ETHANOL PRODUCTION FROM HARDWOOD SAWDUST VIA SEPARATE HYDROLYSIS AND SIMULTANEOUS SACCHARIFICATION FERMENTATION METHODS

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Received: 12 January 2025, Revised:24 March 2025, Accepted: 9 April 2025, Published: 30 April 2025, Publisher: UTP Press, Creative Commons: CC BY 4.0
DOI: https://doi.org/10.61762/ijbrvol14iss1art002

ABSTRACT

The global demand for ethanol as a renewable fuel source has prompted the exploration of alternative feedstocks to mitigate concerns related to food shortages, imbalances in the food chain, increased food prices, and indirect land use. This study investigates the feasibility of producing ethanol from cellulose, specifically utilising hardwood sawdust. Two distinct methods, separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF), were evaluated for their efficiency and environmental impact. Factors such as sugar concentration, ethanol yield, theoretical ethanol yield, yield efficiencies, and sugar conversion were analysed to assess the performance of each method. Findings show that 11.17 and 7.60 g of ethanol can be realised from 100 g of sawdust, following the SHF and SSF procedures, respectively. The sugar concentration decreases with time during fermentation with *Saccharomyces cerevisiae*. The choice between SHF and SSF methods for ethanol production depends on factors such as yield, cost-effectiveness, and environmental considerations. Despite its longer time requirement in this study, SSF emerges as a more efficient and environmentally sustainable approach compared to SHF, albeit at a higher cost.

Keywords: *biofuel, enzyme hydrolysis, ethanol, fermentation, Saccharomyces cerevisiae, sawdust*

INTRODUCTION

Synthetically, ethanol (ethyl alcohol) was first prepared in 1826 by Henry Hennel in Britain and S.G. Serullas in France, with Michael Faraday later demonstrating its production through acid-catalysed hydration of ethylene in 1828 [1]-[2]. It is utilised in the production of automotive blend fuel, antiseptic solutions, chemicals (e.g., ethylene, dyes, detergents, acetaldehydes, acetic acid and glycol), cleaning solutions, alcoholic beverages and pharmaceutical products (e.g. cough syrup) [3]. Schill [4] reported that woody biomass is estimated to be up to 70-80% of all terrestrial biomass on earth, and 70 gal of ethanol can be generated for every ton of wood. Hardwood can be differentiated from softwood based on its amount of lignin, cellulose and hemicellulose contents. According to Tran et al. [5], hardwood contains 18-25 wt.% lignin, 45-55 wt.% cellulose and 24-40 wt.% hemicellulose, while softwood consists of 25-35 wt.% lignin, 45-50 wt.% cellulose and 25-35 wt.% hemicellulose. Optimisation of ethanol production from sawdust is an approach keyed into by several researchers [6]-[11]. The most notable design, modelling and simulation of a bioethanol plant utilising sawmill industry sawdust as feed using ProSimPlus was the one carried out by Alio et al. [12]. Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are two different processes used in ethanol production [13]. In SHF, the steps of hydrolysis and fermentation are carried out separately. It involves breaking down the complex carbohydrates into simple sugars in a separate hydrolysis step and then fermenting the resulting sugars into ethanol by yeast or bacteria in a separate fermentation step. Meanwhile, in SSF, hydrolysis and fermentation occur simultaneously in the same reactor. In such situations, enzymes are added to the fermentation vessel along with the substrate, allowing for the breakdown of complex carbohydrates into simple sugars while fermentation occurs. A significant number of studies employed the SSF and SHF techniques in the production of ethanol/bioethanol from different types of sawdust, as listed in Table 1.

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Table 1 Literature report on ethanol synthesis from sawdust

