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Research article

Enhancing Napier Grass Degradation Efficiency through Microwave Pretreatment and Cellulase Enzyme Application

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Abstract: Napier grass is a promising energy crop for renewable sugar-based carbon production, where effective pretreatment is essential to enhance its biomaterial value. The objective of this research is to determine the optimal conditions for Napier grass pretreatment and achieve the highest level of efficiency in analyzing the initial lignocellulose content, total dissolved solids, and reducing sugars. The research applies Napier grass from the Pak Chong variation in the province of Phra Nakhon Si Ayutthaya, Thailand. Napier grass was pretreated with 0.5% sulfuric acid (v/v) at 140°C for 60 minutes, yielding a maximum cellulose content of 89.62%. This treatment effectively removed hemicellulose and lignin, with only 5.22% and 0.58% remaining, respectively. When the pretreated Napier grass is subjected to thermal and cellulase enzyme degradation and then treated with a microwave at 700 watts for 15 minutes, the initial total dissolved solids amount is 8.13 g/L, with total sugars and reducing sugars at 7.03 g/L and 91.34 mg/g dry weight, respectively. Adding 20 U of cellulase enzyme for 48 hours significantly enhances the degradation rate, resulting in a total sugar content of 13.95 g/L and reducing sugar content of 165.61 mg/g dry weight of Napier grass. The findings of this research are crucial for advancing biomass energy as a sustainable and environmentally friendly renewable energy source. Methane emissions commonly produced during fermentation can be minimized, thereby lowering the carbon footprint of biofuel production. Enhancing the degradation efficiency of Napier grass increases sugar yield, thereby improving its suitability for bioethanol and biofuel production.

Keywords: Biomass energy; Napier grass pretreatment; Ethanol production; Cellulase enzyme; Microwave pretreatment

1. Introduction

Napier grass, scientifically known as *Pennisetum purpureum*, originates from the African region and belongs to the Gramineae family[1-3]. It is also commonly referred to as Napier Grass or Elephant Grass. Napier grass is widely cultivated due to its large stems and leaves, which provide high nutritional value for animal feed. It grows rapidly, has a high yield per acre, and can be harvested year-round for 5-7 years per planting cycle. It thrives well in tropical climates, is a perennial plant with clump-forming and erect stems, and propagates through cuttings similar to sugarcane. There are several varieties of Napier grass, including the common Napier and hybrid varieties. When fully grown, the grass

can reach a height of up to 4 meters and can form dense clumps. Commonly studied and cultivated varieties include Giant Napier (*Pennisetum purpureum* x *P. glaucum* Hybrid cv. King grass), Pakchong 1 (Pennisetum purpureum x P. glaucum Pakchong1), and Alfalfa Napier (Pennisetum purpureum x P. glaucum Hybrid), which, at 45 days of growth, have volatile solid contents of 21.4%, 18.2%, and 23.0%, respectively [2, 4, 12] Additionally, another variety, the Dwarf Napier *(P. purpureum*), has been studied and found to have a total solid content of 23.1% per fresh weight, volatile solid content of 21.5% per fresh weight, and a carbon-to-nitrogen ratio of 59.1:1 [1, 3, 8]. Based on these characteristics, Napier grass is suitable for microbial degradation processes, serving as a substrate for ethanol production. Research indicates that the ethanol yield from Napier grass after microbial degradation, following pretreatment, ranges between 6 and 45 g/L, depending on various production system factors [3, 7, 14]. Napier grass, also known as Elephant Grass, originates from the African region and belongs to the Poaceae (formerly Gramineae) family. This grass is widely cultivated due to its high nutritional value, making it an excellent source of animal feed, thanks to its large stems and leaves. Additionally, Napier grass thrives well in tropical climates, grows in clumps, and propagates through cuttings similar to sugarcane. Napier grass is primarily composed of cellulose, hemicellulose, and lignin, which are resistant to degradation. Cellulose is a polysaccharide chain consisting of D-glucose units linked by β-1,4 glycosidic bonds, containing over 10,000 glucose units. Hemicellulose is a branched polysaccharide composed of various sugars, including hexoses (glucose, galactose, and mannose) and pentoses (xylose and arabinose). Lignin, an organic polymer consisting of phenylpropane units, exceeds 10,000 units and acts as a binding agent for cellulose and hemicellulose fibers, contributing to the structural rigidity and resistance to degradation of the grass [2]. Pretreating Napier grass before converting it to biogas is crucial to breaking down its rigid lignocellulosic structure, allowing enzymes or microorganisms to access and digest it more easily. Pretreatment methods include chemical (acid-alkaline), biological, thermal, and combined treatments. Chemical pretreatment (acid-alkaline) is particularly effective for rapid structural breakdown. This research aims to determine the optimal conditions for decomposing Napier grass and maximizing the efficiency of preliminary lignocellulose analysis, as well as the total dissolved solids, reducing sugar content, and total sugar content [3, 6]. This knowledge will enhance the process of ethanol production from Napier grass [4-5]. Therefore, the objective of this research is to investigate suitable conditions for the decomposition of Napier grass to achieve maximum efficiency in analyzing the preliminary lignocellulose content, total dissolved solids, reducing sugar content, and total sugar content. This study aims to advance the potential of ethanol production processes from Napier grass.

