH CONSTANT TEMPERATURE OF 125 °C

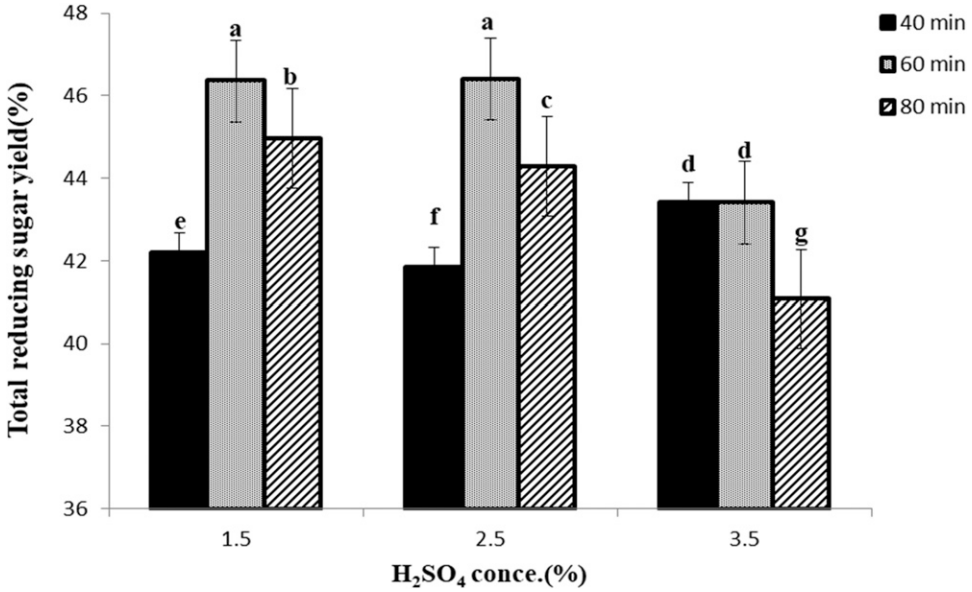
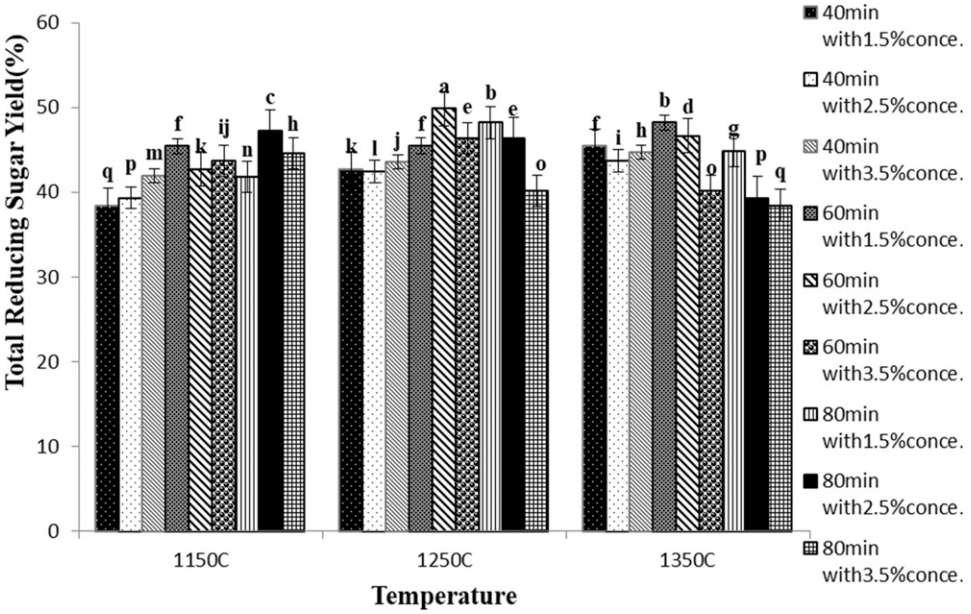


Figure 8
INTERACTION EFFECT OF TEMPERATURE, HYDROLYSIS TIME AND H_2SO_4 CONCENTRATION ON TOTAL REDUCING SUGAR YIELD

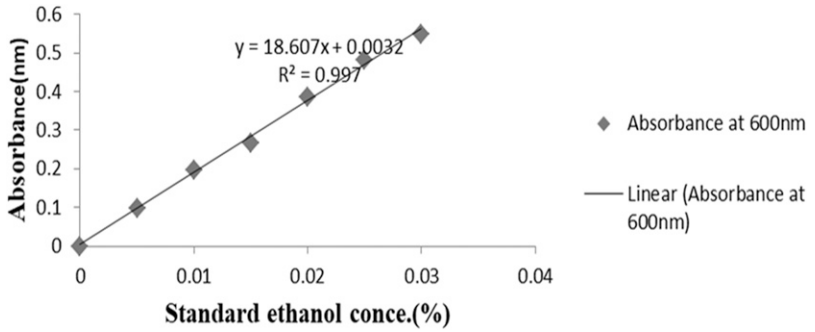


yield (49.83%) was achieved at 2.5% H_2SO_4, 125 °C, and 60 minutes of hydrolysis. In contrast, the lowest yield (38.44%) was obtained at 3.5% H_2SO_4, 135 °C, and 60 minutes. These results are consistent with findings from sugarcane bagasse hydrolysis, where higher acid concentrations, elevated temperatures, and longer hydrolysis times produced lower sugar yields (Timung et al., 2016). This reduction is attributed to sugar degradation into inhibitory compounds such as hydroxymethylfurfural (HMF), furfural, formic acid, and acetic acid, which also negatively affect microbial growth during fermentation (Rajan and Carrier, 2014).

