

Intensification of Hydrolysis Treatment of Waste Plantain Peels Using Ultrasonic Waves for Enhanced Production of Reducing Sugars and Bioethanol

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Abstract

This work focuses on enhancing hydrolysis and bioethanol production from waste plantain peels by using ultrasound acoustic cavitation, comparing its effectiveness to that of hot-acid hydrolysis (HAH) and enzymatic hydrolysis (EH). Statistical response surface methodology (RSM) was employed to examine the enhancement of ultrasonic aided-dilute acid hydrolysis (UADAH) and HAH parameters for the extraction of reducing sugars. The highest reducing sugar yield was obtained as 166.89 ± 0.75 mg/g, 115.03 ± 0.87 mg/g and 131.04 ± 0.29 mg/g from UADAH, HAH and EH respectively. Ultrasound cavitation enhanced the reducing sugar yield with a shorter processing time of 46 min. Notably, the processing time required for UADAH method was reduced threefold and twenty-fold compared to that of HAH and EH, respectively. Also, the UADAH process resulted in a maximum bioethanol yield of 76%. Therefore, ultrasonic irradiation is a promising technology for 2G biofuel production from waste plantain peels owing to its effectiveness in attaining maximum yield within a shorter processing time and with minimal solvent usage.

Keywords

acoustic cavitation, hydrolysis intensification, optimization

1 Introduction

Every inhabitant of this planet relies on renewable energy to meet fuel requirements in a sustainable manner. Renewable energy has become increasingly significant in addressing both the growing energy demand and the environmental pollution caused by the combustion of fossil fuels [1]. This heightened focus on renewable energy has driven the development of alternative technologies for biofuel production [2, 3]. Therefore, it is imperative to develop processes capable of delivering high product yields within a short time frame to enable economically viable scale-up. Biomass, as a renewable resource, has been extensively exploited for biofuel production [4, 5]. Among the various biofuels considered for energy generation, bioethanol appears particularly promising. Its use as an alternative automotive fuel is primarily due to its effectiveness as a substitute or additive for gasoline [6–8].

There has been a greater emphasis in the usage of fruit peels as feedstock for second – generation biofuel production for the past few decades [9]. This is mainly due to the problem associated with shelf - life of processing of fruits

and vegetable waste. India is the largest producer of plantain (*Musa paradisiaca*) being the most prominent fruit crop [10]. The external appearance of plantain is slightly bigger showing resemblance to unripe bananas. The plantains are enriched with starch [11, 12]. The main by-product of banana processing industry is the peel, which represents approximately 30% of the fruit [11, 13]. Due to the presence of large quantities of nitrogen and phosphorus, plantain peel as a by-product poses environmental challenges when improperly disposed of [14]. However, plantain peel holds promise as a bioethanol feedstock owing to its rich lignocellulosic content, offering both an effective waste management solution and a sustainable energy source [1, 5, 15]. Nevertheless, the recalcitrant nature of lignin makes the degradation of lignocellulosic material difficult. As such, a pretreatment step is required prior to its conversion into fermentable sugars [15, 16]. Being a lignocellulosic biomass, plantain peel predominantly comprises a cell wall structure of cellulose, hemicellulose, and lignin [5, 16, 17]. Compared with other agricultural

residues such as sugarcane bagasse and corn stalks, plantain peel contains significantly lower lignin content, which notably shortens the hydrolysis time of lignocellulose and reduces both energy consumption and production costs [11, 18, 19]. Furthermore, the high proportion of glucose and xylose that can be extracted from the cellulose and hemicellulose fractions of plantain peel, coupled with the ability of microorganisms to convert these sugars into ethanol, are key factors driving the utilization of this co-product in biofuel production [17]. In comparison with the conventional fossil fuels and biofuels, the use of waste plantain peel as feedstock for biofuel production is characterized as low cost, low carbon, and environmentally sustainable [20].

Various technologies such as acid hydrolysis, alkali hydrolysis, and steam explosion have been employed for the pretreatment of lignocellulosic biomass [21]. However, due to the high processing cost of enzymes and the economic limitations associated with the use of commercial enzymes for bioethanol production, alternative methods are being explored. In this context, the application of ultrasound has emerged as an effective approach for the fractionation of lignocelluloses for bioethanol production [22–24]. Ultrasonication is an emerging technique capable of intensifying saccharification, achieving higher reducing sugar yields while offering substantial improvements in economic feasibility, reductions in processing time, temperature, chemical usage, and overall energy consumption [25]. Ultrasound induces the formation, growth, and implosive collapse of bubbles in liquids, generating localized regions of high pressure and intense heat within a very short duration [26, 27]. This phenomenon enhances mass transfer, disrupts biomass structure, and facilitates better penetration of acids into the substrate, thereby improving hydrolysis efficiency. The micro-mixing effects induced by ultrasound promote uniform contact between acid and biomass, enhancing reaction kinetics and reducing hydrolysis time. Consequently, this technique can minimize the requirement for high acid concentrations, potentially lowering chemical costs [23]. Several studies have reported the effective application of cavitation in biomass pretreatment and its subsequent role in efficient biofuel production. For instance, Joshi and Gogate [5] and Subhedar et al. [23] demonstrated hydrolysis for fermentable sugar production followed by fermentation, wherein the application of ultrasound resulted in significant process intensification. Ciggin et al. [28] used ultrasound assisted lime pretreatment to reduce the time of lime pretreatment. Previous studies showed that ultrasonication can

be used to intensify the process of biofuel production [28]. However, to best of our knowledge, none of the research has been carried out on utilizing plantain peels for biofuel production using ultrasound waves. Thus, the present study aims to identify the suitable acid for hydrolysis and to intensify the ultrasonic aided-dilute acid hydrolysis (UADAH) process for production of reducing sugar from plantain peel. Three different hydrolysis processes were investigated on reducing sugar production and were optimized using response surface methodology (RSM). The bioethanol yields obtained from different hydrolysis process were investigated.

2 Experimental

2.1 Materials

Fully ripened plantain fruits were collected from local market and peels were removed manually. The peels were cut into small pieces and dried in a hot air oven at 50 °C. The dried peels were ground to fine powder and screened through ASTM 20–40 mesh size (particle size is passing through ASTM-20 mesh and retained in the 40 mesh) to obtain an average particle size range. The sample was stored in an air – tight container for further usage [16].