2. Materials and Methods

2.1 The preparation of Napier grass

The Napier grass should be cut into pieces of 4-5 cm. Then, it should be dried in an oven at 70 ˚C for 48 hours. After drying, the grass should be finely ground using a sample grinder. The ground material should be sieved using an automatic sample sieving machine to ensure the particle size is less than or equal to 125 microns. Finally, the sieved grass powder should be packed in plastic bags and stored in a desiccator at room temperature to maintain its dryness [3, 5-6].

2.2 The determination of the Moisture Content of Napier Grass

Fresh Napier grass, aged 5 years, is chopped into small pieces, approximately 1-2 cm long, using a knife. A portion of the sample is used to study the moisture content by drying the container in an electric oven at 105°C for 2–3 hours for moisture determination. Then, remove the container from the hot air oven and place it in a desiccator. After that, weigh the sample and dry it again in the same manner as in step 1, until the weight difference between the two consecutive weighings is no more than 1-3 milligrams. Weigh the treated sample accurately to a known weight and place it in a moisture determination container of known weight. Then, dry it in an electric oven at 105°C for 5–6 hours. Please remove it from the oven and place it in a desiccator. After that, weigh the sample, dry it again for approximately 30 minutes, and repeat the process until the weight difference between the two consecutive weighings is no more than 1–3 milligrams. Calculate the moisture content using the formula according to equation 1 [7-9]. A portion of the Napier grass sample is used to determine the yield, standard wet basis moisture content (as per equation 2), and standard dry basis moisture content (equation 3) [10-11]. This is done by weighing the sample with a four-decimal-place balance to record

the initial weight. Then, the sample is dried in a hot air oven at 105°C for 24 hours. The experiment was performed in triplicate [6,12].

$$Moisture\ content\ (\%) = \frac{100 \times (Initial\ sample\ weight - Final\ sample\ weight)}{Initial\ sample\ weight} \quad (1)$$

$$Wet\ basis\ moisture\ content\ (\%) = \frac{100 \times (Weight\ of\ water\ in\ sample)}{Total\ weight\ of\ sample} \quad (2)$$

$$Dry\ basis\ moisture\ content\ (\%) = \frac{100 \times (Weight\ of\ water\ in\ sample)}{Weight\ of\ dry\ matter\ sample} \quad (3)$$

2.3 Study on Optimal Conditions for Napier Grass Pretreatment

The sample was analyzed for initial lignocellulosic content, then pretreated using a 0.5% sulfuric acid solution and a 0.5% sodium hydroxide solution at temperatures of 80, 100, 120, and 140°C for durations of 30 and 60 minutes. The pretreated samples were analyzed for initial lignocellulosic content, total dissolved solids, reducing sugars, and total sugars. The best pretreatment conditions were selected based on these analyses. The selected pretreated sample was then subjected to microwave treatment at 700 watts for durations of 5 and 15 minutes. Cellulase enzyme was added at concentrations of 10 and 20 U for 48 hours. Samples were collected every 2 hours to measure total dissolved solids, total sugars, and reducing sugars [5,19]. The experimental data were then statistically analyzed to determine the optimal conditions. The experiments were conducted in triplicate to ensure accuracy and reliability.

2.4 Analysis of Reducing Sugars and Total Sugars

2.4.1 Analysis of Reducing Sugars

The sample solution was pipetted into a test tube at 1 milliliter. DNS reagent was added 1 ml to the test tube. The mixture samples were heated in a boiling water bath at 100°C for 10 minutes. The test tube was immediately cooled by placing it in an ice bath. Add 10 milliliters of distilled water to the cooled mixture. The absorbance of the resulting solution was measured at 540 nanometers using a spectrophotometer. The experimental results were compared to a standard curve prepared using glucose solutions with concentrations ranging from 0 to 2.0 mg/mL [5, 10, 16].

2.4.2 The analysis of total sugar content

The total sugar content is measured by preparing a sample with varying concentrations of glucose solution (1 mL each), adding a 5% phenol solution (1 mL), and mixing thoroughly using a Vortex mixer. Then, 5 ml of concentrated sulfuric acid is added and left to stand for 10 minutes. After that, the sample is incubated in a water bath at 30 °C for 20 minutes. The absorbance at 490 nanometers is then measured. The standard graph of sugar concentration versus absorbance values is then created on these measurements [5, 15, 17-18].

3. Results and Discussion

3.1 Yield results obtained from Napier grass

The experiment investigated the yield (%) of Napier grass by cutting it into 4-5 cm pieces and then drying it at 70°C for 48 hours. Subsequently, the grass was finely ground and sieved to a particle size of ≤125 microns using automated grinding and sieving equipment. The experiment yielded an average production of 6.81 ± 2.98% of fresh Napier grass, indicating the maximum production achievable through chemical processing. The study demonstrated the potential to enhance the yield efficiency of Napier grass through controlled temperature and precise timing, coupled with automated grinding and sieving, ensuring reliable and accurate data. Therefore, this study is crucial for developing more efficient production processes for Napier grass in the future.

3.2 Moisture content of Napier grass

The study on the moisture content of Napier grass includes both the standard dry basis moisture (%db) and wet basis moisture (%wb). Fresh Napier grass was partially cut into pieces approximately 1-2 cm in size and oven-dried in containers at 105°C for 2-3 hours. Subsequently, the samples were removed from the oven and placed in desiccators to cool, after which they were weighed to determine their moisture content.

