
Microwave-assisted alkali pretreatment of itchgrass for fermentable sugar production

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Abstract Itchgrass, a relatively abundant weed in the Philippine cropland, was explored as a potential source of fermentable sugars. Microwave-assisted alkali pretreatment was used as a pretreatment method for itchgrass. The results showed that the treatment with high NaOH concentration (5% w/v), exposed to high microwave irradiation power (300 W), and subjected to a long reaction time (9 min) produced the most reducing sugar after pretreatment and saccharification. Additionally, the characterization of the pretreated itchgrass showed that 47.83% of the original lignin content and 41.02% of the original hemicellulose were removed after the pretreatment. Moreover, the microwave-assisted alkali pretreatment of itchgrass produced more reducing sugar after pretreatment and saccharification compared to the itchgrass pretreated with conventional heating alkali pretreatment. Overall results suggest that itchgrass is a good potential source of fermentable sugars, especially when pretreated using microwave-assisted alkali pretreatment.

Keywords: Itchgrass, Fermentable sugars, Microwave, Alkali, Delignification

Introduction

Itchgrass (*Rottboellia conchinesis* (Lour.) W.D. Clayton) – commonly known as “sagisi” in the Philippines – is an annual grass that is native to tropical Southeast Asia. The grass is mainly characterized by its long, sharp, siliceous, and fragile hairs that can penetrate the skin, which causes itching, hence giving rise to its common name. It has a pale green color, prop roots, cylindrical spike inflorescences and seeds, and can grow to as high as 3 m tall if left uncontrolled. Its seeds can spread over a large area via farm machinery, road construction equipment, water, wind, and birds, among others. The grass is typically found in farms and crop plantations, but it can also spread to other managed habitats (Rojas-Sandoval and Acevedo-Rodriguez, 2022). Itchgrass is one of the major weed infestations on mung bean, banana, cassava, citrus, cowpea, papaya, groundnut, pineapple, rice, sorghum, and soybean plantations in the Philippines.

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Infestations can result in at least a 50% reduction in crop yields (Rojas-Sandoval and Acevedo-Rodriguez, 2022).

Itchgrass has a higher carbon dioxide fixation rate and water and nutrient uptake than the crops; hence, it can outcompete them (Awan *et al.*, 2014; Chauhan and Bajwa, 2015). Combined with the difficulty in handling due to its irritating hairs and the difficulty of removing it from an infested plantation without damaging the crops, itchgrass can easily reproduce and produce large amounts of biomass. Due to its relative abundance, it can be a promising source of fermentable sugars, which can be utilized for bioethanol, biobutanol, bioplastics, and biogas production, among others.

Itchgrass is a lignocellulosic biomass that contains the bulk of its fermentable sugars in its alpha-cellulose. Due to the recalcitrant structure of the lignocellulosic biomass, brought about by the intertwining lignin, alpha-cellulose, and hemicellulose, this makes it impenetrable to hydrolytic enzymes. Hence, it must undergo pretreatment to expose the alpha-cellulose to hydrolytic enzymes that convert it to fermentable sugars. This can be achieved by solubilizing or breaking the lignin structure, selectively removing the hemicellulose, reducing the crystallinity of the alpha-cellulose, or any combination of these approaches (Wardani *et al.*, 2020; Yin *et al.*, 2021; Shukla *et al.*, 2023).

One of the promising methods of lignocellulosic biomass pretreatment is microwave-assisted alkali pretreatment. Coupling microwave irradiation with alkali pretreatment provides a synergistic effect as the microwave irradiation allows the chemical reaction between the alkali solution and the biomass to proceed at elevated temperatures for a shorter reaction time (Li *et al.*, 2016) while using an alkali solution enhances the amount of heat generated during microwave irradiation (Rodríguez *et al.*, 2015; Shang *et al.*, 2019). Several studies (Janker-Obermeier *et al.*, 2012; Singh *et al.*, 2014; Rubab *et al.*, 2023) that utilized microwave-assisted alkali pretreatment in their respective lignocellulosic biomass of interest obtained favorable results in terms of lignin and hemicellulose removal.

