IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

ISO 3297:2007 Certified ⁙ Impact Factor 8.066 ⁙ Peer-reviewed / Refereed journal ⁙ Vol. 10, Issue 7, July 2023

DOI: 10.17148/IARJSET.2023.10741

Enhancement of Reducing Sugar Content in *Artocarpus heterophyllus* (jackfruit) Seed Through Dilute Acid Pretreatment

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Abstract: The effect of varied concentrations of dilute acid on the reducing sugar content of jackfruit seeds (JFS) and its potential as a raw material for bioethanol production was investigated in this study. Raw JFS is known to be rich in starch, which can be converted into fermentable sugar using dilute acid hydrolysis by utilising microbial activity. The reducing sugar concentration of the native and pre-treated biomass sample was determined by the 3,5-dinitro salicylic acid using a UV-VIS spectrophotometer.

Dilute acid pretreatment (DAP) was carried out at varied acid concentrations (1 – 5%, v/v) and solid biomass loading of 10% w/v at a constant temperature of 121℃ for 15, 30, and 45 minutes. After pretreatment, the highest reducing sugar yield (2.01 ± 0.03 mg/g) was obtained at 1% (v/v) hydrolysed for 15 mins. There was a 57.03% increase in reducing sugar yield after pretreatment with dilute acid.

Keywords: Bioethanol, pretreatment, reducing sugars *Artocarpus heterophyllus*.

I. INTRODUCTION

Bioethanol as an alternative fuel has received much attention recently due to the dwindling fossil fuel reserve [1]. Bioethanol is produced through sugar fermentation of carbohydrate sources such as starch and cellulose in an anaerobic environment by utilising microbial enzymes. The feedstocks for bioethanol production can be categorized into three main groups: sugar, starch, and cellulose [2]. Sugar-rich feedstocks can be directly converted to bioethanol by the fermentation process while the feedstocks containing starch/cellulose must be hydrolysed for the subsequent fermentation process [3].

The hemicellulose and lignin fraction forms a protective sheath that encloses the cellulose, preventing enzymatic breakdown of cellulose to release reducing sugars [4]. Therefore, the conversion of cellulosic biomass requires chemical pretreatment using dilute acids to break the recalcitrance of cellulosic biomass, enabling easier enzyme mobility to target areas, and enhancing reducing sugar production for fermentation to bioethanol [5]. Physical and chemical pretreatment maximizes reducing sugar yield by increasing the biomass sample's surface area [4], and enables the dissolution of lignin by changing the cellulose's crystalline structure, thereby improving the reducing sugar yield for further enzymatic hydrolysis [6] [7].

Bioethanol production from cellulosic biomass is strategic to achieve the mandatory E20 blending target under the Ethanol Blending Program (EBP) by Govt. of India (20% replacement of fossil fuels with bioethanol for transport by 2030), in a sustainable way [8]. Different raw materials have been considered to increase our bioethanol production. Artocarpus heterophyllus (jackfruit) is a tropical fruit abundantly found throughout India [9]. The seeds make up a quarter of the total fruit weight [10]. They have a high carbohydrate content [11] and is considered a potential candidate as raw material for bioethanol production.

The present study investigated the effect of different concentrations of dilute acid and hydrolysis time on reducing sugar content using jackfruit seeds (JFS). Different factors including acid concentrations, temperature, biomass loading, and hydrolysis time were considered to maximize the sugar yield. Sindhu *et al*., 2011 studied the effect of biomass loading on acid pretreatment of sugarcane tops and reported that biomass loading ranging from 10% to 15% w/w resulted in almost similar reducing sugar yield, with higher biomass loading (> 25%) resulting in a decrease in reducing sugar yield which could be due to increased inaccessibility of the acid to target areas. Kim et al. 2008 also found that solid loading in dilute acid pretreatment could vary from 5% to 15% dry biomass.

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According to Saha & Bothast, 1999, pretreatment at higher temperatures (> 160°C) facilitated the formation of inhibitory compounds, which becomes more apparent as temperature increases; it has also been observed that a higher concentration of acid is more likely to result in the production of more inhibitors due to the corrosive nature of the acid [12]. Thus, in the present study, the acid pretreatment of JFS (10% biomass loading, w/v) was performed at low concentrations of acid (1-5%, v/v) at a relatively lower temperature of 121°C for 15 – 45 minutes in order to minimize the formation of inhibitory compounds. The total reducing sugar of native and pretreated biomass was determined by the 3,5-dinitrosalicylic acid (DNS) method. The surface morphological features were evaluated by Scanning Electron Microscope (SEM).