This process was repeated until the weight difference between consecutive weighings did not exceed 1-3 milligrams. Samples adjusted to a precise weight were then oven-dried in an electric oven at 105°C for 5-6 hours. After removal from the oven, they were placed in desiccators to cool and be weighed. This oven-drying process was repeated in 30-minute intervals until the weight difference between consecutive weighings did not exceed 1-3 milligrams. The moisture content was calculated according to the standard formula. The results indicate that the standard dry basis moisture content of Napier grass is 93.19 ± 3.45%, and the wet basis moisture content is 1.21 ± 0.36%.

3.3 Results of the optimal conditions for Napier grass pretreatment and saccharification

This study investigated the optimal conditions for decomposing Napier grass using 0.5% sulfuric acid and 0.5% sodium hydroxide solutions. The experiments were conducted at four different temperatures—80, 100, 120, and 140°C—for durations of 30 and 60 minutes. The results showed that, as both temperature and treatment time increased, the lignocellulosic content, total reducing sugars, and total sugars in the solubilized solids also increased. These results effectively stimulate the decomposition process of Napier grass, enhancing its efficiency. The analysis results of cellulose, hemicellulose, and lignin content from Napier grass treated with 0.5% sulfuric acid solution at temperatures of 80, 100, 120, and 140°C for 30 minutes showed that untreated Napier grass had cellulose, hemicellulose, and lignin contents of 33.19 ± 5.65%, 43.24 ± 7.29%, and 12.32 ± 2.33%, respectively. Treated at 80°C, the contents were 40.65 ± 2.62%, 20.65 ± 3.65%, and 6.98 ± 1.69%, respectively. At 100 °C, the contents were 51.18 ± 5.36%, 19.65 ± 1.98%, and 5.32 ± 1.52%, respectively. At 120 °C, the contents were 64.22 ± 5.32%, 12.63 ± 2.06%, and 2.1 ± 0.98%, respectively. At 140°C, the contents were 75.39 ± 5.95%, 9.65 ± 2.25%, and 1.95 ± 1.29%, respectively, as shown in Figure 2. All conditions showed statistically significant differences at a 95% confidence level ($P \leq 0.05$), as shown in Table 1. The treatment temperature with 0.5% sulfuric acid had a statistically significant effect on the cellulose content in Napier grass at the 95% confidence level ($P = 0.000252$). The F value (17.88) was higher than F crit (3.89), confirming that the difference between each temperature group was significant. Therefore, it can be concluded that increasing the treatment temperature had a clear effect on the change in cellulose content in Napier grass. The findings underscore the effectiveness of 0.5% sulfuric acid treatment in enhancing cellulose enrichment and reducing hemicellulose and lignin content in Napier grass. These results are pivotal for advancing biomass conversion technologies, particularly in biofuel production and biorefinery processes. Future studies could further explore optimal treatment conditions and scale-up strategies to maximize the efficiency and sustainability of biomass utilization in industrial applications.

Table 1. Analysis of Variance in Cellulose Content (%) of Napier Grass Treated with 0.5% Sulfuric Acid for 30 Minutes at Various Temperatures.

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	5789.99	2	2894.99	17.88	0.000252*	3.89
Within Groups	1943.38	12	161.95			
Total	7733.37	14				

Note*: Cellulose content (%) of Napier grass treated with 0.5% sulfuric acid by volume at temperatures of 80, 100, 120, and 140 °C for 30 minutes differs significantly statistically at a confidence level of 95% ($P \leq 0.05$).

The analysis of cellulose, hemicellulose, and lignin content from Napier grass treated with 0.5% sulfuric acid at temperatures of 80°C, 100°C, 120°C, and 140°C for 60 minutes yielded the following results: At 80°C: Cellulose content was 45.33% ± 5.35, hemicellulose was 17.32 ± 5.22%, and lignin was 4.33±1.95%. At 100°C: Cellulose content increased to 58.65 ± 4.66%, hemicellulose decreased to 12.32±2.65%, and lignin decreased further to 2.11± 0.39%. At 120°C, Cellulose content continued to increase to 72.98% ± 6.32, hemicellulose decreased to 7.65± 2.63%, and lignin decreased significantly to 1.03 ± 0.59%. At 140°C, cellulose content reached its highest at 89.62 ± 8.33%, hemicellulose decreased to 5.22 ± 1.11%, and lignin was lowest at 0.58 ± 0.21%, as shown in Figure 2. Statistical analysis confirmed significant differences ($P \leq 0.05$) in cellulose,

hemicellulose, and lignin content across all treatment temperatures, indicating that temperature variation has a significant impact on the chemical composition of Napier grass treated with sulfuric acid. This study found that treating Napier grass with 0.5% sulfuric acid at different temperatures (80°C, 100°C, 120°C, and 140°C) for 60 minutes significantly impacted the cellulose content, which consistently increased from 45.33% at 80°C to 89.62% at 140°C. Meanwhile, hemicellulose decreased from 17.32% at 80°C to 5.22% at 140°C, and lignin decreased from 4.33% at 80°C to 0.58% at 140°C, as shown in Table 2. These results demonstrate the ability to modify the chemical composition of Napier grass, which is crucial for producing biofuels and refining biomass materials industrially [11-14]. The study provides scientifically robust insights and is beneficial for advancing more efficient technologies in the future.