Hence, this study utilized microwave-assisted alkali pretreatment in destroying the recalcitrant structure of the itchgrass to be able to produce fermentable sugars. Specifically, this study investigated the effects of NaOH concentration, microwave irradiation power, and reaction time on the reducing sugar after pretreatment and saccharification. Additionally, the pretreated itchgrass was characterized to determine the extent of delignification. Moreover, microwave-assisted alkali pretreatment was compared with conventional heating alkali pretreatment in terms of reducing sugar after pretreatment and saccharification.

Materials and methods

Collection and preparation of itchgrass

The mature itchgrass stalks were collected from a rice farm in Cordon, Isabela, Philippines. All the parts of the itchgrass stalk were used except for its roots, damaged stems, and withered leaves.

The collected itchgrass was thoroughly washed with tap water to remove dirt and other foreign particles. The washed itchgrass was then air-dried and sun-dried for a day to remove residual water. The partially dried itchgrass was cut into approximately 1 cm long strands and further dried for an additional day in an oven set at 105 °C to obtain a bone-dry mass.

The dried itchgrass was further reduced in size using a 600 W knife mill to increase the surface area, partially break the cellulose's crystalline structure, and expose more active binding sites (Brodeur *et al.*, 2011).

The milled itchgrass was homogenized using a series of U.S. Standard Testing Sieves with pore sizes of 1000 µm, 500 µm, and 250 µm. The itchgrass powder with a particle size of -1000 / +250 µm was used. The prepared itchgrass was stored in air-tight plastic bags.

Experimental design

The study followed a two-level factorial experimental design in a completely randomized design (CRD) for three factors: Sodium Hydroxide (NaOH) Concentration (A), Microwave Irradiation Power (B), and Reaction Time (C). These factors were varied, as shown in Table 1.

Table 1. The process conditions varied during the microwave-assisted alkali pretreatment of itchgrass

Parameters	Unit	Low Level (-)	High Level (+)
NaOH Concentration	% w/v	1	5
Microwave Irradiation Power	W	100	300
Reaction Time	min	1	9

The variations in the three factors resulted in eight treatments with varying combinations of NaOH concentration, microwave irradiation power, and reaction time (Table 2). The experimental matrix consisted of randomized and triplicated trial runs for each treatment, totaling 24 runs.

Table 2. The experimental matrix used in the study

Treatment	NaOH Concentration (% w/v)	Microwave Irradiation Power (W)	Reaction Time (min)
T1	1	100	1
T2	1	100	9
T3	1	300	1
T4	1	300	9
T5	5	100	1
T6	5	100	9
T7	5	300	1
T8	5	300	9

Microwave-assisted alkali pretreatment

About 1.00 g of the prepared itchgrass was measured in a beaker using an analytical balance. About 20.00 mL of the prepared NaOH solution was added to the biomass to obtain a 20:1 liquid-to-solid ratio. The reaction mixture was homogenized through slight agitation until the itchgrass was completely soaked in the alkaline solution. The reaction mixture was then subjected to microwave irradiation using a 1200 W Samsung ME-711K Microwave Oven.

Immediately after the pretreatment, the temperature of the reaction mixture was read and recorded using an infrared thermometer. The reaction mixture was then filtered using vacuum filtration to separate the pretreated itchgrass from the pretreatment hydrolysate.

The pretreatment hydrolysate was collected for analysis, while the pretreated itchgrass was washed with approximately 1,000 mL of tap water, followed by 300 mL of distilled water, until it reached a neutral pH. The washed, pretreated itchgrass was dried at 105 °C for 24 h. The dried, pretreated itchgrass was stored in airtight plastic bags.

Alkali pretreatment with conventional heating

The process conditions in the conventional heating alkali pretreatment were based on the microwave-assisted alkali pretreatment experiment, which yielded the highest reducing sugar yield after pretreatment and enzymatic hydrolysis.

About 1.00 g of the prepared itchgrass was measured in a capped test tube using an analytical balance. About 20.00 mL of the NaOH solution was added to the itchgrass to obtain a 20:1 liquid-to-solid ratio. The reaction mixture was homogenized through slight agitation until the itchgrass was completely soaked in the alkaline solution. The reaction mixture was then subjected to conventional

heating using a 2100 W LabTech Shaking Water Bath set at the corresponding temperature (84.13 °C) from the microwave-assisted alkali pretreatment experiment.

Additionally, two reaction times were investigated: the original corresponding reaction time (9 min) from the microwave-assisted alkali pretreatment experiment and a prolonged reaction time of 60 min.