Sulfuric acid, acetic acid, formic acid and hydrochloric acid, potassium dichromate (analytical grade) was purchased from Sisco Research Laboratories (SRL) Pvt. Ltd, Chennai, India for performing hydrolysis of waste plantain peels. For enzymatic hydrolysis (EH), amyl glucosidase and cellulase enzyme were supplied by Merck Pvt. Ltd, Chennai, India. The different media composition required for Yeast Peptone Dextrose (YPD) medium namely dextrose, peptone and yeast extract were provided by Himedia Pvt. Ltd. in Mumbai, India. The yeast strain *Saccharomyces cerevisiae* was obtained from MTCC Chandigarh - India.

2.2 Experimental procedure

2.2.1 Steam explosion pretreatment

About, 0.25% (v/v) sulfuric acid concentration with 17% solid loading was added in an autoclave. The pressure was maintained at 103421 Pa and temperature of 121 °C for 60 min [29]. After the steam explosion, the sample was cooled down to room temperature and neutralized by continuous washing. The solid residue was filtered from mixture and it was dried in an oven for further treatment.

2.2.2 Selection of acid

The influence of different acids in acid hydrolysis was investigated based on the experiments performed using acetic acid, formic acid, hydrochloric acid and sulfuric

acid. These acids were selected based on their effectiveness in hydrolyzing biomass to release reducing sugars. The different acids were prepared at a concentration of 1% (v/v) and kept constant. Further, the acid which produced maximum yield was employed in hydrolysis treatment.

2.2.3 Hydrolysis process

Enzymatic hydrolysis (EH)

The EH was conducted in two major stages namely liquefaction and saccharification. Plantain peels (10 g) were mixed with 100 mL distilled water and the mixture was treated with 1 $\mu\text{L/g}$ of Termamyl (α -Amylase from *Bacillus licheniformis*) 120 L at 90 °C and pH of 7 for 1 h followed by denaturing process which was done by incubating the mixture at 96 °C for 10 min. The mixture was then cooled at room temperature. The saccharification process was carried out by addition of 2.3 $\mu\text{L/g}$ amyloglucosidase at 50 °C, pH 4.5 for 24 h followed by addition of 4 $\mu\text{L/g}$ cellulase at 55 °C, pH 5.5 for 2 h [30, 31].

Hot-acid hydrolysis (HAH)

HAH was conducted in a magnetic stirrer at different temperature (50, 60, 70 °C), time period (60, 120, 180 h) and solid/solvent ratio (1:5, 1:10, 1:15, g/mL).

Ultrasonic aided-dilute acid hydrolysis (UADAH)

Ultrasonic Processor VCX-750, (Sonics and material Inc, USA) operating at 20 kHz nominal frequency with maximum power of 750 W, was used for this study, with a tip diameter of 13 mm (Ti-6Al-4V). Continuous mode of duty cycle was applied for hydrolysis process. The reaction was carried out in a water-cooled jacketed beaker and cold water was circulated in order to maintain the temperature of the reaction at 60 °C. The schematic experimental set is depicted in Fig. 1. The process parameters taken into consideration for maximizing reducing sugar yields were acid concentration, solid to liquid ratio, amplitude and time.

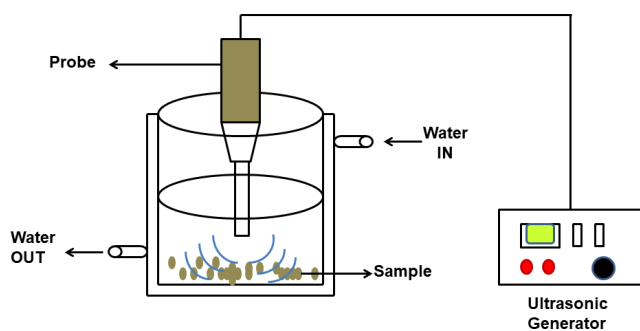


Fig. 1 Schematic representation of the experimental setup

2.2.4 Fermentation

The fermentation studies were performed using baker's yeast (*Saccharomyces cerevisiae*) for ethanol production. Experiments were conducted in a shaking water bath operating at 120 rpm with 5 g/L hydrolysate subjected to 3% yeast culture at 30 °C. pH was fixed at 4 and the samples were withdrawn from fermentation broth at regular time intervals of 12–96 h. The fermentation broth was centrifuged and the supernatant was analyzed quantitatively by potassium dichromate method for the presence of ethanol. 1 mL of cell – free sample and 2 mL of 0.115 M $\text{K}_2\text{Cr}_2\text{O}_7$ was mixed and 9 mL of distilled water was added to the mixture. The mixture was kept in a boiling water bath for 10 min. The samples were cooled and the absorbance was measured at 600 nm using UV–Visible spectrophotometer (Shimadzu, UV-2600). Ethanol yield was calculated as the ratio of ethanol produced in the experiment to the theoretical ethanol.

2.2.5 Analysis

The reducing sugar concentration was analyzed using dinitrosalicylic acid (DNS) assay method [32]. An Elico double beam UV-VIS Spectrophotometer (Model SL-210, Telangana, India) was employed to determine absorbance at 540 nm.

2.2.6 Response surface methodology (RSM)

RSM is a statistical mathematical tool that correlates the multiple independent process variables and dependent responses and also determines the optimum condition for maximum yield [33]. In this study, Design-Expert (version 11.1.2.0, Stat-Ease, Inc., Minneapolis, USA) [34] was used to analyze the regression statistical approach. Box-Behnken design was used for statistical analysis of experimental values obtained in the case of HAH and UADAH. The experimental levels of process variables for hydrolysis were selected based on the preliminary experiments. RSM levels was fixed with four factors, each varied at three levels: Acid concentration – 0.20% v/v (–1), 1.10% v/v (0) and 2% v/v (+1), solvent volume – 5 mL/g (–1), 10 mL/g (0), 15 mL/g (+1), temperature – 50 °C (–1), 60 °C (0), 70 °C (+1) and time – 60 h (–1), 120 h (0), 180 h (+1).

The quadratic polynomial equation for performing the statistical approach of experimental values is given below [33]:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1 X_1 + \beta_{22} X_2 X_2 + \beta_{33} X_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \dots \quad (1)$$

Where Y is dependent variable (response), β_0 is the intercept coefficient of model, $\beta_1, \beta_2, \beta_3$ are linear coefficient, $\beta_{11}, \beta_{22}, \beta_{33}$ are quadratic coefficient, $\beta_{12}, \beta_{13}, \beta_{23}$ are interaction coefficients, X_1, X_2, X_3 are independent variables [35].