Sawdust Types	Microorganisms	Methods	Reference
Pinus patula and *Pinus oocarpa*	*Saccharomyces cerevisiae* and *Trichoderma viride*	Pre-treatment, enzymatic hydrolysis and fermentation	[14]
Eucalyptus	*Saccharomyces cerevisiae*	Autohydrolysis	[15]
Palm wine	*Saccharomyces cerevisiae* and *Trichoderma viride*	Hydrolysis and fermentation	[16]
Chopped wood	*Saccharomyces cerevisiae*	Saccharification of Cellulose	[17]
Eucalyptus	*Saccharomyces cerevisiae*	SHF, SSF and pre-saccharification SSF (PSSF)	[18]
Lignocellulosic biomass	*Aspergillus niger* and *Saccharomyces cerevisiae*	Saccharification of pre-treated biomass	[19]
Spent sawdust matrix	Maitake mushroom (*Grifola frondosa*)	SSF	[20]
Mahogany wood	Yeast	Design batch bioreactor	[21]
Eucalyptus grandis	*Saccharomyces cerevisiae*	SSF, PSSF and SHF	[22]
Timber mill	*Saccharomyces cerevisiae*	Enzymatic hydrolysis, SHF and SSF	[23]
Wood particles	*Trichoderma viride* and *Saccharomyces cerevisiae*	Enzymatic hydrolysis	[24]
Tropical origin	*Saccharomyces cerevisiae* and *Saccharomyces chevalieri*	SHF	[13]
Pleurotus spp. spent mushroom sawdust substrate	*Saccharomyces cerevisiae*	Sugar separation and hydrolysis	[25]
Wood residue	*Trichoderma reesei* and *Saccharomyces cerevisiae*	SSF	[10]
Reduced size sawdust	Yeast	SHF	[26]
Lignocellulosic sawdust substrate	*Saccharomyces cervisae, Trichoderma viride, Trichoderma koninji* and *Trichoderma harzianum*	SSF and SHF	[27]
Khaya senegalensis (red wood), *Terminalia superba* (black wood) and *Gmelina arborea* (white wood)	*Saccharomyces cerevisiae*	Acid hydrolysis	[28]
Ecalyptus globulus and *Cupressus lusitanica*	White rot fungi and *Saccharomyces cerevisiae*	Enzymatic hydrolysis	[29]
Hard and softwood (mahogany)	Wild yeast and *Saccharomyces cerevisiae*	Enzymatic hydrolysis	[30]
Saccharified cellulose sawdust from 20 trees	*Saccharomyces cerevisiae*	SSF and SHF	[31]
Black carob tree (*Prosopis nigra*)	*Novozymes* S. A.	Enzymatic hydrolysis	[9]
Pinus pseudostrobus L.	*Saccharomyces cerevisiae*	SSF and SHF	[32]
Cellulose sawdust	*Aspergillus niger* and *Saccharomyces cerevisiae*	Enzymatic hydrolysis	[33]
Brachystegia eurichoma wood	*Saccharomyces cerevisiae*	Hydrolysis, fermentation and purification	[34]
Furniture market sawdust	*Saccharomyces cerevisiae*	Enzymatic saccharification and Enzymatic hydrolysis	[35]
Wood mill sawdust	*Aspergillus ochraceus* and *Saccharomyces cerevisiae*	Stationary, shaking fermentation and saccharification	[36]
Willow (*Salix* sp.)	*Wickerhamomyces anomalus, Saccharomyces cerevisiae* and *Pichia stipitis*	SSF	[37]
Lumber mill sawdust	*Saccharomyces uvarum* and *Pachysolen tannophilus*	Acid hydrolysis	[38]
Milicia excelsa wood	*Saccharomyces cerevisiae, Staphylococcus aureus*, Micrococcus spp, Actinomycetes spp., *Clostridium cellulovorans*, Bacillus spp, *Lactobacillus plantarum, Aspergillus niger*, Rhizopus spp and *Mucor mucedo*	Fermentation	[39]
Eucalyptus grandis	*Saccharomyces cerevisiae*	PSSF	[40]
Waste sawdust	*Saccharomyces cerevisiae*	Acid hydrolysis	[41]
Waste sawdust	White rot fungi (*Lentinus squarrosulus*)	Micro-carrier bioreactor	[42]
Rubber sawdust waste of furniture manufacturers	*Scheffersomyces shehatae*	SSF	[43]
Hardwood	*Saccharomyces cerevisiae*	SSF and SHF	Current study & [2]