Saccharification

The saccharification was done using enzymatic hydrolysis. The cellulase enzyme used for enzymatic hydrolysis was obtained from the National Institute of Microbiology and Biotechnology (BIOTECH), University of the Philippines Los Baños. It was extracted from the fungi strain *Trichoderma reesei* RUT C30 and has an effective volumetric activity of 450 U/mL.

The enzymatic hydrolysis followed the standard procedural steps outlined in the Enzymatic Saccharification of Lignocellulosic Biomass protocol prescribed by the National Renewable Energy Laboratory (NREL/TP-510-42629). About 0.15 g of the pretreated itchgrass from each trial run was measured and placed in a 20 mL scintillation vial. A volume of 5.00 mL of 0.1 M sodium citrate buffer (pH 4.8~5.0) was added to the vial. A volume of 0.1 mL of 2% (w/v) sodium azide solution was also added to prevent the possible growth of unwanted microbes. The appropriate volume of distilled water was calculated before adding the enzymes, ensuring that 10 mL of the final solution was achieved after adding the required enzyme volume. A control group was also added, which included: unpretreated itchgrass (C1), blank without itchgrass (C2), and blank without enzyme (C3).

The vial set-ups were then placed in a water bath shaker maintained at 50°C and 50 rpm. Before the addition of the enzyme, the vial set-ups were equilibrated for 30-60 min to reach the desired optimum temperature and allow the pretreated itchgrass to be completely soaked in the citrate buffer.

After equilibrating, the cellulase enzyme was added to obtain a 705 U/g loading. The enzymatic hydrolysis was stopped after 72 h by heating the vial set-ups over boiling water for 5 min. The vial set-ups were then cooled down to room temperature using a cold-water bath. The hydrolysate was separated from the itchgrass using a centrifuge operated at 3000 rpm for 10 min.

Measurement of reducing sugar

The amount of reducing sugar in the hydrolysates recovered from the pretreatment and saccharification was estimated using the Dinitrosalicylic Acid (DNS) Method with glucose as a standard. Approximately 3 mL of DNS reagent

was added to 3 mL of hydrolysate. The resulting solution was heated over boiling water for 5-15 min until color development occurred. The solution was then cooled down to room temperature. After that, 1 mL of Rochelle's Salt was added. The absorbance of the solution was read using a spectrophotometer set at 550 nm wavelength (Miller, 1959). The concentration of the hydrolysate was then calculated by comparing it to a standardization curve consisting of various known concentrations of glucose solutions.

Characterization of raw and pretreated itchgrass

The treatment that produced the highest reducing sugar yield was characterized to determine the extent of pretreatment. About 25 g of the raw and pretreated itchgrass samples were sent to the Central Analytical Services Laboratory (CASL) at BIOTECH-UPLB for characterization. The characterization includes the profiling of the following biomass components: alpha-cellulose, hemicellulose, acid-soluble lignin, acid-insoluble lignin, extractives, moisture, and ash.

Statistical tools

Mean was used to summarize the measured reducing sugar yield, alpha-cellulose, hemicellulose, and lignin. A three-way Analysis of Variance (ANOVA) was used to check the significant difference among the various combinations of NaOH concentration, microwave irradiation power, and reaction time. Tukey's Honest Significant Difference Test was used for its post-hoc test. The omega squared was also computed to determine the effect size of the three factors and their interaction. The result of the omega squared was interpreted based on Field (2013). The Pareto chart of effects was generated using Stat-Ease Design Expert v.13. All hypothesis testing was done at $\alpha = .05$.

Results

Reducing sugar of the pretreatment hydrolysate

The pretreatment hydrolysate of T8 (5% w/v NaOH, 300 W, and 9 min) had the highest reducing sugars among the itchgrass treatments (Figure 1). On the other hand, the pretreatment hydrolysate of T1 (1% w/v NaOH, 100 W, and 1 min) had the lowest reducing sugar.