3 Results and analysis

3.1 Chemical composition analysis

The sample was subjected to steam explosion pre-treatment in order to reduce the lignin content present in the plantain peels. The results illustrated in Fig. 2 confirmed that plantain peels are rich in cellulose/hemicelluloses compared to lignin content and therefore can be converted to reducing sugar. The values thus obtained were closely in agreement with the work done by Igbokwe et al. [36]. Also, Ighodaro [13] reported that the cellulose content of plantain peels before treatment was 35.36% while that of plantain peels after treatment was 44.88%.

3.2 Selection of acid for hydrolysis

The efficiency of various acids like acetic acid, formic acid, hydrochloric acid, sulfuric acid and propanoic acid on reducing sugar yield was studied under constant experimental conditions - acid concentration 1% v/v, solid/liquid ratio 1:10, temperature 60 °C and time 60 min and the results are shown in Fig. 3. The results revealed that the use of sulfuric acid resulted in 63.12 mg/g reducing sugar yield, acetic acid and formic acid produced lower yield of 32.18 and 36.29 mg/g, respectively. Propanoic acid and hydrochloric acid yielded 50.25 and 49.73 mg/g, respectively. Therefore, sulfuric acid was used for further HAH and UADAH processes.

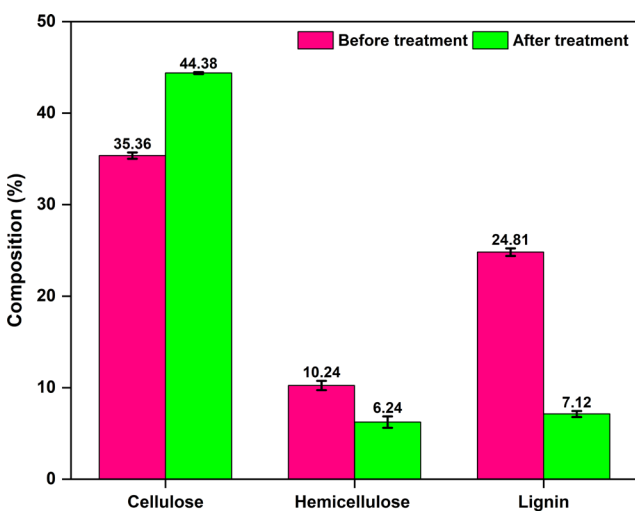


Fig. 2 Chemical composition analysis of plantain peels before and after steam treatment

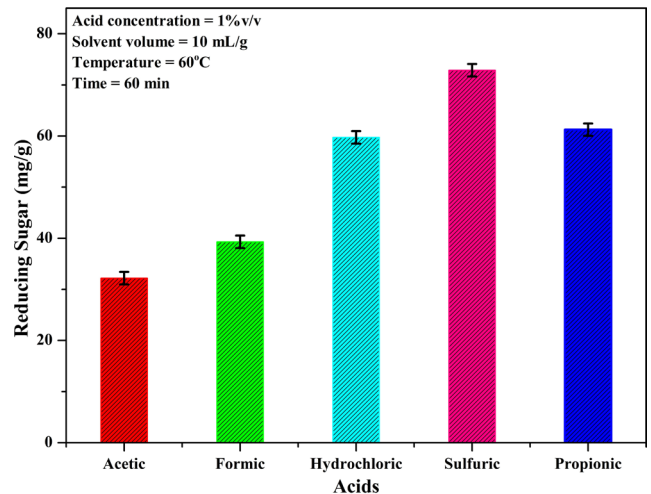


Fig. 3 Comparison of acid efficiency for reducing sugar production

3.3 Ultrasonic aided-dilute acid hydrolysis (UADAH)

3.3.1 Influence of UADAH variables on reducing sugar

The effect of UADAH process variables on the reducing sugar was investigated and results are depicted in Fig. 3. It can be seen that, on increasing the acid concentration from 0.2 to 1.1% (v/v), the reducing sugar yield elevated from 126.40 mg/g to 166.37 mg/g (shown in Fig. 4 (a)) as increasing the concentration led to faster initial conversation rate and high reducing sugar production up to 1.1%. On the contrary, when loading was further increased to 2% (v/v), the reducing sugar slightly decreased probably owing to the corrosive nature of acid that caused further degradation of monosaccharides to furfurals and 5-hydroxymethyl furfurals which turn out to be fermentation inhibitors [37].

The effect of solvent volume added to per gram of sample was studied by varying the ratio over the range of 1:5 to 1:15 g/mL. Increasing the solid/liquid ratio from 1:5 to 1:10 g/mL resulted in higher production (166.37 mg/g) of reducing sugar (Fig. 4 (c)). This could be attributed to the increase in viscosity and dampening effect of ultrasonic energy due to higher amount of solids present in the same processed liquid [5].

Solid/liquid ratios above 1:10 g/L showed no significant change in reducing sugar yield which might be because of the attainment of saturation point beyond which there could be no solid solvent interaction. The impact of higher amplitude of ultrasound treatment has both advantages and disadvantages of its own (shown in Fig. 4 (b), (c)). While increasing the amplitude from 70 to 82%, disruption of the strong solute matrix interactions and active sites on the biomass matrix leads to faster rate of diffusion [38, 39]. This corresponds to the increasing trend of reducing sugar recovery of 166.37 mg/g (shown in Fig. 4 (b), (c)).