On the other hand, low yields were also observed under mild conditions (low temperature, low acid concentration, and short hydrolysis time), which provide insufficient severity for efficient breakdown of lignocellulosic biomass. As noted by Lenihan et al. (2010), such conditions limit the release of five- and six-carbon sugars necessary for subsequent ethanol production.

3.4. Bioethanol Yield: For fermentation, the hydrolysate that produced the maximum reducing sugar yield (49.83%) was selected. Ethanol concentration was quantified using the potassium dichromate method, with measurements taken at 600 nm. A calibration curve was constructed from standard ethanol solutions (Figure 9), and ethanol yield was calculated using Equation (13).

Figure 9
THE CALIBRATION CURVE OF STANDARD ETHANOL



3.4.1. Effect of Fermenting Microbes and Fermentation Time on Ethanol Yield: The interaction between fermenting microorganisms and fermentation duration in days had a significant effect on ethanol yield ($p \leq 0.01$). Among the two microbes tested, *Fusarium oxysporum* produced the highest ethanol yield (32.65%), while the lowest yield (20.57%) was obtained with *Saccharomyces cerevisiae* (Table 2). Ethanol yield also varied with fermentation time. The highest yield (30.97%) was recorded at 3 days of fermentation. Yield decreased substantially at 5 days (22.8%) but increased again at 7 days, reaching 26.6%.

The maximum ethanol yield (51.78%) was obtained from hydrolyzed biomass fermented with *Fusarium oxysporum* after 7 days, whereas the lowest yield

Table 2
EFFECTS OF FERMENTATION DAYS AND FERMENTING MICROBES ON ETHANOL YIELD (%)

Treatments	**EY (%)**	**SE**
Microbes	***	
S. cerevisiae	20.57 b	±0.46
F. oxysporum	32.65 a	±0.46
LSD	1.44	
Fermentation days	***	
3 days	30.97 a	± 0.57
5 days	22.8 c	± 0.57
7 days	26.06 b	± 0.57
LSD	1.76	
CV	2.17	

Table Notes: EY = Ethanol Yield, SE = Standard Error, LSD = Least Significant Difference CV= Coefficient of Variation, Values are expressed as mean and means in different letters are statically significant according to LSD test and (***) indicates highly significance (significant at $P < 0.01$).

(0.34%) was recorded with *Saccharomyces cerevisiae* at the same fermentation period (Figure 10). Ethanol yield from *S. cerevisiae* decreased with prolonged fermentation, while yield from *F. oxysporum* increased steadily over time. These findings suggest that *F. oxysporum* is more effective in ethanol production due to its broad conversion capacity, as it can metabolize both hexose and pentose sugars. However, fermentation with *F. oxysporum* requires longer durations (≥7 days) to complete. This agrees with previous reports showing that *F. oxysporum* produces a wide range of biomass-degrading enzymes, enabling utilization of diverse sugars, but with slower fermentation rates (Coates and Meyers, 2000).

By contrast, *S. cerevisiae* completes fermentation within a short period (approximately 3 days), but yields are lower because it primarily ferments hexose sugars and cannot metabolize pentoses such as xylose. This limitation, along with its reliance on prior chemical or enzymatic pretreatment for cell wall breakdown, reduces its suitability for lignocellulosic biomass fermentation (Coates and Meyers, 2000). Overall, the study highlights the potential of filamentous fungi such as *F. oxysporum*, as well as genetically modified microorganisms, for improving lignocellulose-based ethanol production.

3.5. FT-IR Characterization of Produced Bioethanol: The FT-IR spectrum of the distilled bioethanol was recorded in the wavenumber range of 4000–400 cm^{-1}. Characteristic absorption bands corresponding to alcohol functional groups were observed. A broad peak at 3500–3200 cm^{-1} indicated O–H stretching vibrations, while absorption in the range of 1260–1050 cm^{-1} confirmed C–O stretching.