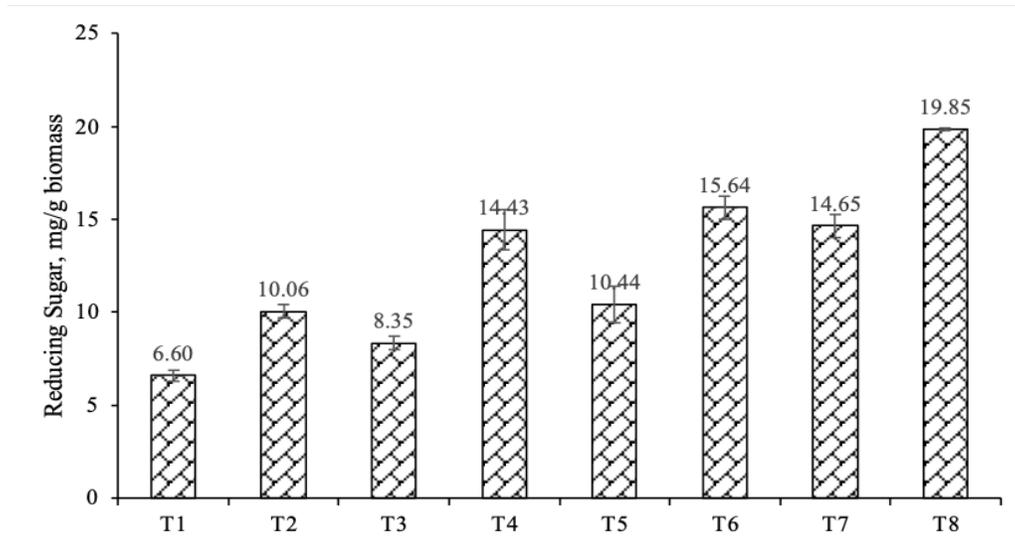


Figure 1. The reducing sugar from the pretreatment hydrolysates

Effect of NaOH concentration, microwave irradiation power, and reaction time on the reducing sugar of the pretreatment hydrolysate

The results showed that there was a significant difference ($p < .05$) in the reducing sugar of the pretreatment hydrolysates between the treatments that received 1% w/v NaOH and those that received 5% w/v NaOH (Table 3). Similarly, there was a significant difference ($p < .05$) in the reducing sugar of the pretreatment hydrolysates between the treatments exposed to 100 W and 300 W microwave irradiation power. Likewise, there was a significant difference ($p < .05$) in the reducing sugar of the pretreatment hydrolysates between the treatments subjected to 1 min and 9 min reaction times. While all three factors had a large effect as indicated by the ω^2 , the effect of NaOH concentration was the highest, followed by reaction time, and lastly by microwave irradiation power.

It was also found that the interaction between the NaOH concentration and microwave irradiation power (AB), the interaction between the microwave irradiation power and reaction time (BC), and the interaction among the three factors (ABC) were significant ($p < .05$). However, their effects were relatively very small compared to the main three factors.

The Pareto chart of effects showed that all three main factors had a positive effect on the reducing sugar of the pretreatment hydrolysate (Figure 2). Hence, increasing the NaOH concentration, microwave irradiation power, and reaction time within the specified range (as defined in Table 2) increased the reducing sugar of the pretreatment hydrolysate.

Table 3. The effect of NaOH concentration, microwave irradiation power, and reaction time on the reducing sugar of the pretreatment hydrolysates

Source	Sum of Squares	df	Mean Square	F	p	ω^2
Overall Model	403.471	7	57.639	140.636	<.001	
A	167.600	1	167.600	408.937	<.001	0.407
B	0.59853	1	79.217	193.286	<.001	0.192
C	0.00213	1	149.260	364.188	<.001	0.363
AB	1.971	1	1.971	4.809	0.043	0.004
AC	0.271	1	0.271	0.662	0.428	0.000
BC	2.588	1	2.588	6.315	0.023	0.005
ABC	2.564	1	2.564	6.257	0.024	0.005
Residuals	6.557	16	0.410			