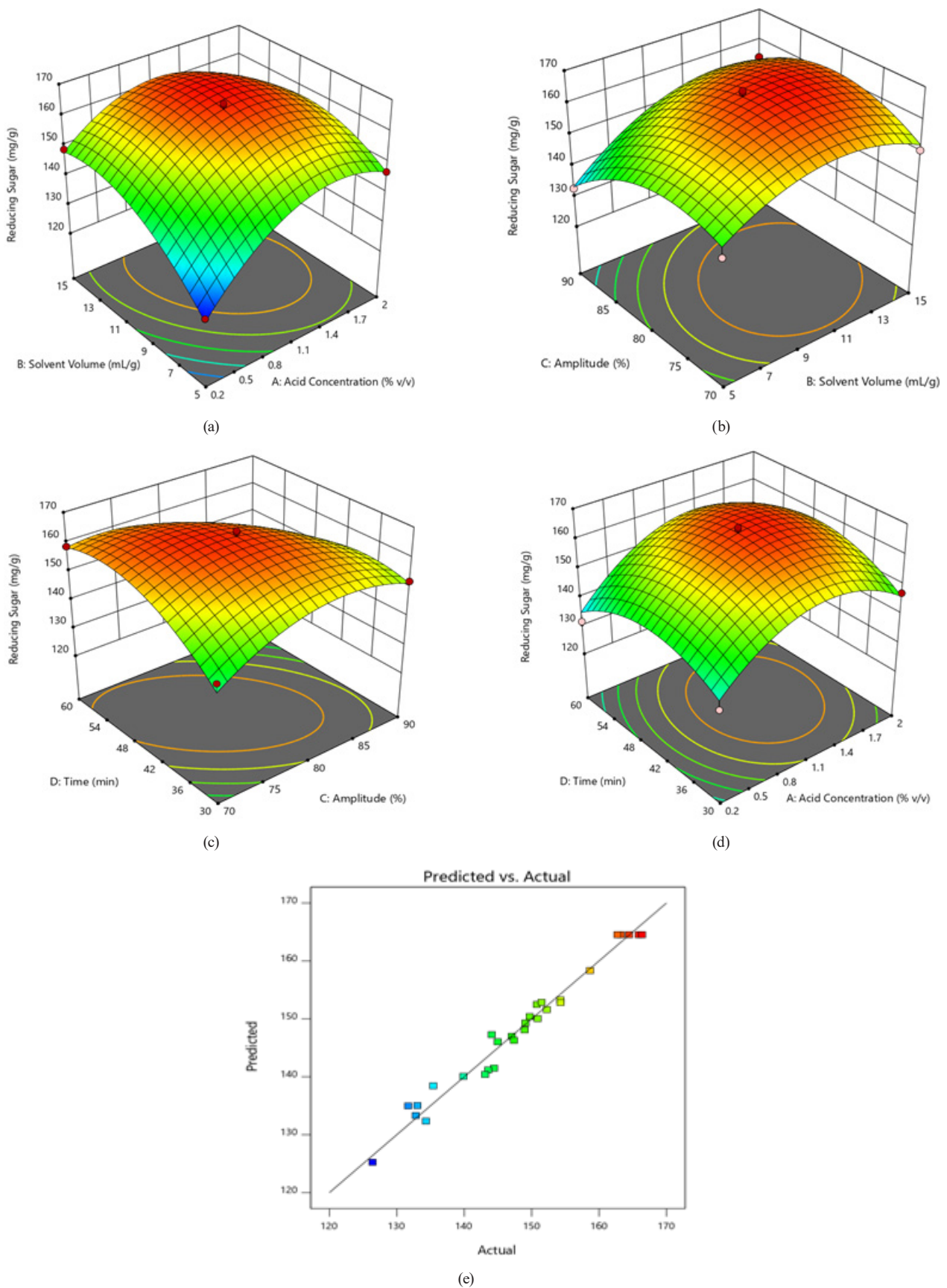


Fig. 4 Influence of UADAH process variables on reducing sugar production (a) Interactive effect of acid concentration and solvent volume, (b) Interactive effect of solvent volume and amplitude, (c) Interactive effect of amplitude and time, (d) Interactive effect of acid concentration and time, (e) Predicted vs. Actual values of the model fit

On the other hand, at higher amplitudes (above 82%) and a constant power of ultrasonic irradiation, the cavitation intensity decreases. This correlates to the fact that as the amplitude increases; the vapor pressure of the solvent also increases [40]. Hence vaporous, cavitation bubbles are generated, giving less intense bubble, which collapses [41]. Therefore, desired sonication effects are reduced which leads to reduction in yield (shown in Fig. 4 (b), (c)).

The conversion rate of reducing sugar increased from 126.40 mg/g to 166.37 mg/g as the acid treatment time increased from 30 min to 45 min (Fig. 4 (d)). After 45 min the reducing sugar content decreased to 131.68 mg/g. This might be due to longer period of sonication that causes its decomposition and structural changes. Harun et al. [42] reported that the production of reducing sugars from water

hyacinth biomass improved with an increase in ultrasonic treatment time to 30 min. Wang and Zhang [43] studied the effect of ultrasonic treatment on the recovery of xylan from a corncob biomass suspended in an alkaline solution. The rate of recovery of xylan was reported to increase with an increase in time until 30 min; however, no enhancement was observed beyond 30 min. The experimental results are summarized in Table 1.

3.3.2 Model fitting

Pareto analysis of variance (ANOVA) was used to investigate the adequacy of statistical model. The significance of model and each independent variable on the response was verified using *F*-test and *p*-value, with all associated results are given in Table 2 and also the developed

Table 1 Experimental values of UADAH

S. No	Acid concentration (% v/v)	Solvent volume (mL/g)	Amplitude (%)	Time (min)	Reducing sugar (mg/g)	
					Exp	Pre
1	1.1 (0)	15.0 (+1)	90 (+1)	45 (0)	154.27	153.28
2	1.1 (0)	5.0 (-1)	70 (-1)	45 (0)	144.08	147.28
3	1.1 (0)	15.0 (+1)	80 (0)	30 (-1)	151.46	152.86
4	1.1 (0)	10.0 (0)	90 (+1)	60 (+1)	133.05	135.05
5	1.1 (0)	10.0 (0)	80 (0)	45 (0)	164.45	164.53
6	0.2 (-1)	10.0 (0)	80 (0)	60 (+1)	131.68	134.98
7	0.2 (-1)	10.0 (0)	90 (+1)	45 (0)	134.32	132.35
8	2.0 (+1)	10.0 (0)	70 (-1)	45 (0)	149.71	150.40
9	2.0 (+1)	10.0 (0)	80 (0)	60 (+1)	150.89	150.06
10	1.1 (0)	10.0 (0)	90 (+1)	30 (-1)	152.24	151.62
11	2.0 (+1)	5.0 (-1)	80 (0)	45 (0)	147.05	146.98
12	0.2 (-1)	15.0 (+1)	80 (0)	45 (0)	148.96	148.10
13	1.1 (0)	10.0 (0)	80 (0)	45 (0)	162.37	164.53
14	1.1 (0)	5.0 (-1)	90 (+1)	45 (0)	132.81	133.31
15	1.1 (0)	15.0 (+1)	80 (0)	60 (+1)	154.29	152.81
16	1.1 (0)	10.0 (0)	70 (-1)	60 (+1)	158.66	158.35
17	0.2 (-1)	10.0 (0)	70 (-1)	45 (0)	143.55	141.18
18	2.0 (+1)	15.0 (+1)	80 (0)	45 (0)	149.08	149.30
19	0.2 (-1)	5.0 (-1)	80 (0)	45 (0)	126.40	125.25
20	2.0 (+1)	10.0 (0)	80 (0)	30 (-1)	147.38	146.29
21	1.1 (0)	10.0 (0)	70 (-1)	30 (-1)	144.42	141.49
22	1.1 (0)	10.0 (0)	80 (0)	45 (0)	165.89	164.53
23	1.1 (0)	10.0 (0)	80 (0)	45 (0)	163.22	164.53
24	2.0 (+1)	10.0 (0)	90 (+1)	45 (0)	144.96	146.05
25	1.1 (0)	15.0 (+1)	70 (-1)	45 (0)	150.78	152.49
26	1.1 (0)	10.0 (0)	80 (0)	45 (0)	166.37	164.53
27	1.1 (0)	5.0 (-1)	80 (0)	60 (+1)	143.09	140.41
28	1.1 (0)	5.0 (-1)	80 (0)	30 (-1)	139.88	140.08
29	0.2 (-1)	10.0 (0)	80 (0)	30 (-1)	135.40	138.45