Enzymatic saccharification is not often adopted for ethanol extraction from sawdust, as seen in Table 1. Considering the high glucose yield of mushroom spent medium, Hassan et al. [44] affirmed that the forgone method is suitable for bioethanol synthesis using biomass. *Saccharomyces cerevisiae* happens to be the most widely used microorganism for the fermentation of wood sawdust. Bioethanol production may rise to 137 billion litres in 2026 [45], and sawdust's contribution to this output is presumably considerable. It competes favourably with banana peel, corn stalks, grass clippings, rice husk and cassava waste, to mention a few, as a sustainable and emission-proof feedstock for ethanol mass production [46]-[49]. Given the economic importance of ethanol and the abundance of this forest biowaste, the current study aimed at producing ethanol from hardwood sawdust. SSF and SHF technologies are still mildly employed for the target substrate and biofuel product (Table 1), and only a handful of studies utilised 'hardwood' sawdust. Thus, the objectives of the research are to synthesise ethanol from the biomass via SHF and SSF methods, establish the conditions for acid and enzyme hydrolysis of pre-treated hardwood sawdust, and lastly, determine the sugar outputs and the ethanol yield from fermentation by *Saccharomyces cerevisiae*. Amongst other methods that may be employed, SHF and SSF both present some unique advantages. For instance, SHF allows for better control over each step of the process, potentially leading to higher ethanol yields and enzyme optimisation for specific substrates to improve efficiency. On the other hand, SSF requires a shorter processing time than SHF and reduced contamination risk since hydrolysis and fermentation occur simultaneously in a single vessel [50]-[51].

MATERIALS AND METHOD

Materials

In this study, the following equipment was used: DT-500B electronic weighing balance, KW-1000DC/Iron water bath, T-24/29lab 500 mL Borosilicate Pyrex glass separating funnel, Ex-10/23 250 mL Pyrex glass Soxhlet extractor, 1000 mL distillation flask, reflux condenser, 80 × 250 mm cellulose thimble, 500 mL FF-500/3UM (24/29) Pyrex glass round-bottom flask, 10 and 25 mL pipettes, 100 and 250 mL beakers, electric heater, 10 mL measuring cylinder, Jenway 3015 pH meter and mortar and pestle. Accordingly, ethanol was produced from 100 g hardwood sawdust using 70 mL n-hexane, 0.4 mL sulphuric acid (H_2SO_4), 5 g *Saccharomyces cerevisiae*, 10 g potato dextrose agar (PDA) and 7 mL distilled water.

Methods

Specifically, SHF and SSF techniques were employed for the production of ethanol. These complementary methods allow for a comprehensive comparison of process efficiency, as SHF separates hydrolysis and fermentation into distinct stages. At the same time, SSF combines both processes into a single step, potentially reducing time and operational costs.

Separate Hydrolysis and Fermentation

Initially, *Saccharomyces cerevisiae* was cultured. Essentially, the PDA solution was prepared by dissolving 10 g of PDA in 250 mL of water in a conical flask in accordance with Afe [34]. An autoclave was used to sterilise the mixture at 121^{o}C for 5 minutes, as recorded by Chukwuemeka et al. [24] and Bakare et al. [28]. The sterilised solution was poured into petri dishes and allowed to solidify before introducing *Saccharomyces cerevisiae* into the dish using a sterilised inoculating loop. Next, the petri dishes were sealed and incubated at 25^{o}C for 48 hours. After culturing the media, sawdust was prepared for hydrolysis. Initially, the sawdust was sieved to create a uniform particle size of 1 mm (using 16 mesh Tyler equipment) and dried for 12 hours to remove moisture. Particle size distribution is also an important physical property of the sawdust biomass that has been examined [10],[26],[52]. In furtherance of the investigation, a 250 mL beaker was filled with 100 g dry sawdust and 100 mL H_2SO_4 was added in the ratio of 1:1, as used in Nwakaire et al. [53]. A spontaneous reaction producing lignin and generating heat and bubbles was observed. The pH of the acidic solution was then adjusted to make it suitable for *Saccharomyces cerevisiae* fermentation. About 200 mL of water (pH = 9.7) was then added to the acidic solution and stirred thoroughly. Furthermore, pH was measured (which reads 1.37), indicating the need for further dilution. Hence, the solution was gradually diluted with water until the pH reached an optimal range of 4.5 [1],[7],[50]. If required, the pH was further adjusted using 9 M sodium hydroxide (NaOH).

Based on the preceding findings, ethanol was subsequently produced from cellulose derived from sawdust. A NaOH solution [22] was added dropwise to the acidic cellulose substrate to achieve a pH of 4.87. Afzal et al. [27] used 2.5% NaOH as sawdust pre-treatment chemical. Hydrogen peroxide and levulinic acid are other pre-treatment chemicals that can be employed [35],[38]. Subsequently, the solution was filtered to separate the cellulose substrate (filtrate) from the lignin residue using a Buchner funnel. The sugar content of the filtrate was then measured. Cultures of *Saccharomyces cerevisiae* were later dissolved in distilled water, and the culture solution was added to the cellulose substrate to initiate fermentation. Its sugar content was monitored over time to assess the conversion rate to ethanol. Fermentation was conducted in a shaker incubator at 30^{o}C for 48 to 72 hours [29]. A 5-day fermentation period is often reported [45]. The fermented solution was distilled using a distillation bath to separate ethanol from water, and the ethanol produced was collected as distillate.