Table 2. Analysis of Variance (ANOVA) of Lignocellulose Content in Napier Grass Treated with 0.5% Sulfuric Acid for 60 Minutes under Various Treatments

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	9852.72	2	4926.36	37.57	4.2852E-05	4.26
Within Groups	1180.39	9	131.16			
Total	11033.11	11				

Note: *The lignocellulose content of Napier grass treated with 0.5% sulfuric acid (volume by volume) at temperatures of 80, 100, 120, and 140 °C for 60 minutes shows statistically significant differences at the 95% confidence level ($P \leq 0.05$).

The analysis of cellulose, hemicellulose, and lignin content in Napier grass treated with 0.5% sodium hydroxide solution at temperatures of 80, 100, 120, and 140 °C for 30 minutes revealed the following results: Napier grass treated at 80 °C contained 35.26 ± 3.35%, 37.32 ± 4.65%, and 8.36 ± 1.65% of cellulose, hemicellulose, and lignin, respectively. At 100 °C, the content was 40.25 ± 6.33%, 28.32 ± 4.09%, and 7.65 ± 3.54%, respectively. At 120 °C, the content was 49.63 ± 4.19%, 17.32 ± 4.11%, and 5.32 ± 1.52%, respectively. At 140 °C, the content was 56.65 ± 8.19%, 12.48 ± 2.29%, and 2.29 ± 1.05%, respectively. All conditions showed statistically significant differences at a 95% confidence level ($P \leq 0.05$), as shown in Table 3. The results indicate that the cellulose content increases significantly as the temperature rises, while the hemicellulose and lignin contents decrease. This demonstrates that higher temperatures in sodium hydroxide treatment can significantly increase the cellulose content in Napier grass.

Table 3. Analysis of Variance (ANOVA) of Lignocellulose Content in Napier Grass Treated with 0.5% Sodium Hydroxide for 30 Minutes under Various Treatments

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	3136.02	2	1568.01	21.08	0.000402*	4.26
Within Groups	669.61	9	74.40			
Total	3805.63	11				

Note: *The lignocellulose content of Napier grass treated with 0.5% sodium hydroxide (weight by volume) at temperatures of 80, 100, 120, and 140 °C for 30 minutes shows statistically significant differences at the 95% confidence level ($P \leq 0.05$)

The analysis of cellulose, hemicellulose, and lignin content in Napier grass treated with 0.5% sodium hydroxide solution at temperatures of 80, 100, 120, and 140 °C for 60 minutes revealed the following results: Napier grass treated at 80 °C contained 48.32 ± 4.29%, 18.62 ± 2.96%, and 6.12 ± 1.29% of cellulose, hemicellulose, and lignin, respectively. At 120 °C, the content was 57.62 ± 5.65%, 15.32 ± 2.29%, and 1.78 ± 0.65%, respectively. At 140 °C, the content was 72.66 ± 7.09%, 11.32 ± 2.48%, and 1.28 ± 0.19%, respectively. All conditions showed statistically significant differences at a 95% confidence level ($P \leq 0.05$), as shown in Table 4.

Table 4. Analysis of Variance (ANOVA) of Lignocellulose Content in Napier Grass Treated with 0.5% Sodium Hydroxide for 60 Minutes under Various Treatments

ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	5492.39	2	2746.19	35.41	5.43E-05	4.26
Within Groups	697.9291	9	77.55			
Total	6190.319	11				

Note: *The lignocellulose content of Napier grass treated with 0.5% sodium hydroxide (weight by volume) at temperatures of 80, 100, 120, and 140 °C for 60 minutes shows statistically significant differences at the 95% confidence level ($P \leq 0.05$).

The result shows that using sulfuric acid to pretreat Napier grass is more effective than using sodium hydroxide. Increasing the treatment time also enhances the effectiveness of the pretreatment. Statistically significant results were obtained for removing hemicellulose and lignin at a 95% confidence level ($P \leq 0.05$). Napier grass treated with 0.5% sulfuric acid (by volume) at 140°C for 60 minutes yielded the highest cellulose content of 89.62 ± 8.33%, and the maximum removal of hemicellulose and lignin was 5.22 ± 1.11% and 0.58 ± 0.21%, respectively, as shown in Figure 1.