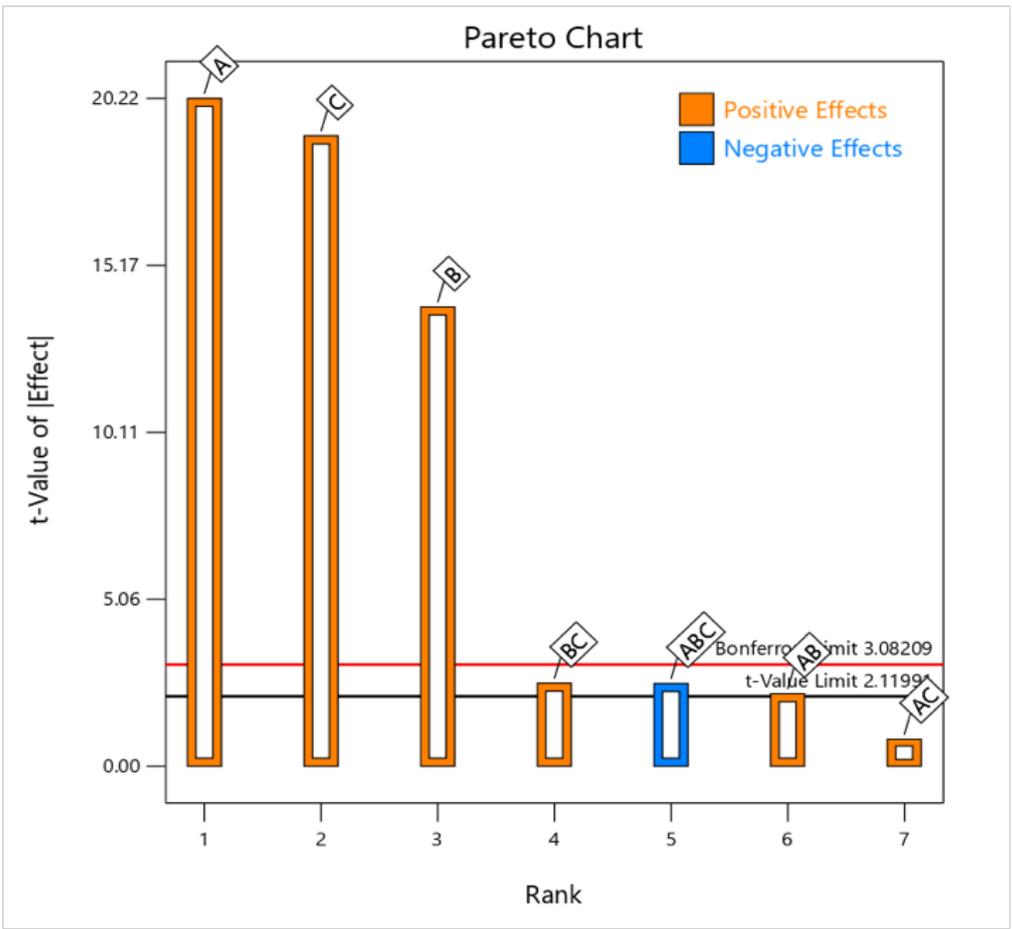


Figure 2. The Pareto chart of effects on the reducing sugar of the pretreatment hydrolysate

Reducing sugar of the saccharification hydrolysate

It was found that the saccharification hydrolysate of T8 (5% w/v NaOH, 300 W, and 9 min) had the highest reducing sugars among the itchgrass treatments (Figure 3). Moreover, it was found to be not significantly different ($p>.05$) from the reducing sugar of T6 (5% w/v NaOH, 100 W, and 9 min). On the other hand, the lowest reducing sugar was produced by T1 (1% w/v NaOH, 100 W, and 1 min). Additionally, the reducing sugar of T1 was not significantly different ($p>.05$) from the reducing sugar of the control (C1). It was also found that little to no reducing sugar was released in the prepared blank without itchgrass (C2) and the blank without enzyme (C3), indicating that no saccharification occurred in these controls.

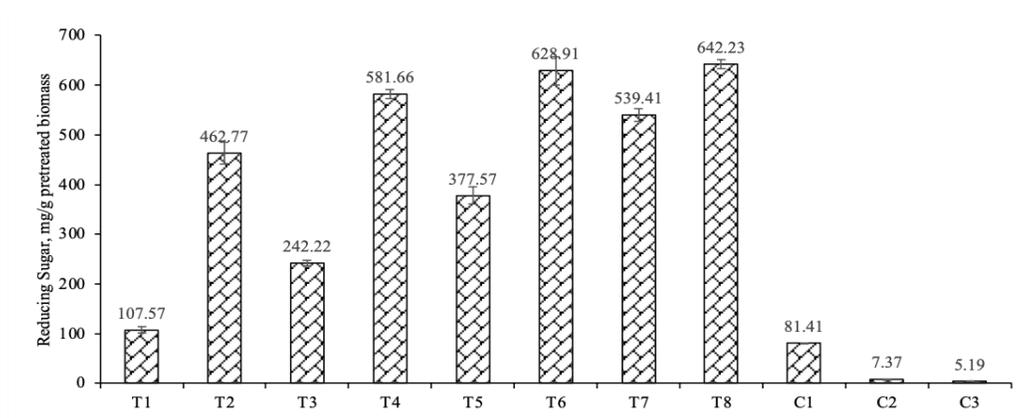


Figure 3. The reducing sugar from the saccharification hydrolysates

Effect of NaOH concentration, microwave irradiation power, and reaction time on the reducing sugar of saccharification hydrolysate