Table 2 Analysis of variance (ANOVA)

Source	Sum of squares	Coefficient estimate	DF	Mean square	F-value	p-value Prob > F
Model	3217.54	164.53	14	229.82	38.16	< 0.0001
X_1	393.99	5.73	1	393.99	65.42	< 0.0001
X_2	475.40	6.29	1	475.40	78.94	< 0.0001
X_3	130.35	-3.30	1	130.35	21.64	0.0004
X_4	0.065	0.073	1	0.065	0.011	0.9190
$X_1 X_2$	105.37	-5.13	1	105.37	17.50	0.0009
$X_1 X_3$	5.02	1.12	1	5.02	0.83	0.3768
$X_1 X_4$	13.07	1.81	1	13.07	2.17	0.1629
$X_2 X_3$	54.46	3.69	1	54.46	9.04	0.0094
$X_2 X_4$	0.036	-0.095	1	0.036	0.005	0.9394
$X_3 X_4$	279.39	-8.36	1	279.39	46.39	< 0.0001
X_{12}	1114.95	-13.11	1	1114.95	185.13	< 0.0001
X_{22}	527.08	-9.01	1	527.08	87.52	< 0.0001
X_{32}	516.90	-8.93	1	516.90	85.83	< 0.0001
X_{42}	522.85	-8.96	1	522.85	86.82	< 0.0001
Residual	84.31		14	6.02		
Lack of Fit	74.12		10	7.41	2.91	0.1577
Pure error	10.20		4	2.55		
Std. Dev.		2.45		R^2		0.9745
Mean		147.97		Adj. R^2		0.9489
C.V%		1.66		Pred. R^2		0.8659
PRESS		442.85		Adep. Pre.		22.25

DF: degree of freedom, PRESS: prediction error sum of squares

model was significant with experimental values which is depicted in Fig. 4 (e). The p -value evaluation was carried out to find the significance of model coefficients, individual and interacting terms on the response. The higher significant variables had less p -value. The p -value less than 0.0001 showed that the predicted model was highly significant. The R^2 value of 0.9745 when closer to 1, indicates the better correlation between the predicted model and experimental results. It also demonstrates the accuracy of the developed model [44]. The adjusted R^2 (0.9489) and predicted R^2 (0.8659) indicated the adequacy of model. The adequate precision is the measure of signal-to-noise ratio and when it is higher than 4, it is said to be desirable [45]. The results of present study were found to be 22.25, which showed that the signal is adequate.

The coefficient of variation (C.V%) indicates the average value of residual variation and higher the C.V% values have lowered the reliability [30]. The C.V% value of present study, 1.66%, shows high reliability of the experiments. The "Lack of Fit" p -value 0.1577 indicates insignificant term. There was only 0.1577% chance that it might exist due to noise.

3.3.3 Optimization

The UADAH optimum condition was determined using numerical optimization in order to obtain maximum yield. It was found that, acid concentration of 1.2%, solvent volume of 12 mL/g, amplitude of 80% and time of 46 min resulted in maximum yield. Under optimum condition, the predicted reducing sugar yield was 167.12 mg/g. To validate the predicted value, experiments were carried out in triplicate and the yield obtained was 166.89 ± 0.75 mg/g. It can be observed that the experimental yield thus obtained (166.89 ± 0.75 mg/g) was in close agreement with statistically predicted value.

3.4 Comparison of hydrolysis process

The UADAH process was compared to HAH and EH for maximum reducing sugar yield and the results are given in Fig. 5. The UADAH process yielded 166.89 ± 0.75 mg/g, HAH process gave 115.03 ± 0.87 mg/g and EH produced 131.04 ± 0.29 mg/g of reducing sugar yield. The UADAH process showed a maximum yield compared to other methods (EH and HAH), this could be attributed to the disruption of polymeric structure and the polysaccharides

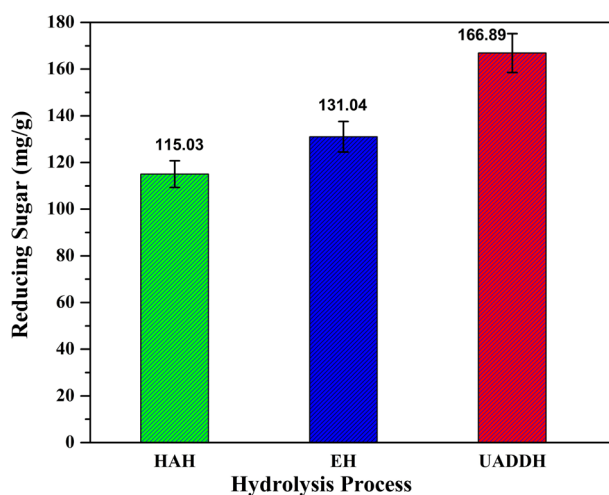


Fig. 5 Comparison of effectiveness of UADAH process with HAH and EH

glycosidic bonds present in the plantain peel via acid hydrolysis as well as ultrasonic cavitation. The micro-bubbles formed due to ultrasonic waves allowed more accessibility of solvent thus favoring the hydrolysis process [45, 46]. In addition, destruction of rigid protein matrix (hydrophobic) and amylose-lipid complex surrounding granules via acoustic cavitation enhanced the productivity [47]. Hence, ultrasonic cavitation helps in enriching the activity of catalyst and lowering the limiting barrier of diffusion around the substrate which leads to intensification of hydrolysis rate of reaction [23].

Thus, reduction in process time compared to HAH and EH. It is important to note that processing time of UADAH process was 3 folds lower than that of HAH and more than 20 folds than that of EH. When consider about cost implication of EH and HAH processes could impact negatively due their lower yield as well as longer processing time on the basis of techno economic analysis. The current study results show that reduction of hydrolysis processing time by UADAH which is significant result noticing in economical point of view and helps in energy saving as well. The result of present study was in agreement with previous studies using sugarcane bagasse, spent citronella [48], sweet lime peel [1] and rice hull biomass hydrolyzed by sonication assisted process.

