

## Comparative Evaluation of Pretreatment Strategies on Enzymatic Saccharification of *Hylocereus polyrhizus*'s Pericarps for Bioethanol Production

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**Abstract:** In order to evaluate the possibility of bioethanol production from an indigenous fruit waste - pericarps of dragon fruit, the efficacy of several pretreatment methods on enzymatic digestibility of pericarps were investigated. Following that, the simultaneous saccharification and fermentation (SSaF) of pretreated pericarps to ethanol was conducted by using instant dry yeast. From our study, it was observed that the total sugar yields obtained after enzymatic hydrolysis of thermally pretreated pericarps (0.6 g/g) were higher than untreated pericarps powders (0.28 g/g). By using thermally pretreated pericarps as biomass resource, ethanol concentration of 1.5 g/L was achieved after 80 h of cultivation process. The ethanol yield of 27% (% w/w initial dry weight of biomass) as recorded from the present study is lower compared to the theoretical yield of ethanol from pure glucose solution (51% w/w glucose). Nevertheless, these results indicate that by applying thermal pretreatment, the pericarps of dragon fruit could be considered as an economically viable lignocellulosic material for production of fermentable sugars related to bioethanol production.

**Key words:** Bioethanol · Fruit peel · Pretreatments · Simultaneous saccharification and fermentation (SSaF)

### INTRODUCTION

Billions kilograms of waste are generated by food processing plants every year. Often, this waste is disposed off in landfills or as low value animal feeds. The dragon fruit industry is no exception. Usually the pericarps of dragon fruit are disposed off after the meat is consumed. One alternative method to utilise this dragon fruit waste is to produce fuel grade ethanol from the fermentable sugars and polysaccharides in the pericarps. Bioethanol is a form of renewable energy that can be produced through the fermentation of various lignocellulosic biomass and it has the advantages of increasing energy availability, decreasing air pollution and diminishing atmospheric CO<sub>2</sub> accumulation [1]. The complex structure of lignocellulosic biomass limits the biomass utilization for energy production [2]. Hence, pretreatment step is necessary in improving the saccharification efficiency for ethanol production [3]. In

the present study, four pretreatment methods (physical, chemical, thermal and combined treatment) were employed. Following that, the biomass-to-ethanol conversion process was conducted through simultaneous saccharification and fermentation (SSaF) technique. Simultaneous saccharification and fermentation (SSaF) is defined as a process which combines enzymatic saccharification and yeast (*Saccharomyces cerevisiae*) fermentation [4]. When these two processes are simultaneously accomplished in a same unit, the glucose formed during cellulose hydrolysis is immediately assimilated by yeast to convert it into ethanol and hence reducing the inhibitory effect of glucose over the culture of yeast [5].

The objectives of this study were to: (i) evaluate the effects of chemical, thermal and combined pretreatments on the structures of pericarp and the subsequent yield on reducing sugar, (ii) investigate the conversion of pretreated pericarps to bio-ethanol through SSaF process.

## MATERIALS AND METHODS

**Preparation and Pretreatment of Feedstock:** Dragon fruit pericarps were by courtesy of a commercial dragon fruit facility. The pericarps were stored frozen (-20°C) prior to any cleaning process. The cleaned pericarps were dried overnight at 60°C in an oven. The dried pericarps were then powdered to attain the particle size in between 150 µm and 300 µm.

In the present study, four different pretreatment techniques were applied to pre-treat the dried dragon fruit pericarps. In thermal pretreatment, the pericarps were autoclaved at 121°C, 15 psi for 15 min while chemical pretreatment process involved solutions of acid (5 N H<sub>2</sub>SO<sub>4</sub>) or alkaline (5 N NaOH). In combined pretreatments, the pericarps were treated by (i) thermal followed by chemicals; and (ii) chemicals followed by thermal treatments.

### Hydrolysis and Saccharification Process:

Saccharification of treated pericarps was done by using 1% w/v of dried pericarps in solution containing sterile distilled water, filter-sterilised pectinase (Pectinex ® ULTRA SP-L from novozymes, Denmark) and cellulase (Celluclast ® 1.5 L from novozymes, Denmark). The cultivation process was conducted in 250-mL Erlenmeyer flask at room temperature in the static condition with intermittent mixing. Culture broth was withdrawn at pre-determined time intervals for the analysis of reducing sugar concentration.