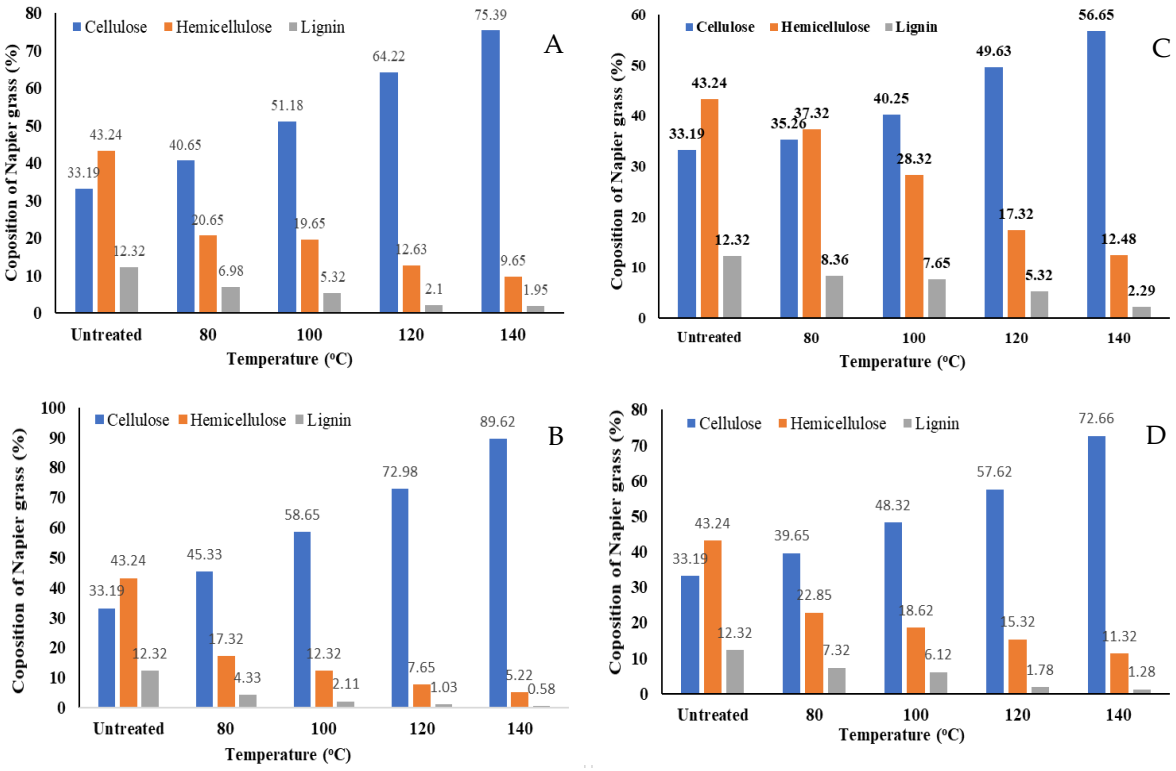

Figure 1. Napier grass treated under different conditions. A) Treated with 0.5% sulfuric acid for 30 minutes. B) Treated with 0.5% sulfuric acid for 60 minutes. C) Treated with 0.5% sodium hydroxide for 30 minutes. D) Treated with 0.5% sodium hydroxide for 60 minutes.

The solution of Napier grass, treated with 0.5% sulfuric acid and 0.5% sodium hydroxide, was tested at temperatures of 80, 100, 120, and 140 °C for 30 and 60 minutes and then analyzed for total dissolved solids, total sugar content, and reducing sugar content, the following results were observed: For 0.5% sulfuric acid at temperatures of 80, 100, 120, and 140 °C for 30 minutes, the total dissolved solids were 2.5, 3.7, 5.62, and 6.81 °Brix, the total sugar content was 2.12, 3.11, 3.75, and 5.22 g/L, and the reducing sugar content was 1.98, 2.85,

3.02, and 4.25 g/L, respectively. For 60 minutes, the total dissolved solids were 3.29, 4.45, 6.27, and 7.32 °Brix, the total sugar content was 2.57, 3.75, 4.01, and 6.38 g/L, and the reducing sugar content was 2.19, 3.04, 3.65, and 4.91 g/L, respectively. For 0.5% sodium hydroxide at temperatures of 80, 100, 120, and 140 °C for 30 minutes, the total dissolved solids were 1.92, 2.45, 3.63, and 5.33 °Brix, the total sugar content was 1.48, 2.11, 3.09, and 4.56 g/L, and the reducing sugar content was 0.85, 1.57, 2.44, and 3.29 g/L, respectively. For 60 minutes, the total dissolved solids were 2.32, 2.97, 4.12, and 6.05 °Brix, the total sugar content was 1.91, 2.45, 3.44, and 5.26 g/L, and the reducing sugar content was 1.12, 1.99, 3.01, and 4.23 g/L, respectively. As shown in Figure 2, the results indicate that higher temperatures and longer treatment times increase the total dissolved solids, total sugar content, and reducing sugar content. Furthermore, sulfuric acid treatment proved to be more effective than sodium hydroxide treatment. This research aims to minimize the use of chemicals, as high concentrations of chemicals can impact the conversion of biomass to furfural [9, 20]. The pretreatment of biomass was noted to be effective at breaking bonds, but high concentrations of chemicals do not lead to the desired main products.