3.5 Fermentation

Fermentation was carried out using hydrolysate obtained from UADAH, HAH and EH at optimum condition. The ethanol production started from 12 h of fermentation process and ethanol yield was calculated for every 12 h of the process, the results are illustrated in Fig. 6. Fermentation using *S. cerevisiae* at a pH lower than 4,

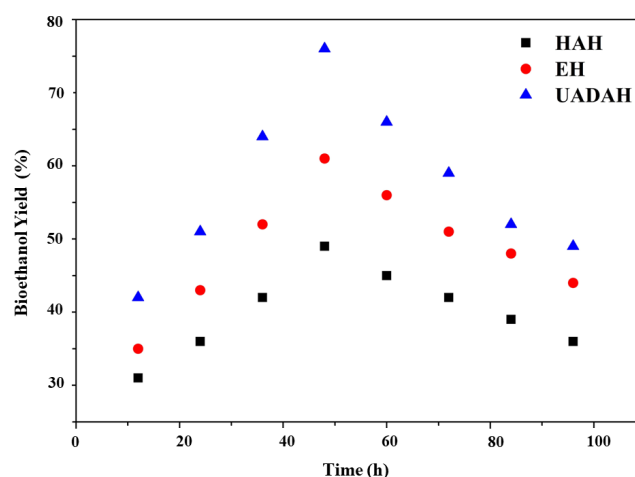


Fig. 6 Bioethanol yield on different hydrolysis process

produced acetic acid while pH higher than 5 resulted in butyric acid during bioethanol production process. John et al. [29] also reported that maximum bioethanol production occurred at pH 4. So, in the current study, fermentation process was carried out at pH 4. Bioethanol yield increased up to a fermentation period of 48 h and then it started to decline. This could be due to the reaction of alcoholic groups with acetic acid and other by-products of fermentation process. The maximum bio – ethanol yield of 76%, 49% and 61% was obtained from UADAH, HAH and EH respectively, with fermentation duration of 48 h and pH 4 as shown in Fig. 6. Velmurugan and Muthukumar [25] stated that generation of fermentation inhibitors noticeably decreased in UADAH treatment. This could be the reason for the maximum yield obtained from UADAH. The present study bioethanol yield was compared with other sources such as 35.86% from mixed fruit sample, 26.50% from mango pulp and 28.45% from banana pulp [49] and found that higher yield (76%) was obtained from plantain peel.

4 Conclusion

An in-depth study into hydrolysis of waste plantain peel biomass has been carried out to obtain maximum reducing sugar and bio – ethanol yield. For acid hydrolysis, five different acids have been investigated, and sulfuric acid was found to release maximum reducing sugar and further, HAH and UADAH were carried out with sulfuric acid. UADAH process resulted in the highest yield of 166.89 ± 0.75 mg/g under the optimum condition of 1.2% acid concentration, 12 mL/g solvent volume, 80% amplitude and 46 min processing time. The bio-ethanol yield was obtained as 76%, 49% and 61% from UADAH, HAH

and EH respectively. Hence, in the present study, it is concluded that ultrasonic cavitation assisted acid hydrolysis

intensified the reducing sugar yield and bioethanol production in less processing time.