II. METHODS AND MATERIALS

A. Feedstock collection and preparation: JFS was obtained from the local market of Shillong. The seeds were cut into small pieces, dried, grounded, and passed through 1 mm sieve. The sample was stored at room temperature for further use. JFS was analysed for moisture and ash content.

B. Reducing sugar analysis: The reducing sugar content of native and pretreated biomass was determined by the method of Miller, 1959 [13]. The reducing sugar content of native and pre-treated feedstock was determined by the 3,5-dinitrosalicylic acid (DNS) method using a UV-VIS spectrophotometer. Standard for calibration curve was prepared by adding 3 ml of DNSA reagent to a series of standard using glucose as stock solution (0 – 500 µg).

Distilled water was used as blank instead of sugar. The contents of the tubes were boiled for 5 minutes and to these, 1 mL of 40% of Rochelle Salt was added while still warm. After cooling, the colour formed was estimated spectrophotometrically at 540 nm against blank. 1.5 mL of the sample was run in parallel and the concentration of the sugar was estimated using the standard graph. The percentage of reducing sugar was arrived at using Eq. (1):

$$Reducing\ sugar\ yield\ \left(\frac{mg}{g}\right) = \frac{Reducing\ sugar\ concentration,\ mgL^{-1}}{Biomass\ concentration,\ gL^{-1}} \ldots\ldots\ldots\ldots\ldots\ldots \quad \text{Eq. (1)}$$

C. Dilute acid pretreatment (DAP) of JFS using H_2SO_4: The DAH was carried out at a 10% (w/v) solid content in an Erlenmeyer Flask containing 60 ml of varied acid concentrations (1 - 5%, v/v) at a constant temperature and different time periods (15, 30, and 45 mins). The hydrolysate, after treatment, was separated by filtering the contents using muslin cloth. The residue was washed with distilled water till pH was close to neutral and dried at 60°C. DNSA method was used to determine changes in reducing sugar content in the treated samples. All experiments were carried out in triplicates and expressed as mean values ± standard deviation (SD).

D. Scanning electron microscope (SEM): To document and understand the influence of pretreatment on the plant biomass, surface morphological characterization of native and pretreated biomass was done using SEM at a magnification range of ×500. All the scanning electron micrographs were acquired on a JSM-3630 (JEOL) at 10 kv voltage.

III. RESULTS AND DISCUSSION

1. Composition analysis of JFS: The composition analysis of JFS (% dry weight basis) was: Moisture, 60.67% ± 0.88; Ash, 3.4% ± 0.11. The reducing sugar yield of native sample was 1.28 ± 0.16 mg/g. All experiments were performed in triplicates.

2. Effect of dilute acid pretreatment on JFS: The maximum reducing sugar yield of 2.01 mg/g ± 0.03 was obtained at a 1% v/v H_2SO_4 concentration hydrolysed for 15 minutes. In fig. 1., we observe that an increase in acid concentration and hydrolysis time resulted in gradual decrease in the reducing sugar yield.

The highest reducing sugar yield was obtained with lower concentrations of acid with shorter hydrolysis time of 15 minutes. At 30 and 45 mins, the difference in the reducing sugar yield was not significant (Fig. 3). At 45 minutes, there was a gradual increase in the sugar yield and the maximum yield was achieved at 3% v/v acid concentration, which reduces with increase in acid concentration.

The reducing sugar yield of native sample was 1.28 ± 0.01 mg/g which increased to 2.01 ± 0.03 mg/g after treatment. There is 57.03% increase in the yield as a result of pretreatment. The decrease in reducing sugar yield could be due to the increase in production of interferences with increasing acid concentration. Higher acid concentration and longer incubation time further degrades the constituent sugars to inhibitory compounds [14]. Common inhibitor products formed are furans, hydroxymethyl furfural (furfurals), and other organic acids [15].