Figure 10
INTERACTION EFFECT OF MICROBES AND FERMENTATION DAYS ON ETHANOL YIELD

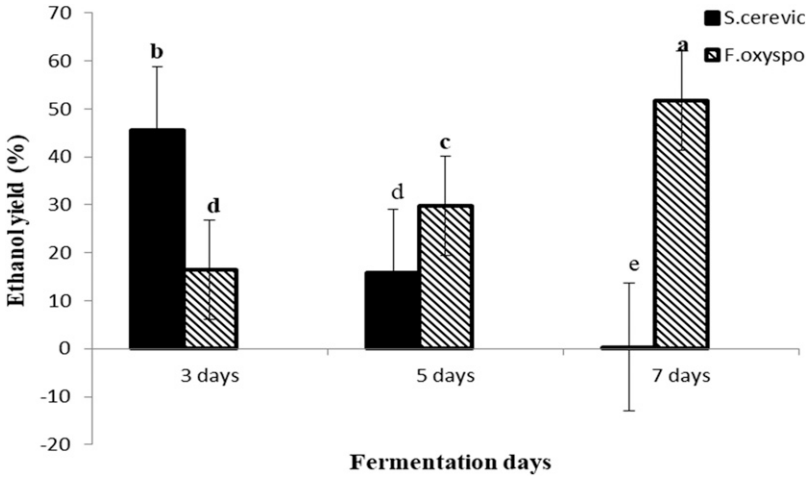
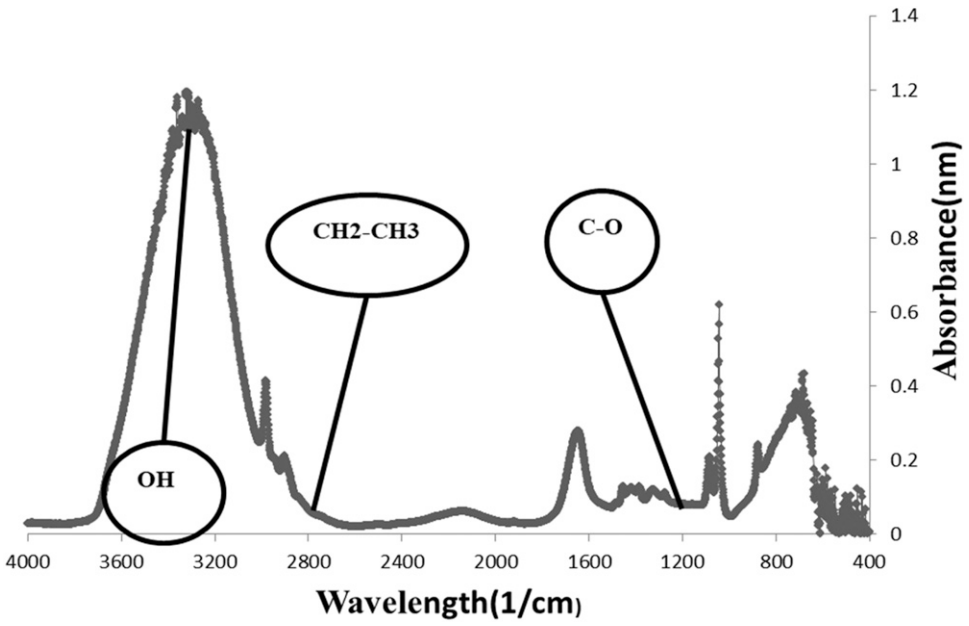


Figure 11
FOURIER TRANSFORM INFRARED SPECTRA OF THE PRODUCED BIOETHANOL FROM SEDGE GRASS



Additional peaks at 2880–2930 cm^{-1} were attributed to symmetric stretching of $–CH_2$ and $–CH_3$ groups (Yu et al., 2007). These spectral features verify that the product obtained from sedge grass hydrolysate was ethanol (Figure 11).

4. Summary and Conclusion

Maximizing fermentable sugar yield from lignocellulosic biomass requires both knowledge of biomass composition and optimization of acidic hydrolysis conditions. In this study, the effects of hydrolysis parameters—temperature, acid concentration, and reaction time—on TRS yield from sedge grass (*Schoenoplectus tabernaemontani*) were investigated, along with the influence of fermentation time and microbial strain (*Saccharomyces cerevisiae* and *Fusarium oxysporum*) on ethanol production. All three hydrolysis factors (temperature: 115, 125, 135 °C; time: 40, 60, 80 minutes; and H_2SO_4 concentration: 1.5%, 2.5%, 3.5%) significantly affected TRS yield.

Sedge grass biomass was found to contain 39.87% cellulose, 42% hemicellulose, 13.07% lignin, and 5.06% extractives—characterized by high cellulose and hemicellulose with relatively low lignin content. The highest TRS yield (49.83%)

was obtained at 125 °C, 2.5% H_2SO_4, and 60 minutes hydrolysis. The maximum ethanol yield was achieved with *F. oxysporum* after 7 days of fermentation. The low lignin content of sedge grass facilitated efficient hydrolysis of cellulose and hemicellulose using dilute acid, requiring shorter reaction times and lower chemical inputs compared to lignin-rich biomasses.

FT-IR analysis of the fermentation product confirmed the presence of O–H, C–H, and C–O functional groups, verifying ethanol production from sedge grass biomass collected from Lake Hawassa. Conditions involving excessively high acid concentrations, extreme temperatures, or extended hydrolysis times reduced TRS yield due to sugar degradation.

In conclusion, sedge grass represents a promising lignocellulosic feedstock for bioethanol production due to its favorable chemical composition and high sugar conversion efficiency. Beyond ethanol, residual biomass is biodegradable and can be utilized as biofertilizer, supporting its potential for integrated biorefinery applications.

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