### Simultaneous Saccharification and Fermentation Process (SSAF):

Upon identifying the putative pretreatment method of pericarps for production of the highest level of reducing sugar, simultaneous saccharification and fermentation process were conducted. Pretreated dried pericarps (1 g) were supplemented with 100 mL solution of (g/L): yeast extract, 10 and peptone, 20. The flasks were autoclaved for 15 min at 121°C. Following that, the filter-sterilised pectinase (0.5 mL) and cellulase (0.5 mL) were added to the flasks. The flasks were inoculated with 10% v/v of yeast solution (*Saccharomyces cerevisia*). The batch ethanol fermentation process was conducted under static condition at room temperature with intermittent mixing. Culture broth was withdrawn at pre-determined time intervals and centrifuged at 9000 rpm for 10 min. The supernatants were used for measurement of reducing sugars and ethanol concentration.

**Analytical Procedures:** All processes were conducted in triplicate and the average values were presented. Samples were withdrawn at pre-determined time intervals. The content of reducing sugars was determined using 3,5-dinitrosalicylic acid method [6] by measuring absorbance at 540 nm. Yields for sugars were calculated using Equation 1 using equation as proposed by Neureiter *et al.* [7].

$$Y = \frac{CV}{W} \quad (1)$$

$C$  is the concentration of reducing sugar (g/L),  $V$  is total volume of the liquid phase (L) and  $W$  is the dry weight of the corresponding lignocellulosic waste (g). In this study, the soluble ethanol concentration was measured by using GC on a capillary column (Zebron ZB-WAX<sub>plus</sub>) and detection was done by a flame ionization detector. The ethanol yield ( $Y$ ) (% w/w initial dry weight of pericarps) was calculated with Equation 2 as proposed by Zhu *et al.* [3].

$$Y = \frac{E0.9}{S0.51} * 100 \quad (2)$$

Where:  $E$  is ethanol concentration (g/L) and  $S$  is initial concentration of dried pericarps (g/L).

**Scanning Electron Microscopy (SEM):** Scanning electron microscopy was conducted to examine surface morphology of different pretreated pericarps samples.

## RESULTS AND DISCUSSION

### Saccharification Efficiency of Different Pretreatment Strategies:

As observed from Table 1, thermally pretreated pericarps had successfully increased the yield of reducing sugar (0.60 g/g) after hydrolytic enzymes loading and it is almost 50% higher than the untreated pericarps (0.28 g/g). High pressure steam in thermal pretreatment is able to degrade lignin and hemicelluloses as well as to increase the surface area without decrystallizing the cellulose [8]. The total surface area of cellulose exposed for downstream enzymatic hydrolysis is significantly increased and thus improve the sugar yield. The maximum glucose has been successfully recovered from enzymatic hydrolysis of thermally pretreated poplar, wheat straw and other lignocellulosic biomass [9, 10]. From SEM analysis, the initially smooth and connected structure of untreated pericarps powders

