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# Enzymatic hydrolysis of lignocellulosic biomass from pineapple leaves by using endo-1,4-xylanase: Effect of pH, temperature, enzyme loading and reaction time.

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**Abstract.** Pineapple leaves; is one of lignocellulosic materials that composed of about 80% of carbohydrate containing lignin, cellulose and hemicellulose. This waste still retains a considerable amount of soluble sugars that can be transformed into valuable fermentable sugars. This research was conducted to study the effect of different pH, temperature, enzyme loading and reaction time in order to identify the best working conditions to produce the highest yield of sugar. Prior to enzymatic hydrolysis, the pineapple leaves were subjected to hydrothermal pretreatment in order to obtain the hydrolysate liquid. Enzymatic hydrolysis was then carried out by using endo-1,4-xylanase and the reducing sugar was analyzed by using dinitrosalicylic (DNS) method. The result has shown that the highest reducing sugar concentration of 70.9 mg/L was obtained both at pH 5.5 and 50 °C. In addition, the best enzyme loading and reaction time were recorded at 0.5% (w/v) and 45 min that resulted 60.1 mg/L and 72.0 mg/L respectively.

## 1. Introduction

As one of the most important fruits in the world, the pineapple also known as *Ananas comosus* is the leading edible member of the *Bromeliaceae*'s family [1]. However, there are many parts of pineapple that are unused such as leaves, outer skin and that can be reused to produce new products. Recently, the waste of pineapple is either used as animal feed or disposed to the soil as a waste has increased and contributed to many environmental issues and energy waste. This waste still retains a considerable amount of soluble sugars, as well as high fiber and low protein contents [2]. For example, in pineapple cultivation, the pineapple leaves can be further processed to produce value-added product in which one of the targeted compounds is monosaccharides such as xylose and glucose.

The enzymatic hydrolysis of lignocellulosic biomass is a type of hydrolytic reaction catalyzed by cellulase and hemicellulase enzymes that act on polysaccharides in the biomass i.e. xylan. This process occurs at mild operating conditions of temperature and pH which leads to the depolymerisation or cleavage of polysaccharides into shorter chain oligomers. Enzymatic hydrolysis contributes more advantages than chemical hydrolysis. It is known that chemical hydrolysis requires high temperature, and operates at high pH condition whereby toxic vapour from HCl or H<sub>2</sub>SO<sub>4</sub> could be released and causes air pollution. As enzymatic hydrolysis can be carried out at low temperature and mild pH conditions, thus it will not incur high production cost and preserve the environment. Besides that, high yield of sugars is able to harvest via enzymatic hydrolysis as compared to chemical hydrolysis.

Endo-1,4-xylanases are the most important among the xylanases due to their direct involvement in cleaving the glycosidic bonds and in liberating short xylooligosaccharides [3]. Xylan is the principle carbohydrate which can be found in hemicellulose and consequently the mode of action of xylanases and their related accessory enzymes are of great interest in understanding the hydrolysis of hemicellulose substrates. Xylanases hydrolyze the  $\beta$ -1,4 bond in xylan, creating xylooligomers which are further reduced to xylose by xylosidases. However, a complete degradation requires the



synergistic action of acetyl esterase to remove the acetyl substituents from the  $\beta$ -1,4-linked D-xylose backbone of xylan [4].

Acid hydrolysis procedure will generate more inhibitors i.e. by adding in organic acids; that can give significant influence in the subsequent process such as fermentation. In addition, further purification process is required to remove these inhibitors [5]. To date, none of researches has reported on the study of operating conditions of enzymatic hydrolysis from pineapple waste for sugar recoveries. Therefore, the current study was carried out to study the effect of pH, temperature, enzyme loading and reaction time that can influence the enzymatic hydrolysis performance, hence quantifying the reducing sugar content from pineapple leaves by using endo-1,4-xylanase.

## 2. Material and Methods

### 2.1 Raw Materials

The pineapple residues (leaves) were obtained from Pekan Pina Sdn Bhd in Kuantan, Pahang. Crude Endo-1,4-xylanase was obtained from Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Malaysia. Anhydrous citric acid (CAS No. 77-92-9), sodium citrate dihydrate (CAS No. 6132-04-3, xylose/D-(+)-xylose (CAS No. 58-86-6), acetic acid (CAS No. 64-19-7), sodium hydroxide (CAS No. 1310-73-2), 3,5-dinitrosalicylic acid (CAS No. 609-99-4), and potassium sodium tartrate tetrahydrate (CAS No. 6381-59-5) were obtained from Sigma Aldrich

### 2.2 Substrate Preparation

Pineapple residues i.e the leaves were separated from the fruits. About 1 kg of pineapple leaves was weighed. The sample was washed by using deionized water. Then, the sample was cut into small pieces and dried in an oven at 70 °C for 24 h. Next, the dried small pineapple leaves were ground by using a grinder. Then, the ground pineapple leaves were undergoing hydrothermal pretreatment by using autoclave for 2 h at 121°C in order to get the pineapple leaves juice, i.e. hydrolysate liquid. After that, the hydrolysate liquid was filtered by using nylon a filter paper 0.45  $\mu$ m and centrifuged at 5000 rpm for 15 min to remove the fine particles.