The results showed that there was a significant difference ($p<.05$) in the reducing sugar of the saccharification hydrolysates between the itchgrass treatments that were pretreated with 1% w/v NaOH and those pretreated with 5% w/v NaOH (Table 4). Similarly, there was a significant difference ($p<.05$) in the reducing sugar of the saccharification hydrolysates between the itchgrass treatments pretreated with 100 W and 300 W microwave irradiation power. Likewise, there was a significant difference ($p<.05$) in the reducing sugar of the saccharification hydrolysates between the itchgrass treatments pretreated with 1 min and 9 min reaction time. It was also found that reaction time had the largest effect, followed by NaOH concentration. On the other hand, microwave irradiation power had a medium effect.

It was also found that the interactions among the factors were all significant ($p < .05$). However, the effects of these interactions were relatively small compared to the three main factors.

Table 4. The effect of NaOH concentration, microwave irradiation power, and reaction time on the reducing sugar of the saccharification hydrolysates

Source	Sum of Squares	df	Mean Square	F	p	ω^2
Overall Model	780270	7	111467	443.01	<.001	
A	236349	1	236349	939.33	<.001	0.301
B	68921	1	68921	273.91	<.001	0.088
C	412496	1	412496	1639.39	<.001	0.525
AB	2303	1	2303	9.15	.008	0.003
AC	43472	1	43472	172.77	<.001	0.055
BC	10120	1	10120	40.22	<.001	0.013
ABC	6609	1	6609	26.27	<.001	0.008
Residuals	4026	16	252			

The Pareto chart of effects showed that all three main factors had a positive effect on the reducing sugar of the pretreatment hydrolysate (Figure 4). Hence, increasing the NaOH concentration, microwave irradiation power, and reaction time within the specified range (as defined in Table 2) increased the reducing sugar of the saccharification hydrolysate.

Characterization of raw and pretreated itchgrass

The results of the characterization showed that microwave-assisted alkali pretreatment reduced the lignin and hemicellulose content of the itchgrass (Table 5). This exposed the alpha-cellulose, as evident in the amount of reducing sugar produced during saccharification.

Table 5. Characterization of raw and pretreated itchgrass

Component	Percent Composition*	
	Raw Itchgrass	Pretreated Itchgrass [†]
Lignin	16.16 ± 0.86	8.43 ± 0.21
Alpha-Cellulose	34.65 ± 2.73	67.87 ± 1.69
Hemicellulose	26.38 ± 2.65	15.56 ± 1.72