Simultaneous Saccharification and Fermentation

During SSF, 100 g of fine sawdust was mixed with 100 mL of 0.4M H_2SO_4, resulting in a pH of 3.1. Perchloric acid is among the acids used to carry out hydrolysis [25]. Calcium hydroxide ($Ca(OH)_2$) was then added dropwise until the pH reached 4.61 [23]. The mixture was later autoclaved at 120^{o}C for 10 minutes and then cooled to approximately 30^{o}C. The sugar content of the mixture was measured. Following this, 2.5 g of cellulose and *Saccharomyces cerevisiae* were added to the mixture. The mixture was placed in a shaker incubator at 150 rpm for 48 to 72 hours at 30^{o}C to complete the fermentation [34], closely above 140 rpm used by Hideno et al. [20]. Sugar content was monitored every four hours to assess fermentation rate and duration. After fermentation, a 100 mL sample of the mixture was distilled in a distillation bath, and the ethanol yield at 78^{o}C was measured. Previously, Trevorah and Othman [23] found that NaOH pre-treated sawdust gave higher ethanol yield than untreated at 60^{o}C following the SSF technique.

Experimental Safety Measures

Several precautionary measures were taken, as would be detailed. Due to the exothermic nature of the hydrolysis reaction, precautions were taken to handle the reactor vessel safely. Handling of H_2SO_4 was done with care to ensure safety. The acid was added to water rather than water being added to acid to prevent potential explosions or skin burns. Protective gear, including overalls, gloves, and goggles, were worn throughout the experiment. When removing hot liquids from the autoclave, caution was exercised to prevent accidents or injuries.

Ethanol Production Calculations

Determining the ethanol yield requires assessing the amount of sugar converted to ethanol during fermentation. It could be done by finding the change in sugar concentration over the fermentation period. Then, a known conversion factor can be harnessed to estimate the amount of ethanol produced. Essentially, the calculation method followed was clearly explained. First, the initial and final sugar concentrations were extracted and used to calculate the change in sugar concentration. A known conversion factor (0.51 g ethanol/g sugar) was assumed and used to estimate the amount of ethanol produced following Equation 1. Furthermore, the ethanol yield, theoretical ethanol yield and the yield efficiencies under both SHF and SSF outcomes were calculated using Equations 2 to 4 [37],[41], respectively.

$$\text{Ethanol produced} = \begin{pmatrix}\text{Change in sugar}\\ \text{concentration}\end{pmatrix}(\text{conversion factor}) \quad (1)$$

$$\text{Ethanol Yield} = \frac{(\text{Ethanol produced})}{(\text{Initial Weight of Cellulose Sawdust})} \times 100 \quad (2)$$

$$\text{Theoretical Ethanol Yield} = \begin{pmatrix}\text{Sugar content of}\\ \text{cellulose sawdust}\end{pmatrix}(\text{conversion factor}) \quad (3)$$

$$\text{Yield Efficiency} = \frac{(\text{Actual Ethanol Produced})}{(\text{Theoretical Ethanol Produced})} \times 100 \quad (4)$$

Yield efficiency was computed to showcase how effectively the process converted the available sugars in the sawdust to ethanol compared to the maximum potential yield. For the intermediate sugar produced, the % sugar yield and the % sugar conversion were respectively calculated for SHF and SSF using Equations 5 and 6 [2],[38].

$$\text{Sugar Yield} = \left(\frac{\text{Initial Sugar Conc.}}{\text{Theoretical Max. Sugar Content}}\right) \times 100 \quad (5)$$

$$\text{Sugar Conversion} = \frac{(\text{Sugar Produced}) - (\text{Sugar Unconverted})}{\text{Sugar Produced}} \times 100 \quad (6)$$

Sugar unconverted is implied here as the final sugar concentration, and the 'sugar produced' is the initial sugar level in the sawdust. The unfermented sugar production/content of the hydrolysate was measured in '°Brix' (i.e., 1 g sugar/100 g solution) [13]. Overall, the designed steps and findings are as contained in Otulugbu [2].