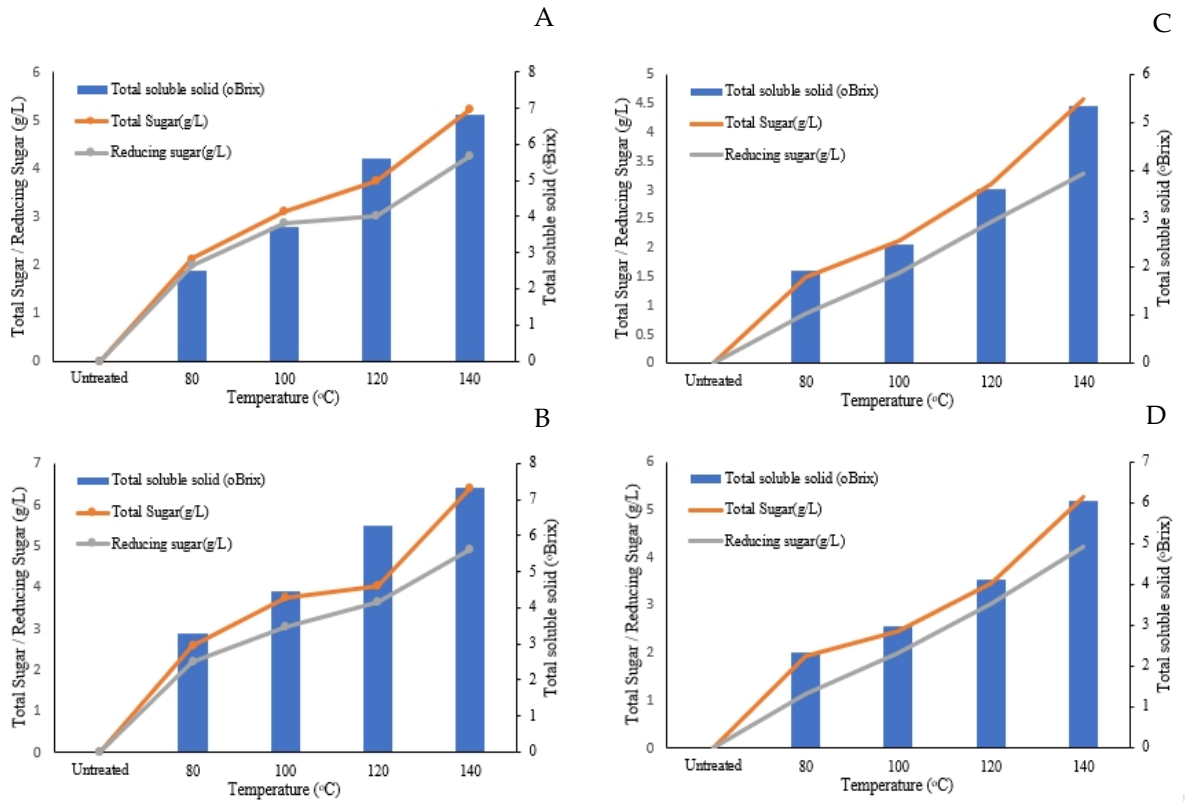

Figure 2. Total Dissolved Solids, Total Sugar Content, and Reducing Sugar Content of Napier Grass Solutions Treated Under Various Conditions A) Treated with 0.5% Sulfuric Acid for 30 Minutes B) Treated with 0.5% Sulfuric Acid for 60 Minutes C) Treated with 0.5% Sodium Hydroxide for 30 Minutes D) Treated with 0.5% Sodium Hydroxide for 60 Minutes.

Napier grass was selected from the above conditions (pretreated with 0.5% sulfuric acid by volume at 140°C for 60 minutes) and subjected to hydrolysis by heat and cellulase enzyme addition. The samples were then pretreated using a microwave at 700 watts for 5 and 15 minutes. Then, cellulase enzyme was added at 10 and 20 U for 48 hours, with samples collected every 2 hours. In these conditions, heat was applied using a microwave at 700 watts. Increasing the duration resulted in higher dissolution and an increase in total sugar and reducing sugar content. The initial total dissolved solids were 7.15 ± 0.39 (5 minutes) and 8.13 ± 1.52 (15 minutes), as shown in Table 5 and Figure 3. The total sugar and reducing sugar were 6.71 ± 0.95 (5 minutes) and 7.03 ± 1.12 (15 minutes) grams per liter, respectively. The initial reducing sugar content was 81.06 ± 5.29 (5 minutes) and 91.34 ± 3.66 (15 minutes) milligrams per gram of dry weight of Napier grass, as shown in Table 6 and Figure 4.

Table 5. Comparison of Total Soluble Solids between Treatments of Treated Napier Grass under Different Incubation Conditions

	Test Value = 0.05					
	T	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
A	232.76	24	.000	7.98	7.91	8.05
B	119.82	24	.000	8.27	8.13	8.41
C	250.63	24	.000	8.35	8.28	8.41
D	125.33	24	.000	8.65	8.51	8.79

Note: Incubation with cellulase enzyme from 0 to 48 hours with statistically significant differences at a 95% confidence level ($P \leq 0.05$). A) Using microwave for heating at 700 Watt, 5 minutes with 20 U of cellulase B) Using microwave for heating at 700 Watt, 5 minutes with 20 U of cellulase C) Using microwave for heating at 700 Watt, 15 minutes with 10 U of cellulase D) Using microwave for heating at 700 Watt, 15 minutes with 20 U of cellulase

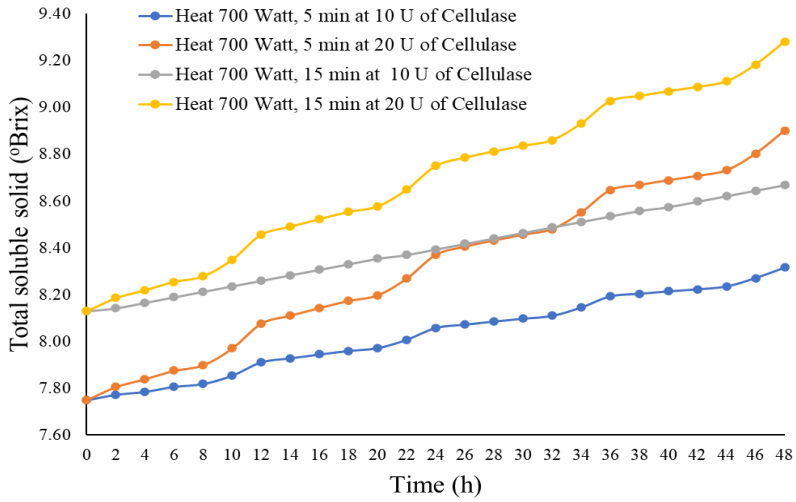

Figure 3. Total Soluble Solids Content of Treated Napier Grass

Table 6. Comparison of Total Sugar Content between Treatments of Treated Napier Grass under Different Incubation Conditions