### 2.3 Enzymatic Hydrolysis: Effect of pH, temperature, enzyme loading and reaction time

The first step was to get the best working condition for pH. Three different pH readings at 4.5, 5.5 and 6.5 were studied. The selection of pH values were made according to other studies [6,7] In order to adjust the required pH, 5 M NaOH and 5 M H<sub>2</sub>SO<sub>4</sub> were used. On the other hand, three different temperatures i.e. 40 °C, 50 °C and 60°C were carried out for the effect of temperature [7,8]. In the second part, the best enzyme loading was determined by evaluating three different enzyme loadings which were set at 0.5% (w/v), 1% (w/v) and 2% (w/v) [13]. Finally, the effect of reaction time was studied at three different durations in which the hydrolysis reaction was set for 15, 30 and 45 min [13].

### 2.4 Reducing Sugar Analysis

#### 2.4.1 Preparation of DNS Reagent

The analysis of reducing sugar concentration in each sample of pineapple leaves was conducted using DNS method [9]. Therefore, 16 g of NaOH was prepared and 10 g of 3,5-dinitrosalicylic acid was added to the alkaline solution. The mixture was heated and stirred on a hot plate stirrer. In a separated beaker, 300 g of sodium-potassium tartrate tetrahydrate was added into 125 mL of distilled water and was heated. Both DNS and salt solutions were mixed and stirred continuously. The mixture was let to cool down to ambient temperature. The mixture was then poured into 1000 mL volumetric flask and diluted to make up to 1 L with distilled water.

#### 2.4.2 Reducing Sugar Analysis by DNS Method

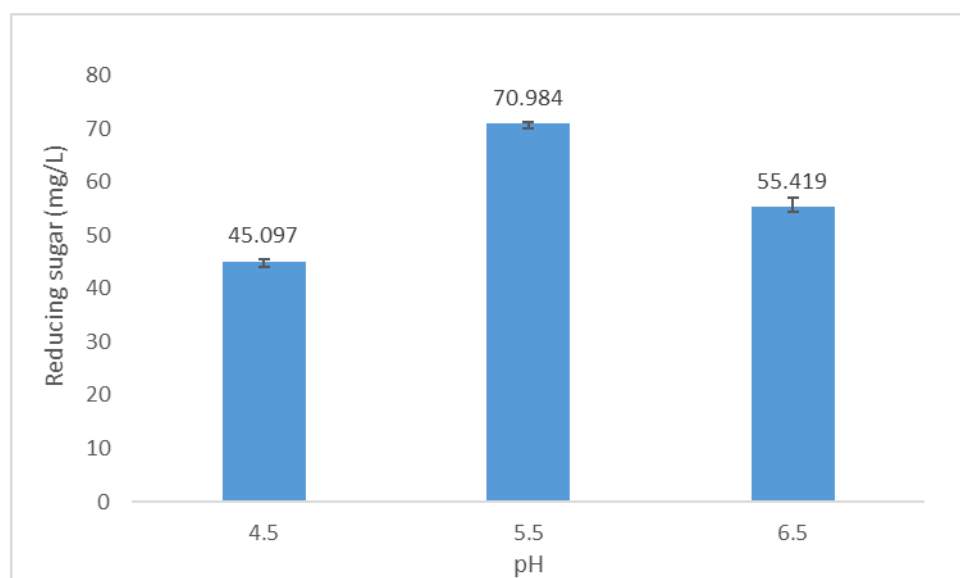
Five mL of sample was put into a test tube. Then, 1 mL of DNS reagent was added to each tube and the solution was thoroughly mixed and boiled at 100 °C until the solution turn to red-brown. The sample was put into cold water bath for 10 min in order to let it cool to room temperature. Then, the reducing sugar content was measured by using UV- visible spectrophotometer at 540 nm.

### 3. Results and Discussion

#### 3.1 The effect of pH

Figure 1 shows that pH 5.5 was the best condition whereby the highest concentration of reducing sugar recorded was about 71 mg/L that is 29% and 57% higher as compared to pH 6.5 and 4.5, respectively. Similar result has been reported whereby pH 5 was found to be the best condition for enzymatic hydrolysis of oil palm fronds by using endo-1,4-xylanase from *Trichoderma longibrachiatum* [8].