RESULTS AND DISCUSSIONS

Sugar Concentration from SHF and SSF of Sawdust

Following the SHF technique of ethanol manufacture, hydrolysis yielded 32.4 g of sugar from 100 g of sawdust. Theoretically, 52.2 g could be produced from the same weight of sawdust. Additionally, the experiment involved monitoring the sugar concentration on a 4-hourly basis to assess the fermentation rate by observing the decrease in sugar concentration over time. The fermentation process was considered complete when no significant change in sugar concentration occurred. The raw data, including Brix values representing the sugar content in the solution, is provided in Table 2.

Table 2 Sugar concentration during ethanol synthesis at the time progression

Time (h)	Sugar Concentration (°Brix) (g)	
	SHF	SSF
0	32.4	24.7
4	32.4	26.1
8	31.7	25.2
12	30.5	28.0
16	25.6	26.3
20	18.3	36.6
24	13.4	25.1
28	11.8	20.3
32	11.4	17.2
36	11.0	13.4
40	10.8	10.4
44	10.5	10.2
48	10.5	9.9
52	10.5	9.8
56	-	9.8
60	-	9.8

Likewise, the procedure for the production of ethanol using 100 g hardwood sawdust and cellulose enzymes and yeast by SSF is discussed. Here, the sugar content obtained is 24.7g < the theoretical value of 52.2 g found in 100 g of cellulose sawdust. Table 2 also reveals the sugar concentration of the solution at 4-hour intervals of time to assess the conversion rate of sugar to ethanol and determine the exact time required in SSF for ethanol production. Figure 1 depicts the raw data in Table 2, showing the resultant trend of the sugar concentration under SHF and SSF analysis.

As shown in Figure 1a, the sugar concentration decreases progressively over time during SH-fermentation. The fermentation process appears to slow down after around 20 hours, and the sugar concentration stabilises around 10.5 °Brix after approximately 44 hours. This stabilisation indicates that fermentation is complete at that point. Farías-Sánchez et al. [32]'s sugar concentration-time SHF plot realised a completely decreasing trend, almost similar to this finding. It is apparent in Figure 1b that due to the simultaneous occurrence of saccharification and fermentation processes, there's an initial period of instability, resulting in the zigzag pattern observed in the graph during the first 20 hours. The maximum sugar concentration of 36.6 °Brix was achieved at the 20-hour mark, indicating the completion of saccharification. Subsequently, a steady state was reached, indicating only fermentation was taking place. The process took 52 hours, after which the remaining sugar content was 9.8 g. Distillation yielded 6.3 mL of ethanol from 100 mL of a 400

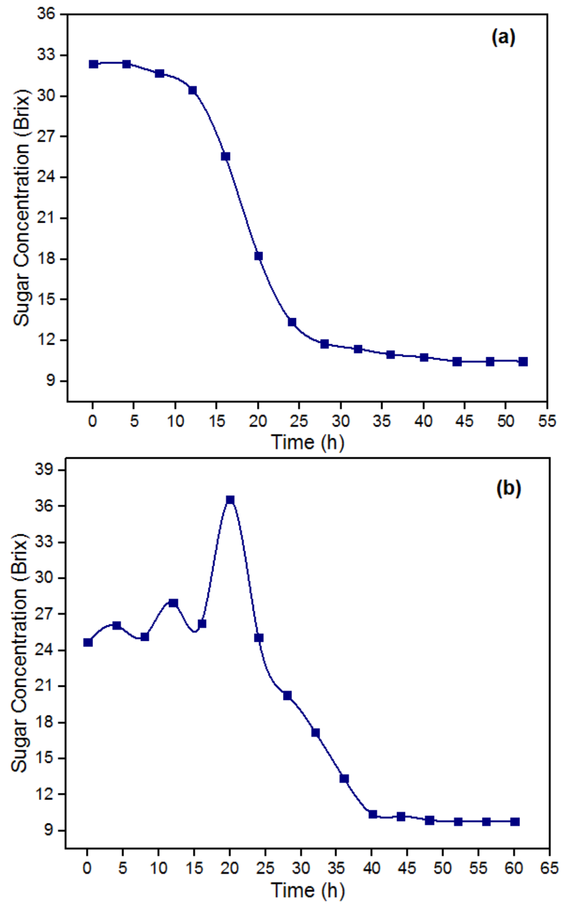

Figure 1 Sugar content against time from (a) SHF and (b) SSF methods

mL solution, resulting in a total ethanol content of 25.2 mL. In a study conducted by Ogundipe and Lu [38], reduced sugar concentration with time showcased a decreasing trend. Figure 2 replicates Figures 1a and 1b with the inclusion of statistical analysis and standard deviations.