References

- [1] John, I., Pola, J., Appusamy, A. "Optimization of Ultrasonic Assisted Saccharification of Sweet Lime Peel for Bioethanol Production Using Box–Behnken Method", *Waste and Biomass Valorization*, 10(2), pp. 441–453, 2019.
<https://doi.org/10.1007/s12649-017-0072-1>
- [2] Mgeni, S. T., Mtashobya, L. A., Emmanuel, J. K. "Bioethanol production from fruit wastes juice using millet and sorghum as additional fermentable sugar", *Cleaner Energy Systems*, 10, 100177, 2025.
<https://doi.org/10.1016/j.cles.2025.100177>
- [3] Srinivasan, S., Venkatachalam, S. "An energy-efficient process for enhanced production of bioethanol from sorghum biomass: a futuristic approach towards circular economy", *Biomass Conversion and Biorefinery*, 15(1), pp. 1581–1592, 2025.
<https://doi.org/10.1007/s13399-024-06083-2>
- [4] Owusu, P. A., Asumadu-Sarkodie, S. "A review of renewable energy sources, sustainability issues and climate change mitigation", *Cogent Engineering*, 3(1), 1167990, 2016.
<https://doi.org/10.1080/23311916.2016.1167990>
- [5] Joshi, S. M., Gogate, P. R. "Intensification of dilute acid hydrolysis of spent tea powder using ultrasound for enhanced production of reducing sugars", *Ultrasonics Sonochemistry*, 61, 104843, 2020.
<https://doi.org/10.1016/j.ultsonch.2019.104843>
- [6] Raina, N., Boonmee, R., Kirdponpattara, S., Narasingha, M., Sriariyanun, M., Phitsuwan, P., Chuetor, S. "Process performance evaluation of different chemical pretreatments of lignocellulosic biomass for bioethanol production", *Industrial Crops and Products*, 211, 118207, 2024.
<https://doi.org/10.1016/j.indcrop.2024.118207>
- [7] Bušić, A., Mardetko, N., Kundas, S., Morzak, G., Belskaya, H., Ivančić Šantek, M., Komes, D., Novak, S., Šantek, B. "Bioethanol Production from Renewable Raw Materials and Its Separation and Purification: A Review", *Food Technology and Biotechnology*, 56(3), pp. 289–311, 2018.
<https://doi.org/10.17113/ftb.56.03.18.5546>
- [8] Krishnan, S., Ahmad, M. F., Zainuddin, N. A., Din, M. F. M., Rezaia, S., Chelliapan, S., Taib, S. M., Nasrullah, M., Wahid, Z. A. "Chapter 9 - Bioethanol production from lignocellulosic biomass (water hyacinth): a biofuel alternative", In: Singh, L., Yousuf, A., Mahapatra, D. M. (eds.) *Bioreactors: Sustainable Design and Industrial Applications in Mitigation of GH Emissions*, Elsevier, 2020, pp. 123–143. ISBN 978-0-12-821264-6
<https://doi.org/10.1016/B978-0-12-821264-6.00009-7>
- [9] Kasaeian, A., Fereidooni, L., Ahmadbeigi, A., Kahedi, S., Koohshoori, M. S., Ghafarian, S., Tajmoussavilangerudi, A., Salaripoor, H. "Review on Bioethanol Production from Fruit Peels", *Waste and Biomass Valorization*, 2025.
<https://doi.org/10.1007/s12649-025-02938-5>
- [10] Padam, B. S., Tin, H. S., Chye, F. Y., Abdullah, M. I. "Banana by-products: an under-utilized renewable food biomass with great potential", *Journal of Food Science and Technology*, 51(12), pp. 3527–3545, 2014.
<https://doi.org/10.1007/s13197-012-0861-2>
- [11] Happi Emaga, T., Andrianaivo, R. H., Wathelet, B., Tchango, T. J., Paquot, M. "Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels", *Food Chemistry*, 103(2), pp. 590–600, 2007.
<https://doi.org/10.1016/j.foodchem.2006.09.006>
- [12] Arun, K. B., Persia, F., Aswathy, P. S., Chandran, J., Sajeev, M. S., Jayamurthy, P., Nisha, P. "Plantain peel - a potential source of antioxidant dietary fibre for developing functional cookies", *Journal of Food Science and Technology*, 52(10), pp. 6355–6364, 2015.
<https://doi.org/10.1007/s13197-015-1727-1>
- [13] Ighodaro, O. M. "Evaluation Study of Nigerian Species of *Musa paradisiaca* Peels: Phytochemical screening, Proximate analysis, Mineral composition and Antimicrobial activities", *Researcher*, 4(8), pp. 17–20, 2012.
<https://doi.org/10.7537/marsrsj040812.04>
- [14] Tchobanoglous, G., Theisen, H., Vigil, S. "Integrated solid waste management: Engineering principals and management issues", McGraw-Hill, Inc., 1993. ISBN 978-0-07-063237-0
- [15] Vyas, P., Kumar, A., Singh, S. "Biomass breakdown: A review on pretreatment, instrumentations and methods", *Frontiers in Bioscience-Elite (FBE)*, 10(1), pp. 155–174, 2018.
<https://doi.org/10.2741/E815>
- [16] Tiwari, G., Sharma, A., Kumar, A., Sharma, S. "Assessment of microwave-assisted alkali pretreatment for the production of sugars from banana fruit peel waste", *Biofuels*, 10(1), pp. 3–10, 2019.
<https://doi.org/10.1080/17597269.2018.1442665>
- [17] Sharma, P., Sharma, N. "RSM approach to pre-treatment of lignocellulosic waste and a statistical methodology for optimizing bioethanol production", *Waste Management Bulletin*, 2(1), pp. 49–66, 2024.
<https://doi.org/10.1016/j.wmb.2023.12.004>
- [18] Yelne, M., Kininge, M. M., Gogate, P. R. "Ultrasound-Assisted Intensified Delignification of Sugarcane Bagasse in the Presence of Metal Salts and Hydrogen Peroxide", *Waste and Biomass Valorization*, 15(8), pp. 4699–4715, 2024.
<https://doi.org/10.1007/s12649-024-02466-8>
- [19] Guo, J., Li, Z., Su, L., Tsang, Y.-F., An, A. K., Yu, C.-F. "Optimization of acid pretreatment and enzymatic hydrolysis on the production of ethanol fuel from waste banana peels", *Energy & Environment*, 29(8), pp. 1354–1364, 2018.
<https://doi.org/10.1177%2F0958305X18777232>
- [20] Kitson-Hytesy, M., Fei-Baffoe, B., Sackey, L. N. A., Miezah, K. "Production of bioethanol from plantain and yam peels using *Aspergillus niger* and *Saccharomyces cerevisiae*", *Biomass Conversion and Biorefinery*, 14(8), pp. 9087–9095, 2024.
<https://doi.org/10.1007/s13399-022-03352-w>
- [21] Son, H., Lee, J., Yoo, H. Y. "Improved Sugar Recovery from Mandarin Peel under Optimal Enzymatic Hydrolysis Conditions and Application to Bioethanol Production", *Processes*, 12(9), 1960, 2024.
<https://doi.org/10.3390/pr12091960>