* % w/w, dry basis

† T8 - 5% (w/v) NaOH, 300 W, and 9 min

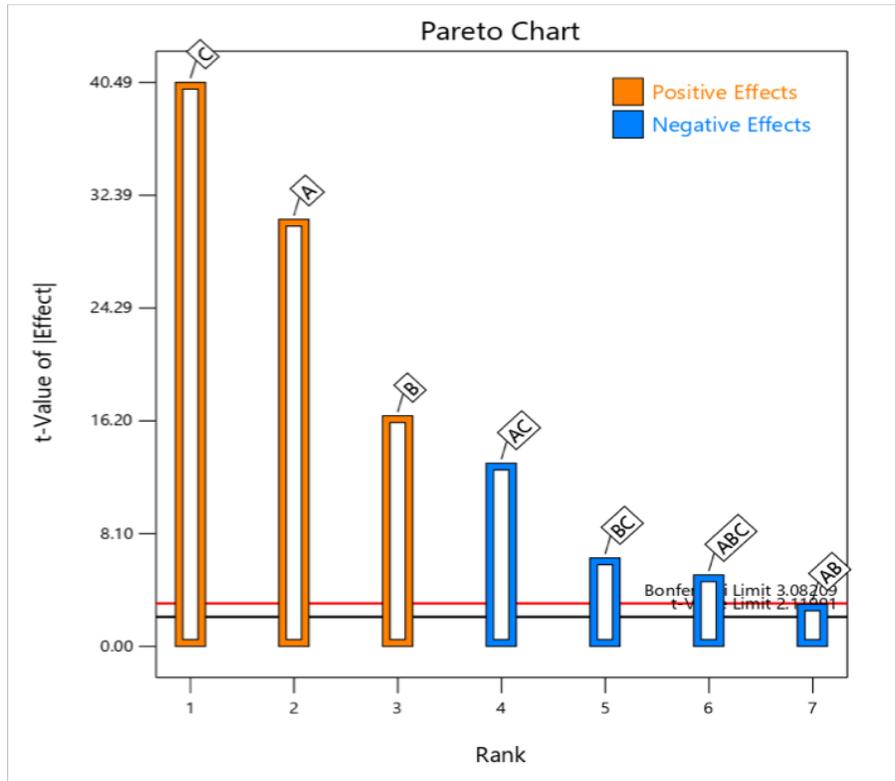


Figure 4. The Pareto chart of effects on the reducing sugar of the saccharification hydrolysate

Comparison with conventional heating alkali pretreatment

The reducing sugar from the pretreatment and saccharification hydrolysates using microwave-assisted alkali pretreatment was significantly higher ($p < .05$) than when using conventional heating alkali pretreatment. Moreover, increasing the reaction time of the conventional heating alkali pretreatment did not significantly increase ($p > .05$) the reducing sugar (Table 6).

Table 6. Comparison of reducing sugar between microwave-assisted alkali pretreatment and conventional heating alkali pretreatment

Condition	Reducing Sugar (mg/g)*	
	Pretreatment	Saccharification
Microwave Pretreatment	19.85 ± 0.11 ^a	642.23 ± 21.00 ^a
Conventional Heating (9 min)	10.88 ± 1.25 ^b	440.28 ± 30.00 ^b
Conventional Heating (60 min)	11.11 ± 2.37 ^b	431.06 ± 6.00 ^b

* Means not sharing the same superscript is significantly different according to Tukey’s Honest Significant Difference Test

Discussion

The itchgrass treatments that received the 5% (w/v) NaOH produced significantly higher ($p < 0.05$) reducing sugars after pretreatment and saccharification than the treatments that received the 1% (w/v) NaOH. Similar results were observed in studies that explored other biomasses such as cassava stem (Kamalini *et al.*, 2018), *Camelina sativa* L. (Gupta *et al.*, 2018), and catalpa sawdust (Jin *et al.*, 2016). NaOH works by swelling the alpha-cellulose, thus decreasing its crystallinity and increasing its internal surface area (Sun *et al.*, 2016). Moreover, NaOH also breaks down the amorphous area of the alpha-cellulose (Utoro *et al.*, 2023). Furthermore, NaOH also removes the lignin and hemicellulose content, thus exposing more alpha-cellulose (Sun *et al.*, 2016). Additionally, since NaOH is highly polar, it increases the absorption of microwave energy during the microwave irradiation process, thus producing more hotspots and increasing the heating rate during the reaction (Rodríguez *et al.*, 2015; Shang *et al.*, 2019). This may enhance the reaction rate due to the elevated reaction temperature from the generated heat. Hence, a higher NaOH concentration used during microwave-assisted alkali pretreatment can increase the itchgrass's susceptibility to enzymatic attacks, resulting in higher reducing sugars during saccharification.

The itchgrass treatments exposed to high microwave irradiation power (300 W) produced significantly higher ($p < 0.05$) reducing sugars after pretreatment and saccharification than those exposed to low microwave irradiation power (100 W). Similar observations were found in studies that explored other biomasses, such as cassava stem (Kamalini *et al.*, 2018), kuma sawdust (Sudiana *et al.*, 2017), mango peel waste (Tiwari *et al.*, 2016), and banana fruit peel (Tiwari *et al.*, 2018). This can be attributed to the higher heat generation and blasting of the lignocellulose structure, as more microwave energy is absorbed by the reaction medium, thereby enhancing the delignification process (Ethaib *et al.*, 2020; Sharma *et al.*, 2022). While increasing the microwave irradiation power beyond 300 W may contribute to a higher effect on delignification, this may also negatively affect the pretreatment as higher microwave irradiation power causes faster evaporation of the reaction medium, which leads to the halting of the chemical reaction (Rodrigues *et al.*, 2011) and the scorching of the lignocellulose biomass.