The error bars in Figure 2 show the variability in ethanol production metrics for both SHF and SSF. Specifically, it represents the standard deviations, showing the changeability in sugar concentration measurements over time for both SHF and SSF processes.

Ethanol Production Metrics

Experimental results in Table 3 indicate that SHF and SSF processes have advantages and limitations in ethanol production from sawdust.

Table 3 Records of various ethanol yield computations

Metrics	Value	
	SHF	SSF
Initial Sugar Conc. (Brix)	32.4	24.7
Maximum Sugar Conc. (g)	32.4	36.6
Final Sugar Conc. (Brix)	10.5	9.8
Change in Sugar Conc. (Brix)	21.9	14.9
Conversion Factor (g/g)	0.51	0.51
Actual Ethanol Produced (g)	11.169	7.599
Initial Weight of Cellulose Sawdust (g)	100	100
Ethanol Yield (%)	11.17	7.6
Theoretical Ethanol Yield (g)	16.524	12.597
Yield Efficiency (%)	67.59	60.32
Theoretical Maximum Sugar Conc. (g)	52.2	52.2
Sugar Yield (%)	62.07	70.11
Sugar Conversion (%)	67.59	73.22

In SHF, 32.4 g of sugar was produced, corresponding to an ethanol efficiency and sugar yield of 67.59%, which is a 20.8 mL ethanol yield. The use of highly concentrated acid for hydrolysis may have destroyed some sugar and produced inhibitors, leading to lower-than-expected sugar conversion and ethanol yield. Additionally, experimental error may have contributed to the deviation from the theoretical yield of 34.1 mL. Fermentation was completed in 44 hours period. In SSF, pre-treatment with dilute H_2SO_4 alone produced 24.7 g of sugar, indicating a sugar yield of 60.32%,

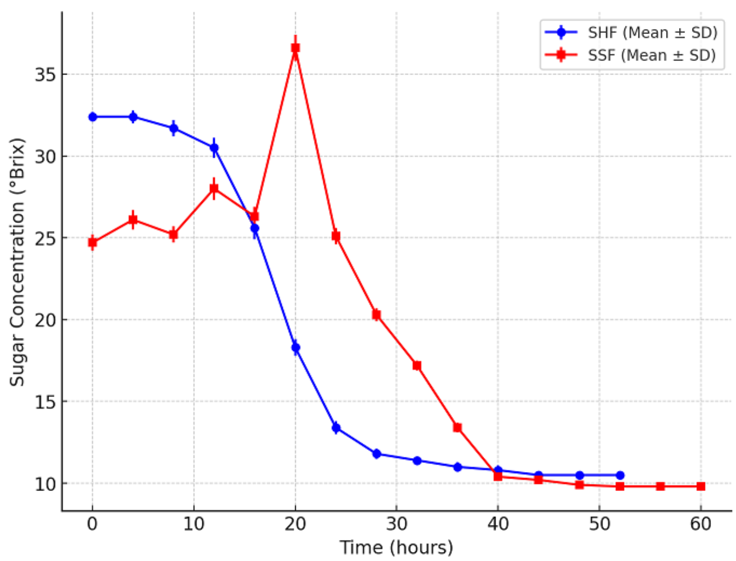

Figure 2 Mean and standard deviation analysis of Figure 1

suggesting that dilute acid pre-treatment alone is inefficient for sugar production from sawdust. However, the addition of cellulose increased sugar production, with a maximum sugar yield of 36.6 g achieved. The simultaneous sugar and ethanol production initially resulted in an unsteady state until a steady state was achieved after maximum sugar production. SSF resulted in a higher ethanol yield of 25.2 mL compared to SHF. Here, fermentation was completed in 52 hours. It is evident that since cellulose enzymes are required for SSF, they are likely more expensive than SHF. Generally, SHF shows promise for higher ethanol yields, but careful consideration of cost-effectiveness is necessary in process selection. Mahogany hardwood utilised by Mathew et al. [30] resulted in 27.67% ethanol greater than those obtained using SHF and SSF methods. Critical comparisons made are represented in Figure 3.