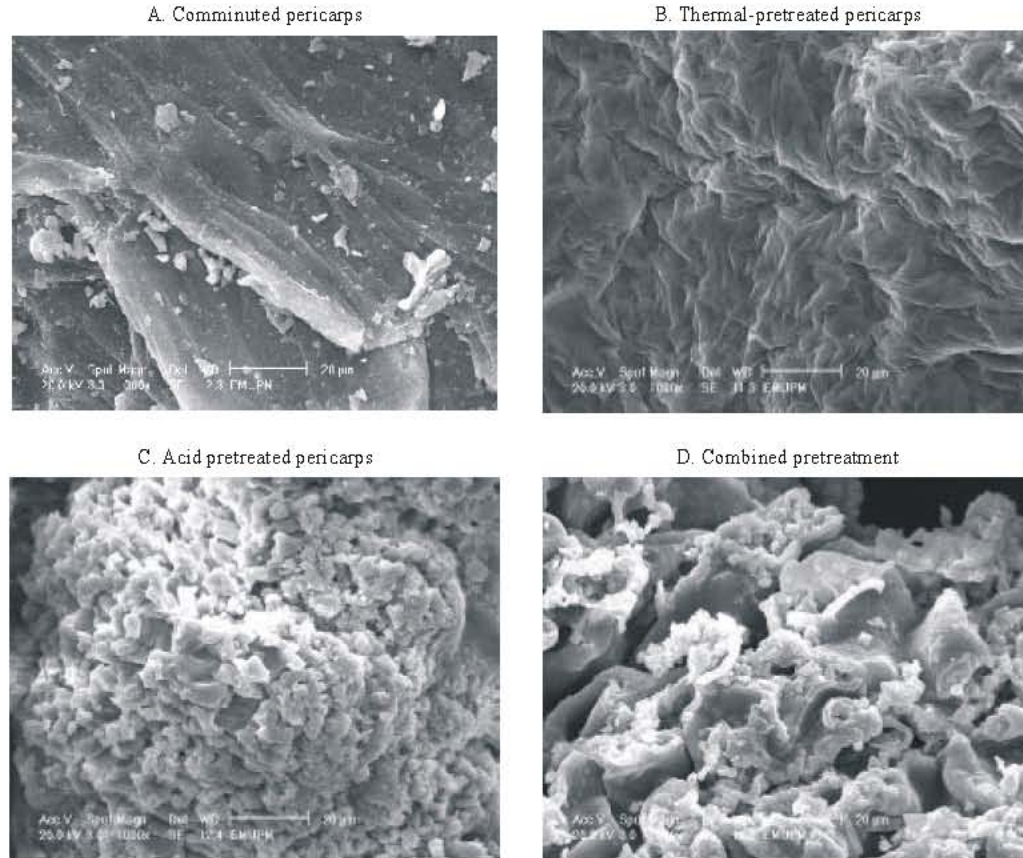


Fig. 1: SEM image of pericarp fibers (magnification: 1000 X)

Table 1: Yield of reducing sugar from pretreated pericarps after enzymatic hydrolysis using commercial enzymes

Methods	Maximum concentration of reducing sugar (g/L)	Yield (g/g)*
Untreated pericarps	2.82 <sup>b</sup>	0.28 <sup>d</sup>
Chemical Treatment		
HCl	2.06 <sup>e</sup>	0.21 <sup>e</sup>
NaOH	2.90 <sup>c</sup>	0.29 <sup>d</sup>
Thermal Treatment	5.98 <sup>a</sup>	0.60 <sup>a</sup>
Combined Treatment		
HCl followed by autoclave	2.24 <sup>e</sup>	0.22 <sup>e</sup>
NaOH followed by autoclave	2.03 <sup>e</sup>	0.20 <sup>e</sup>
Autoclave followed by HCl	2.56 <sup>d</sup>	0.26 <sup>c</sup>
Autoclave followed by NaOH	1.46 <sup>f</sup>	0.15 <sup>f</sup>

\* g of reducing sugar per g of initial dry weight of pericarps

<sup>a-f</sup> mean values in the same column not followed by the same letter are significantly different (P < 0.05).

(Figure 1 A) have become rugged, unsmooth and loosen after thermal pretreatment (Figure 1 B). Hence, this pretreatment can be considered as an effective method in alteration of pericarps structure for conversion of lignocellulosic compound in pericarps to reducing sugars.

In the present study, comminution alone did not increase the yield of glucose as compared with thermally pretreated pericarps (Table 1). Comminution process

might result in the reduction in the cellulose content. For example, cellulose content for particle sizes smaller than 90  $\mu\text{m}$  was 13.4% lower than those of larger particles [11]. Besides, comminution process is unable to remove the lignin which restricts the access of the enzymes to cellulose and inhibit cellulases [12, 13].

Chemical pretreatment approaches have gained significant attentions to increase the cellulose accessibility to hydrolytic attack [14]. However, as shown in Table 1, the yield of reducing sugars from acid and alkaline pretreated pericarps was about 33.3% and 48.3% lower than untreated pericarps. Salt-crystals can be clearly observed on the surface of the acid- (Figure 1 C) and alkaline- pretreated pericarps (figure not shown). Although chemical pretreatments had made the surface structure of pericarps corrugate and porous, the precipitation of salt-crystals confers an additional barrier for the enzymes to reach cellulose, thus decreasing the sugar yields. Same phenomenon were reported when the formation of large amount of sodium chloride salt (NaCl) or gypsum occurred after the neutralization step of chemical pretreatment process [15].