A change in pH above or below the pH optimum will reduce the rate of enzyme reaction considerably. This is because the changes in pH lead to the breaking of the ionic bonds that hold the tertiary structure of the enzyme in place which gives effect to the total net charges of the enzymes. Therefore, the enzyme begins to lose its functional shape, particularly the shape of the active site, such that the substrate will no longer fit into it, causes denatured and unable to catalyze chemical reactions. As a result, the production rate of the product will decrease by reducing of hydrolysis rate. Furthermore, changes in pH may not only effect the shape of the enzyme, but it may also change the shape or change the properties of the substrate so that either the substrate cannot bind to the active site or it cannot undergo catalysis.



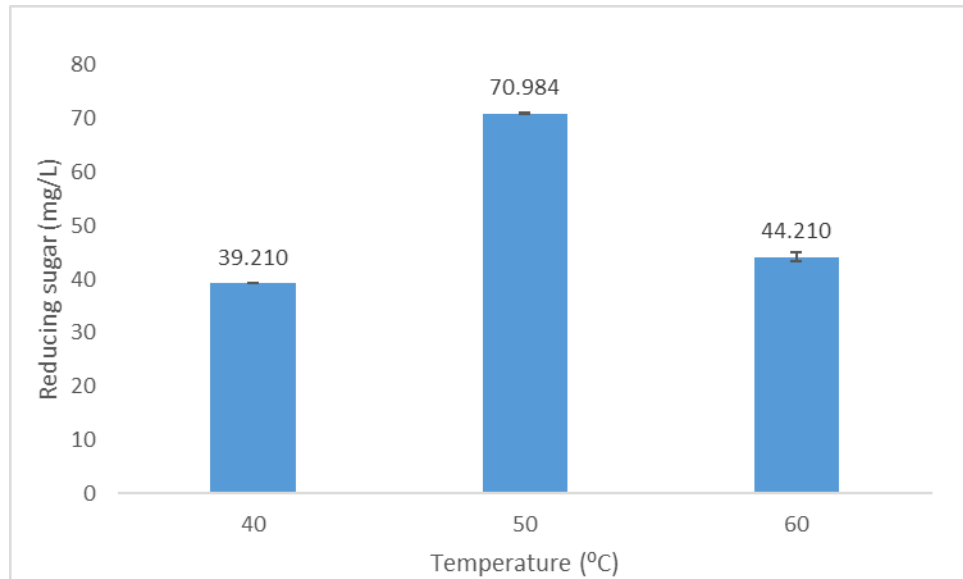
**Figure 1:** Reducing sugar concentration for different pH at 50 °C with 1% (w/v) of endo-1,4-xylanase for 45 min

#### 3.2 The effect of temperature

The other factor that affects the enzymatic hydrolysis performance is temperature. In order to convert the substrate into the product, the enzyme used must collide with and bind to the active site of the substrate. Therefore, enough energy is necessary to make the good orientation of collision by increasing the temperature. Consequently, Figure 2 indicates that 50 °C was the best temperature condition for enzymatic hydrolysis of endo-1,4-xylanase to depolymerize the polysaccharides of pineapples leaves waste by giving the highest concentration of reducing sugar which was 71 mg/L while the lowest concentration of sugar yield was recorded at 40 °C which was 39.21 mg/L. This result was also supported from the finding of previous studies whereby temperature at 50 °C was mentioned to be the best condition enzymatic hydrolysis [8,10].

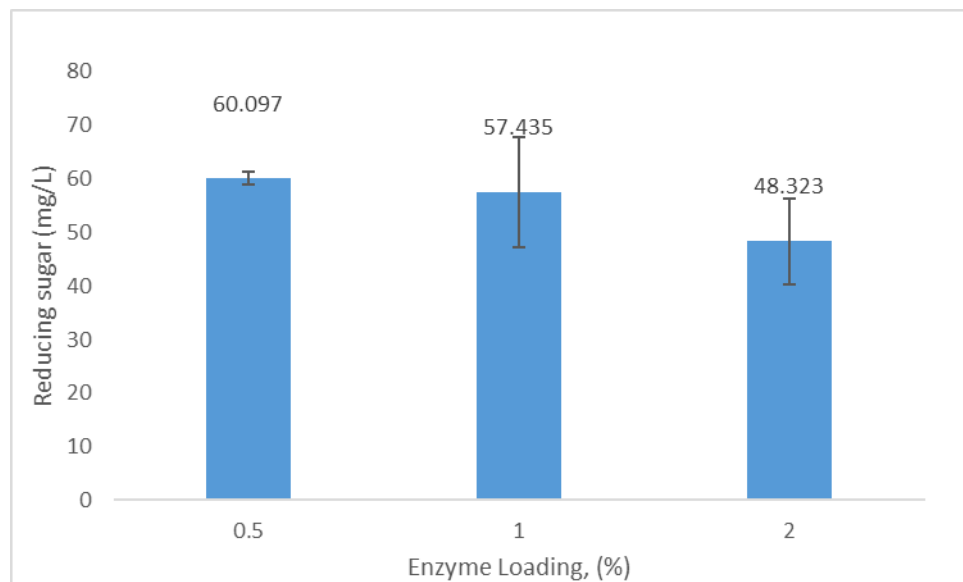
From the figure as well, it can be deduced that temperature at 50 °C has a very significant influence since the reducing sugar concentration measured was higher about 60 – 80% as compared to 40 °C and 60 °C. By increasing the temperature above the optimum value, it can cause the enzyme to

denature hence resulting in termination of reducing sugar production while by decreasing the temperature below the optimum value can cause the enzyme have not enough energy to collide with the substrate [11].