	Test Value = 0.05					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
A	30.08	24	.000	8.28	7.71	8.85
B	21.90	24	.000	8.86	8.029	9.69
C	31.24	24	.000	8.59	8.029	9.17
D	21.67	24	.000	9.44	8.54	10.34

Note: Incubation with cellulase enzyme from 0 to 48 hours with statistically significant differences at a 95% confidence level ($P \leq 0.05$). A) Using microwave for heating at 700 Watt, 5 minutes with 20 U of cellulase B) Using microwave for heating at 700 Watt, 5 minutes with 20 U of cellulase C) Using microwave for heating at 700 Watt, 15 minutes with 10 U of cellulase D) Using microwave for heating at 700 Watt, 15 minutes with 20 U of cellulase

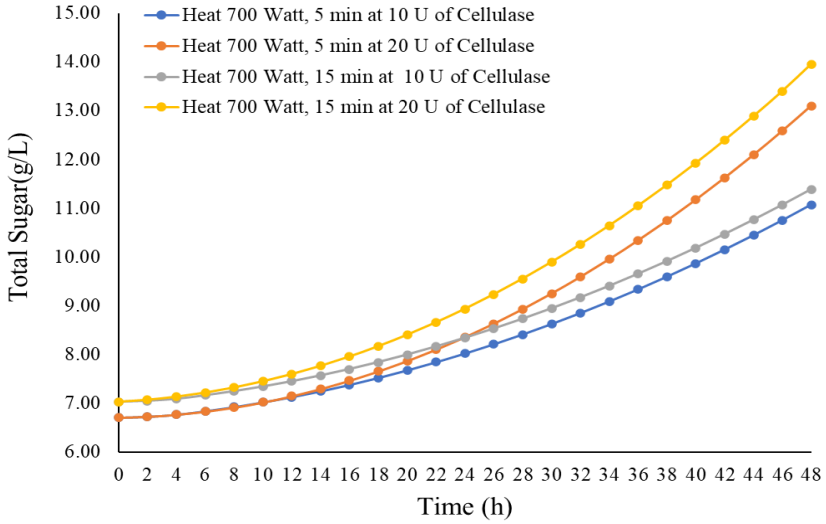

Figure 4. Total Sugar Content of Treated Napier Grass

When Napier grass preheated at 700 watts was treated with 5 U and 10 U of cellulase enzyme, it was found that the hydrolysis rate was reasonable in all conditions. However, there were significant differences at a 95% confidence level ($P \leq 0.05$). The best hydrolysis condition was when 20 U of cellulase enzyme was added and incubated for 48 hours after pretreatment by heating at 700 watts for 15 minutes, resulting in 9.28 degrees Brix, with a total sugar content of 13.95 ± 1.62 grams per liter and a reducing sugar content of 165.61 ± 9.22 milligrams per gram of dry weight of Napier grass. As shown in Table 7 and Figure 5. These findings highlight the effectiveness of microwave pretreatment and enzymatic hydrolysis in enhancing sugar yields from Napier grass, crucial for improving the efficiency of biofuel production processes [14, 20]. Acid pretreatment at high temperatures can produce inhibitory compounds that affect fermentation, including furans (HMF, furfural) derived from six-carbon and five-carbon sugars, phenolic compounds from lignin, and organic acids (acetic acid, formic acid) from hemicellulose. These compounds can inhibit the growth of microorganisms and the production of ethanol, butanol, or biogas. [4, 15, 19]

Table 7. Comparison of Reducing Sugar Content between Treatments of Treated Napier Grass under Different Incubation Conditions with Cellulase Enzyme

	Test Value = 0.05					
	t	**df**	**Sig. (2-tailed)**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	**Upper**
A	83.23	24	.000	87.27	85.16	89.44
B	27.82	24	.000	101.33	93.82	108.85
C	93.01	24	.000	97.55	95.39	99.71
D	24.97	24	.000	117.28	107.58	126.97

Note: Incubation with cellulase enzyme from 0 to 48 hours with statistically significant differences at a 95% confidence level ($P \leq 0.05$) A) Using microwave for heating at 700 Watt, 5 minutes with 20 U of cellulase B) Using microwave for heating at 700 Watt, 5 minutes with 20 U of cellulase C) Using microwave for heating at 700 Watt, 15 minutes with 10 U of cellulase D) Using microwave for heating at 700 Watt, 15 minutes with 20 U of cellulase.