Undeniably, the waste products from SHF can pose environmental hazards, particularly due to the use of highly concentrated H_2SO_4. The energy-intensive process required for distilling and reusing such concentrated acid on a small scale may not be cost-effective, leading to the disposal of the acid. Previously, Adewumi [54] studied the energy involved in the production of ethanol from sawdust. It is crucial to handle and dispose of 18 M H_2SO_4, as also used by Amaefule et al. [13] and Adeeyo et al. [26], with extreme caution, as it is highly corrosive, can cause severe skin burns, and is not safe for consumption. On the other hand, the acid used in SSF is typically in much smaller quantities and may have minimal environmental impact. The dilute acid used in SSF and the enzymatic processes involved may result in less hazardous waste products than SHF. This difference in the scale and nature of acid usage accentuates the environmental considerations when choosing between SHF and SSF processes for ethanol production.

CONCLUSION

Comparisons between separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) methods for ethanol production from cellulose hardwood sawdust were conducted. The study monitored sugar concentration during ethanol synthesis at different time intervals to assess the conversion rate to ethanol. During the study, various equations were used to calculate ethanol yield, theoretical ethanol yield, yield efficiencies, sugar yield, and sugar conversion. The ethanol yield efficiency from sawdust was determined to be 67.59% in SHF and 60.32% in SSF, with the highest value achieved in SHF. While SHF shows promise for higher ethanol yields, careful consideration of cost-effectiveness is necessary in process selection. Both SHF and SSF methods should be further optimised to improve sugar and ethanol yields by refining the acid hydrolysis and fermentation conditions in SSF. Efforts should be made to minimise environmental impact, particularly in SHF, where hazardous waste is generated. Consideration should be given to recycling or safer disposal methods for the acid used in SHF. Further cost-benefit analyses should be conducted to determine the most economically viable approach. Diversification of pre-treatment chemicals and fermentation microorganisms on different wood types are additional research gaps that need to be filled.

ACKNOWLEDGEMENT

The authors would like to express their profound gratitude to all contributors involved in conceptualising the research, developing the methodology, conducting the result analysis, and compiling the literature review. Each author played a significant role in the successful completion of this study. Sincere appreciation is also

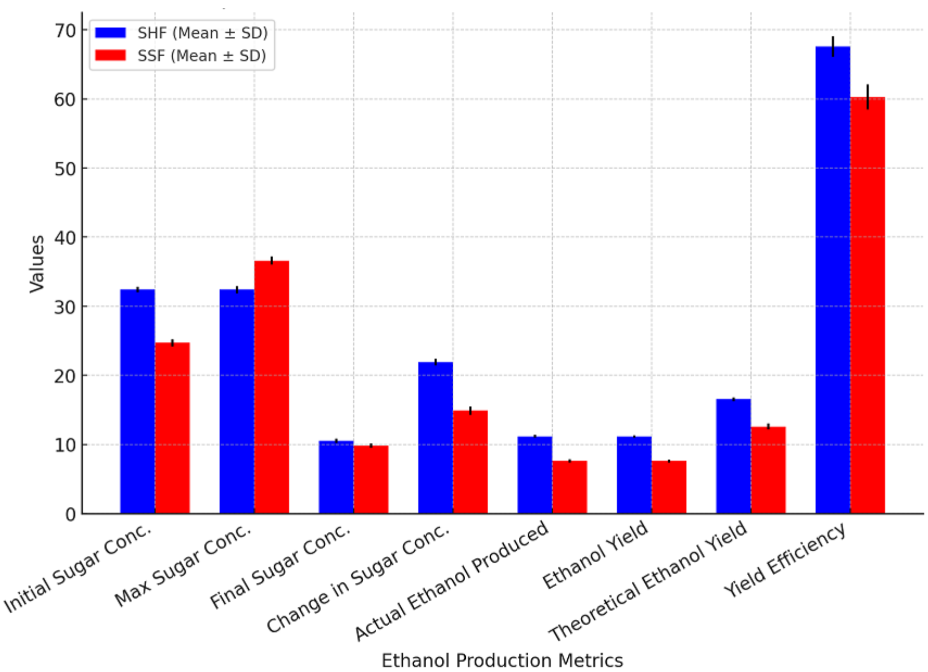

Figure 3 Comparison of ethanol production metrics (SHF vs. SSF)

extended to the University of Maiduguri, Nigeria, and Arcada University of Applied Sciences, Finland, for providing an enabling environment that made this work possible.

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