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Table 1: Reducing sugar yield of JFS after dilute acid pretreatment

Acid concentration (%, v/v)	Reducing sugar yield (mg/g)		
	15 mins	30 mins	45 mins
1	2.01 ± 0.03	1.680 ± 0.03	1.72 ± 0.03
2	1.90 ± 0.05	1.63 ± 0.02	1.74 ± 0.03
3	1.81 ± 0.03	1.53 ± 0.04	1.80 ± 0.03
4	1.74 ± 0.05	1.44 ± 0.03	1.65 ± 0.03
5	1.69 ± 0.03	1.42 ± 0.004	1.48 ± 0.02

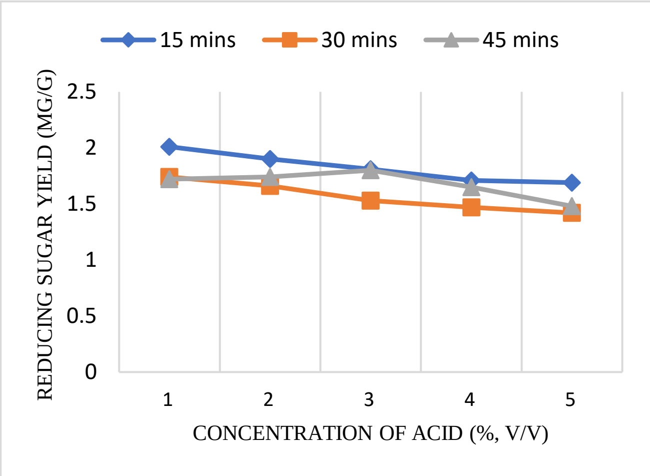
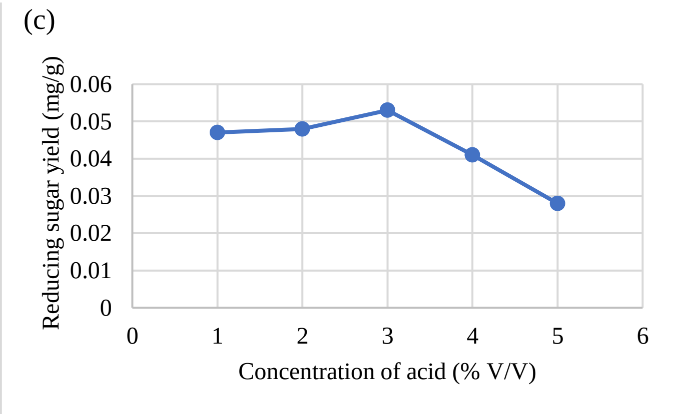


Fig. 1: Reducing sugar yield of JFS at different concentrations of H2SO4 (1 - 5% v/v) hydrolysed for 15, 30 minutes, and 45 minutes.



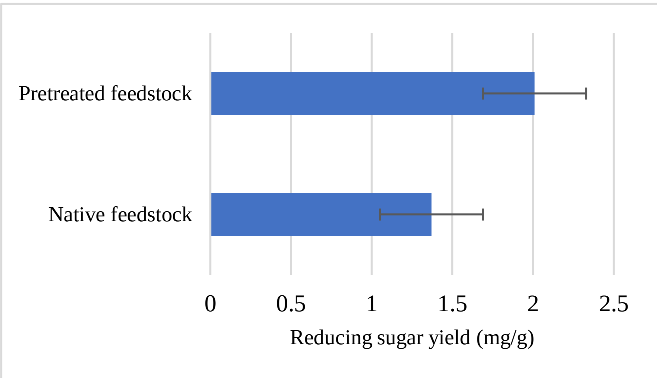


Fig. 2: Comparision of reducing sugar yield of native and pretreated JFS.