The itchgrass treatments subjected to a long reaction time (9 min) produced significantly higher ($p < 0.05$) reducing sugars after pretreatment and saccharification than those subjected to a short reaction time (1 min). Similar results were observed in studies that explored other biomasses such as cassava stem (Kamalini *et al.*, 2018), banana leaf (Gabhane *et al.*, 2014), mango peel waste (Tiwari *et al.*, 2016), banana fruit peel (Tiwari *et al.*, 2018), and *Camelina*

sativa L. (Gupta *et al.*, 2018). This can be attributed to the longer exposure time to microwave irradiation, which intensifies heat generation, thus enhancing the delignification process (Ethaib *et al.*, 2020). Additionally, since the density of polarity within the lignocellulose biomass changes as moisture evaporates, the hotspots shift in location, covering a larger area for the blasting effect (Aguilar-Reynosa *et al.*, 2017). While prolonging the reaction time beyond 9 min may contribute to a higher effect on delignification, this may also negatively affect the pretreatment, as a longer reaction time may reduce the volume of the reaction medium (Pooja and Pajmada, 2017) necessary for the microwave-assisted alkali pretreatment to continue. Additionally, a longer reaction time under harsh conditions promotes the production of degradation products from sugars (Boonsombuti and Luengnaruemitchai, 2013; Klein *et al.*, 2016).

Among the eight itchgrass treatments, treatment T8 produced the highest reducing sugar after pretreatment (19.85 mg/g raw itchgrass) and saccharification (642.23 mg/g pretreated itchgrass) as it received a high NaOH concentration (5% w/v), exposed to high microwave irradiation power (300 W), and subjected to long reaction time (9 min). The characterization of T8 revealed that 47.83% of the original lignin content and 41.02% of the original hemicellulose content of the raw itchgrass were removed after pretreatment, thereby exposing more alpha-cellulose for enzymatic attacks during the saccharification process. Similar results were observed in several studies that utilized microwave-assisted alkali pretreatment (Janker-Obermeier *et al.*, 2012; Singh *et al.*, 2014; Rubab *et al.*, 2023). Therefore, microwave-assisted alkali pretreatment can effectively remove lignin and hemicellulose from itchgrass, increasing its enzymatic susceptibility in saccharification.

Compared to conventional heating alkali pretreatment, microwave-assisted alkali pretreatment of itchgrass produced significantly higher ($p < 0.05$) reducing sugars, as evidenced in the pretreatment and saccharification hydrolysates. Moreover, increasing the reaction time of the conventional heating pretreatment method to 60 min did not significantly increase ($p > 0.05$) the reducing sugar produced. Similar results were observed in the studies of Jassim *et al.* (2013) and Sudiana *et al.* (2017). This is because, in the conventional heating method, heat is transmitted via conduction and convection from the heat source down to the outermost surface and eventually to the innermost layers of the itchgrass biomass. The heat transfer rate is relatively slow, as it is heavily dependent on the thermal diffusivity of the biomass and the reaction medium; hence, longer reaction time is needed to achieve an acceptable degree of delignification. In contrast, the microwave heating method generates heat from the polar molecules and ions within the biomass and the reaction medium, providing rapid and volumetric heating. Consequently, a shorter reaction time is required to have an acceptable degree of delignification (Saini *et al.*, 2015; Loow

et al., 2016). Therefore, microwave-assisted alkali pretreatment was more effective in delignifying and breaking down the recalcitrant structure of the itchgrass than conventional heating alkali pretreatment. Since the result of the microwave-assisted alkali pretreatment was only compared to the conventional heating alkali pretreatment, future studies should also look into its comparison with other irradiation pretreatment methods to be able to further assess its advantages and disadvantages in terms of delignification, removal of hemicellulose, and exposure of alpha-cellulose.

While microwave-assisted alkali pretreatment of the itchgrass yielded favorable results, the process should be further optimized to get the maximum effect of the pretreatment and recover the most fermentable sugars. Through this, itchgrass, usually deemed as an unwanted weed in croplands, can have additional value as a potential source of fermentable sugars.

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