- [22] Kyriakoudi, A., Kalfa, E., Zymvrakaki, E., Kalogiouri, N., Mourtzinou, I. "Recovery of Ellagic Acid from Pomegranate Peels with the Aid of Ultrasound-Assisted Alkaline Hydrolysis", *Molecules*, 29(11), 2424, 2024.
<https://doi.org/10.3390/molecules29112424>
- [23] Subhedar, P. B., Ray, P., Gogate, P. R. "Intensification of delignification and subsequent hydrolysis for the fermentable sugar production from lignocellulosic biomass using ultrasonic irradiation", *Ultrasonic Sonochemistry*, 40, pp. 140–150, 2018.
<https://doi.org/10.1016/j.ultsonch.2017.01.030>
- [24] Kucharska, K., Rybarczyk, P., Hołowacz, I., Łukajtis, R., Glinka, M., Kamiński, M. "Pretreatment of Lignocellulosic Materials as Substrates for Fermentation Processes", *Molecules*, 23(11), 2937, 2018.
<https://doi.org/10.3390/molecules23112937>
- [25] Velmurugan, R., Muthukumar, K. "Utilization of sugarcane bagasse for bioethanol production: Sono-assisted acid hydrolysis approach", *Bioresource Technology*, 102(14), pp. 7119–7123, 2011.
<https://doi.org/10.1016/j.biortech.2011.04.045>
- [26] Senrayan, J., Venkatachalam, S. "Ultrasonic acoustic-cavitation as a novel and emerging energy efficient technique for oil extraction from kapok seeds", *Innovative Food Science & Emerging Technologies*, 62, 102347, 2020.
<https://doi.org/10.1016/j.ifset.2020.102347>
- [27] Villa-Vélez, H. A., Cornelio, M. L., Corrêa, J. L. G., Telis-Romero, J. "Identification of acoustic fields in aqueous biomass solutions of banana waste pretreated by power ultrasound", *Biomass Conversion and Biorefinery*, 8(1), pp. 87–96, 2018.
<https://doi.org/10.1007/s13399-017-0256-8>
- [28] Ciggin, A. S., Yılmaz, F., Perendeci, N. A. "Efficient environmentally friendly enzymatic and ultrasonic pretreatment of lignocellulosic wastes for enhanced methane production", *Biomass Conversion and Biorefinery*, 14(19), pp. 24021–24039, 2024.
<https://doi.org/10.1007/s13399-023-04629-4>
- [29] John, I., Yaragarla, P., Muthaiah, P., Ponnusamy, K., Appusamy, A. "Statistical optimization of acid catalyzed steam pretreatment of citrus peel waste for bioethanol production", *Resource-Efficient Technologies*, 3(4), pp. 429–433, 2017.
<https://doi.org/10.1016/j.refit.2017.04.001>
- [30] Aruwajoye, G. S., Faloye, F. D., Kana, E. G. "Soaking assisted thermal pretreatment of cassava peels wastes for fermentable sugar production: Process modelling and optimization", *Energy Conversion and Management*, 150, pp. 558–566, 2017.
<https://doi.org/10.1016/j.enconman.2017.08.046>
- [31] Noparat, P., Prasertsan, P., O-Thong, S., Pan, X. "Dilute acid pretreatment of oil palm trunk biomass at high temperature for enzymatic hydrolysis", *Energy Procedia*, 79, pp. 924–929, 2015.
<https://doi.org/10.1016/j.egypro.2015.11.588>
- [32] Miller, G. L. "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar", *Analytical Chemistry*, 31(3), pp. 426–428, 1959.
<https://doi.org/10.1021/ac60147a030>
- [33] Senrayan, J., Venkatachalam, S. "A short extraction time of vegetable oil from *Carica papaya* L. seeds using continuous ultrasound acoustic cavitation: Analysis of fatty acid profile and thermal behavior", *Journal of Food Process Engineering*, 42(1), e12950, 2019.
<https://doi.org/10.1111/jfpe.12950>
- [34] Stat-Ease, Inc. "Design-Expert, (version 11.1.2.0)", [computer program] Available at: <https://www.statease.com/software/design-expert/> [Accessed: 15 August 2024]
- [35] Şenol, H., Erşan, M., Görgün, E. "Optimization of temperature and pretreatments for methane yield of hazelnut shells using the response surface methodology", *Fuel*, 271, 117585, 2020.
<https://doi.org/10.1016/j.fuel.2020.117585>
- [36] Igbokwe, P. K., Idogw, C. N., Nwabanne, J. T. "Enzymatic Hydrolysis and Fermentation of Plantain Peels: Optimization and Kinetic Studies", *Advances in Chemical Engineering and Sciences*, 6(2), pp. 216–235, 2016.
<https://doi.org/10.4236/aces.2016.62023>
- [37] Loow, Y.-L., Wu, T. Y., Md. Jahim, J., Mohammad, A. W., Teoh, W. H. "Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline pretreatment", *Cellulose*, 23(3), pp. 1491–1520, 2016.
<https://doi.org/10.1007/s10570-016-0936-8>
- [38] Teke, G. M., De Vos, L., Smith, I., Kleyn, T., Mapholi, Z. "Development of an ultrasound-assisted pre-treatment strategy for the extraction of D-Limonene toward the production of bioethanol from citrus peel waste (CPW)", *Bioprocess and Biosystems Engineering*, 46(11), pp. 1627–1637, 2023.
<https://doi.org/10.1007/s00449-023-02924-y>
- [39] da Silva, A. S., Espinheira, R. P., Teixeira, R. S. S., de Souza, M. F., Ferreira-Leitão, V., Bon, E. P. S. "Constraints and advances in high-solids enzymatic hydrolysis of lignocellulosic biomass: a critical review", *Biotechnology for Biofuels and Bioproducts*, 13(1), 58, 2020.
<https://doi.org/10.1186/s13068-020-01697-w>
- [40] Utekar, P. G., Kininge, M. M., Gogate, P. R. "Intensification of delignification and enzymatic hydrolysis of orange peel waste using ultrasound for enhanced fermentable sugar production", *Chemical Engineering and Processing - Process Intensification*, 168, 108556, 2021.
<https://doi.org/10.1016/j.cep.2021.108556>
- [41] Naveena, B., Armshaw, P., Tony Pembroke, J. "Ultrasonic intensification as a tool for enhanced microbial biofuel yields", *Biotechnology for Biofuels and Bioproducts*, 8(1), 140, 2015.
<https://doi.org/10.1186/s13068-015-0321-0>
- [42] Harun, M. Y., Dayang Radiah, A. B., Zainal Abidin, Z., Yunus, R. "Effect of physical pretreatment on dilute acid hydrolysis of water hyacinth (*Eichhornia crassipes*)", *Bioresource Technology*, 102(8), pp. 5193–5199, 2011.
<https://doi.org/10.1016/j.biortech.2011.02.001>
- [43] Wang, Y., Zhang, J. "A novel hybrid process, enhanced by ultrasonication for xylan extraction from corncobs and hydrolysis of xylan to xylose by xylanase", *Journal of Food Engineering*, 77(1), pp. 140–145, 2006.
<https://doi.org/10.1016/j.jfoodeng.2005.06.056>
- [44] Niju, S., Nishanthini, T., Balajii, M. "Alkaline hydrogen peroxide-pretreated sugarcane tops for bioethanol production—a process optimization study", *Biomass Conversion and Biorefinery*, 10(1), pp. 149–165, 2020.
<https://doi.org/10.1007/s13399-019-00524-z>
- [45] Safari, M., Abdi, R., Adl, M., Kafashan, J. "Optimization of biogas productivity in lab-scale by response surface methodology", *Renewable Energy*, 118, pp. 368–375, 2018.
<https://doi.org/10.1016/j.renene.2017.11.025>

- [46] Mello, L. R. P. F., Mali, S. "A combination of chemical and physical pretreatments in the saccharification of malt bagasse: the effects of ultrasonication in diluted acid medium", *Biomass Conversion and Biorefinery*, 12(12), pp. 5493–5504, 2022.
<https://doi.org/10.1007/s13399-020-01106-0>
- [47] Shewale, S. D., Pandit, A. B. "Enzymatic production of glucose from different qualities of grain sorghum and application of ultrasound to enhance the yield", *Carbohydrate Research*, 344(1), pp. 52–60, 2009.
<https://doi.org/10.1016/j.carres.2008.10.006>
- [48] Timung, R., Naik Deshavath, N., Goud, V. V., Dasu, V. V. "Effect of Subsequent Dilute Acid and Enzymatic Hydrolysis on Reducing Sugar Production from Sugarcane Bagasse and Spent Citronella Biomass", *Journal of Energy*, 2016(1), 8506214, 2016.
<https://doi.org/10.1155/2016/8506214>
- [49] Arumugam, R., Manikandan, M. "Fermentation of Pretreated Hydrolyzates of Banana and Mango Fruit Wastes for Ethanol Production", *Asian Journal of Experimental Biological Sciences*, 2(2), pp. 246–256, 2011.