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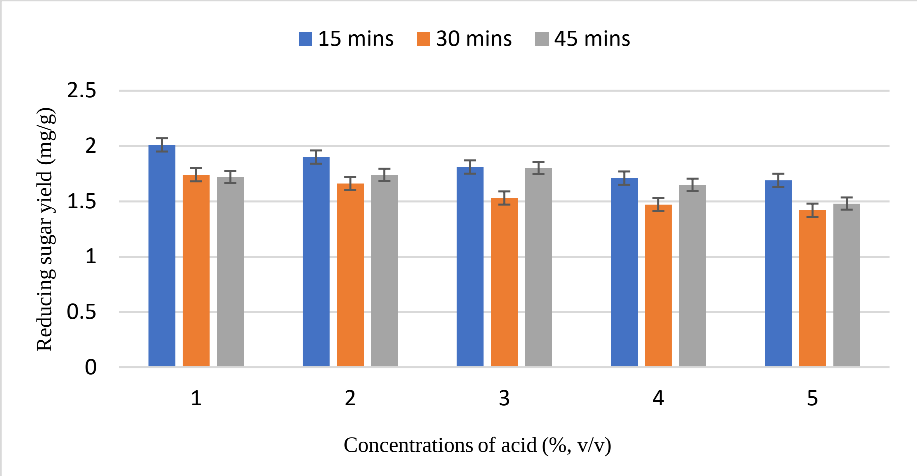
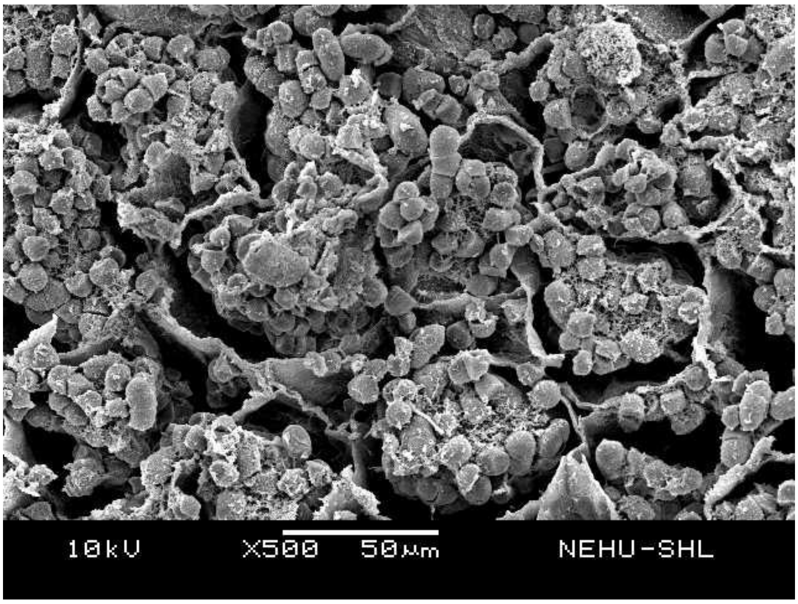


Fig. 3: The effect of varied concentrations of dilute acid (H_2SO_4) and time intervals on the reducing sugar yield.

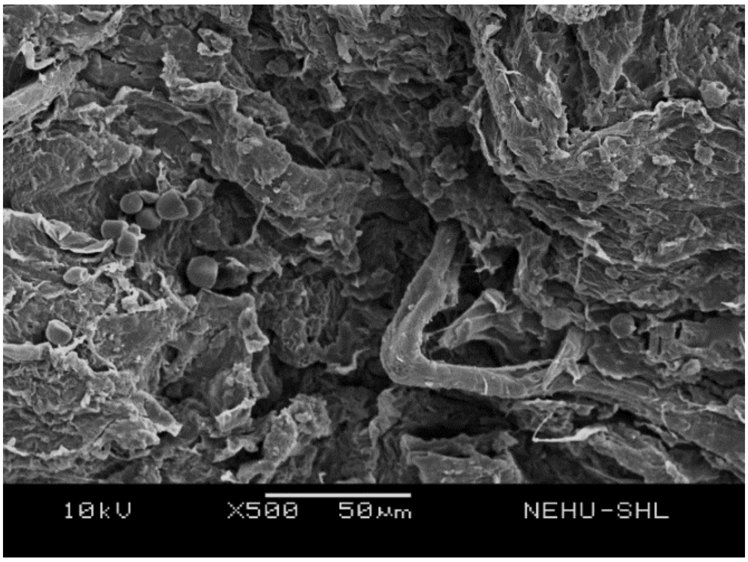
3. Result of SEM: The structural changes that occurred in the seed sample as a result of pretreatment with dilute H_2SO_4 were studied. JFS granules (5 and 10 µm) have smooth bell-shaped surfaces [15]. Fig. (4) shows the morphology of native (a) and pre-treated (b) JFS.

The native JFS had a compact and well-ordered structure indicating a rigid and densely packed structure, whereas the pre-treated JFS had some discernible changes specifically the appearance of abrasion and a rather smooth and continuous surface. This could be due to reduced rigidity of cellulose, as well as some layering and scaling, which was possibly caused by lignin removal or hemicellulose degradation.

The SEM images show that morphological structures of the pre-treated samples were thus destroyed by opening the native structure during pretreatment, which increases the surface area for enzymatic hydrolysis.



(a)



(b)

Fig. 4: SEM images of JFS (a) Native JFS and (b) Pretreated JFS with dilute H_2SO_4

IV. CONCLUSION

Reducing sugars content play an important role in determining the feasibility of a biomass sample for bioethanol production. From the results, the optimum conditions for acid pretreatment of JFS were: 1% v/v H2SO4, 10% w/v solid loading, and hydrolysis time of 15 min. It can thus be concluded that lower acid concentration and shorter time period were found favourable for maximum reducing sugar retrieval on JFS.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Indian Council of Social Science Research (ICSSR), New Delhi and NEHU Non – Net fellowship for financial support to carry out this research work.

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