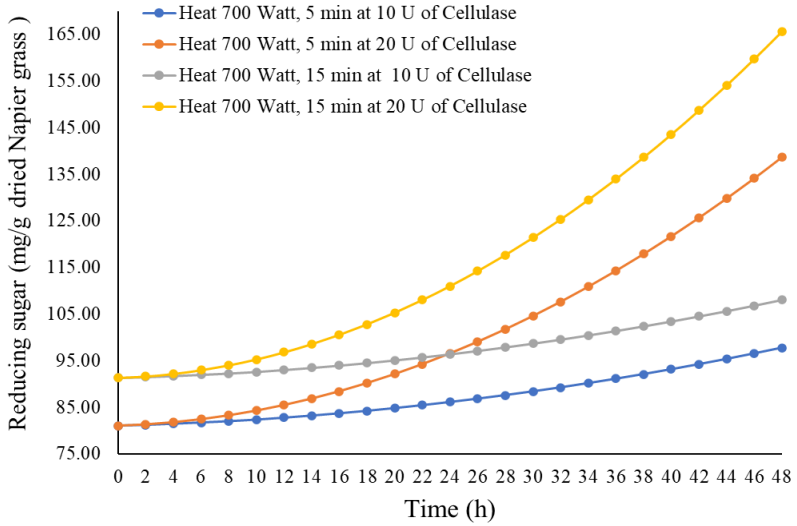

Figure 5. Reducing Sugar Content of Treated Napier Grass

When the best-treated Napier grass was examined under a scanning electron microscope at a magnification of 3,000× and an accelerating voltage of 20 kV, the microstructural images revealed significant surface degradation. The cell walls of the Napier grass appeared to be severely damaged—scattered, torn, and peeled off into thin sheets. The surface morphology was rough and uneven, with numerous holes and gaps visible. The application of microwave pretreatment effectively disrupted the hydrogen bonds within the cellulose fibers, causing the rapid evaporation of intracellular water and resulting in internal pressure buildup and cell rupture, thereby forming porous structures. In addition, the action of cellulase enzymes facilitated further biodegradation by accelerating the hydrolysis of glycosidic bonds in cellulose, resulting in the release of fermentable sugars, including high-purity glucose. The SEM images demonstrated extensive structural breakdown, indicating that the enzymes could efficiently penetrate and access the internal matrix of the biomass. Consequently, such a high degree of pretreatment effectiveness is expected to significantly enhance the production of biofuels, such as bioethanol, biobutanol, or biogas, by improving the digestibility of the biomass and yielding more efficient substrates for microbial fermentation, as shown in Figure 6.

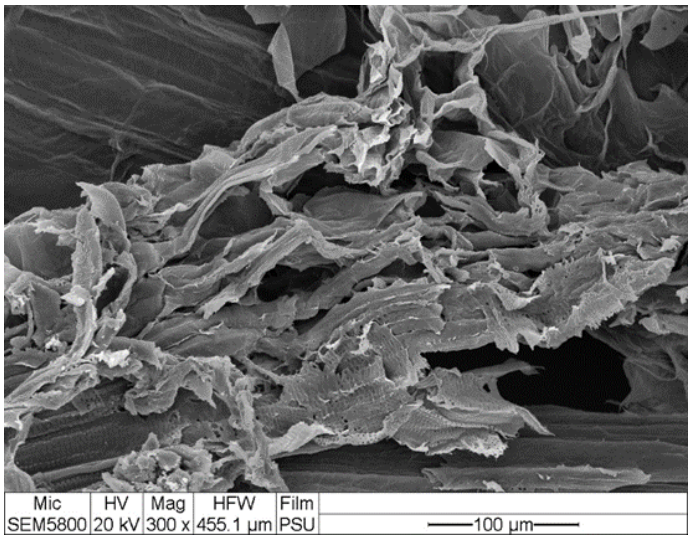

Figure 6. SEM micrograph of napier grass after microwave pretreatment and cellulase hydrolysis showing extensive cell wall bio-degradation

4. Conclusions

Developing an efficient decomposition process for Napier grass revealed that sulfuric acid is more effective than sodium hydroxide in pretreating the grass. Increased processing time significantly improved the pretreatment results, effectively removing hemicellulose and lignin with a 95% confidence level ($P \leq 0.05$). Napier grass treated with 0.5% (v/v) sulfuric acid at 140°C for 60 minutes yielded the highest cellulose content at 89.62 ± 8.33% and achieved the most substantial removal of hemicellulose and lignin, at 5.22 ± 1.11% and 0.58 ± 0.21%, respectively. When the pretreated Napier grass was further decomposed under optimal conditions by heating and the addition of cellulase enzyme, microwave treatment at 700 watts for 15 minutes resulted in an initial total dissolved solids concentration of 8.13 ± 1.52 g/L. The total sugar and reducing sugar concentrations were 7.03 ± 1.12 g/L, with an initial reducing sugar concentration of 91.34 ± 3.66 mg/g of dry Napier grass. After the addition of 20 U of cellulase enzyme for 48 hours, with sampling every 2 hours, the total sugar concentration increased to 13.95 ± 1.62 g/L, and the reducing sugar concentration rose to 165.61 ± 9.22 mg/g of dry Napier grass. Microwave pretreatment of Napier grass, combined with cellulase enzymes, effectively enhanced the degradation of cellulose structures. SEM images revealed cell wall degradation and porosity formation, facilitating better access and enzymatic degradation, which led to the release of fermentable sugars.

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