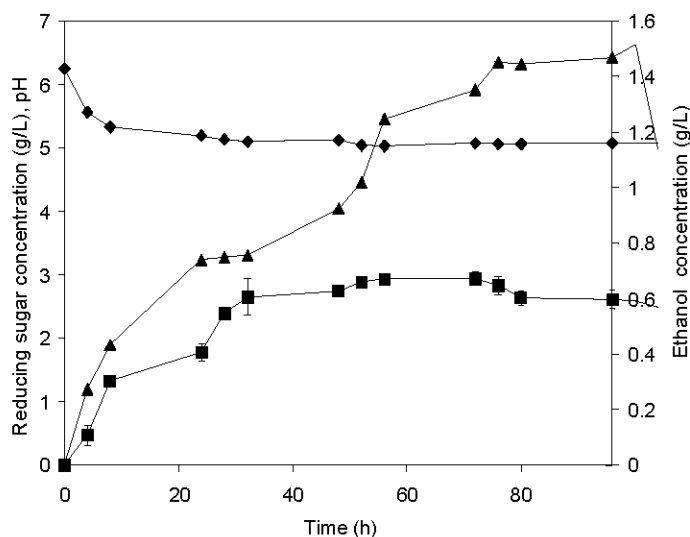


Fig. 2: Time course for the SSaF of the thermal pretreated pericarps to ethanol. Symbols: (◆), pH; (■), glucose; (▲) ethanol. Error bars indicate the mean  $\pm$  standard deviation of three experiments. For data points without error bars, the errors were smaller than the size of the symbols.

Regardless the sequence of pretreatment, all the combined pretreatment techniques tested had made considerably large alteration in the structure of pericarps. A lot of perforation can be seen on the rough surface of the treated pericarps (Figure 1 D). However, sodium chloride salt-crystals were found deposited on the surface of treated pericarps. These salt-crystals remained exist on the pericarps even after autoclaving of the chemically pretreated pericarps during combined pretreatments process. It is because the sodium ions and chloride ions are held very strong by ionic bonding and this confers the salt-crystal structure a very rigid structure and extremely high melting temperature (approximately 800°C) [16].

**Simultaneous Saccharification and Fermentation of Ethanol from Pericarps:** From comparative study on pretreatment techniques, pericarps pretreated thermally were selected for subsequent SSaF study as the highest level of reducing sugars could be obtained from the enzymatic saccharification of thermal pretreated pericarps.

As can be observed from Figure 2, the ethanol content in the medium increased sharply during the cultivation process and attained maximum levels of 1.5 g/L after 80 hours of incubation. The ethanol yield (% w/w initial dry weight of pericarps) is approximately 27% which is lesser as compared with the theoretical yield of ethanol from pure glucose solution, i.e., 51% (g ethanol/g glucose) [17]. As shown in Figure 2, the glucose concentration in production medium didn't decrease significantly. The maximum glucose

concentration of 2.940 g/L was achieved in this medium after 3 days of incubation and decrease gradually to a value of 2.490 g/L in the 4th day. The acidity of cultivation medium containing thermally pretreated pericarps increased before plateau off after 24 h of cultivation process. In general, *Saccharomyces cerevisiae* is an acidophilic organism and, as such, grows better under acidic conditions. The optimal pH range for yeast growth can be varied from pH 4 to 6. The intracellular enzymes of yeast work best at its optimal pH, which is acidic because of the acidophilic nature of the yeast itself. Hence, when the extracellular pH deviates from the optimal level, the yeast cell needs to invest energy to absorb hydrogen ions into the cell for the purpose of maintaining the optimal intracellular pH. If the extracellular pH deviates too much from the optimal range, it may become very difficult for the cell to maintain constant intracellular pH and eventually the enzymes may not function normally. It was reported that when the intracellular enzymes are deactivated due to the deviation of pH, the yeast cell will not be able to grow and make ethanol efficiently [18].

## CONCLUSION

Lignocellulosic by-products from the agro-industrial fields may provide raw materials for producing bioethanol, using chemical and biotechnological approaches. In the present study, experimental data demonstrated a reducing sugars yield of 60% from the conversion of thermally pretreated dragon fruits' pericarps. Under SSaF process

of thermally pretreated pericarps to ethanol, the ethanol yield of 27% (% w/w initial dry weight of pericarps) was achieved. The results revealed that the thermal pretreatment was an efficient pretreatment method of pericarps for its bioethanol production. Moreover, the current study offers an opportunity in exploring the potential of using a locally available fruit waste as biomass feedstock for production of bioethanol as alternative energy source.

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