**Figure 2:** Reducing sugar concentration for different temperatures at pH 5.5 with 1% (w/v) of endo-1,4-xylanase for 45 min

### 3.3 The effect of enzyme loading



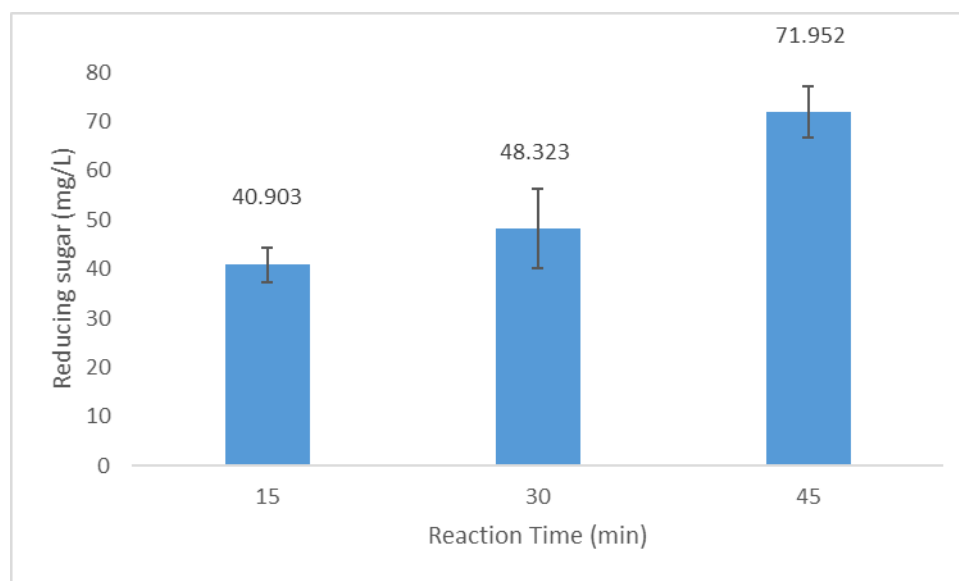
**Figure 3:** Reducing sugar concentration for different enzyme loading at 50 °C and pH 5.5 for 45 min

As for the second part of the experimental work, the best conditions for both pH and temperature were used to determine the best working conditions for the other two parameters; enzyme loading and reaction time. Figure 3 shows the concentration of reducing sugar at three different enzyme loadings i.e. 0.5%, 1.0% and 2.0%, respectively. Therefore, it can be deduced that no significant difference can be obtained from the three enzyme loading values.

In addition, all the values of enzyme loading showed more or less about the same amount of reducing sugar concentration. This presumably due to the amount of substrate available for the enzymatic hydrolysis was limited, hence limiting the reducing conversion. The result also implies that 0.5% (w/v) of enzyme loading was sufficient to hydrolyze the polysaccharides. Further increasing the enzyme loading will not further enhance the enzymatic performance as the excess enzymes that present will not be able to depolymerize the polysaccharides biomass. Such claim has been in consensus with other researchers, that mentioned the addition of more enzyme may result in not getting full hydrolysis effect of the extra enzymes [12]. Due to that reason, 0.5% (w/v) of endo-1,4-xylanase was chosen to be used for the subsequent experimental works as lessen the amount of enzyme would reduce the operational cost.

### 3.4 The effect of reaction time

As can be seen from Figure 4, a significant difference was shown by three different reaction times; 15, 30 and 45 min. These values of reaction time were chosen based on previous work done by [13]. It shows that reaction time for 45 min has given the highest concentration of reducing sugar; 72 mg/L that is 43% and 33% higher than 15 and 30 min, respectively. Furthermore, the trend abides the general rule that is increasing reaction time will increase the production rate. As for 15 and 30 min, the low yield of sugar could be due to less contacting time between the enzymes and substrates. This was empirically proven when the reaction time for 15 min shows the lowest reducing sugar measured which was 40 mg/L. However, further reaction time than 45 min should be studied as well in order to evaluate the efficiency of enzyme activity for a longer period of reaction time. The hydrolysis performance may decrease due to the unavailability of substrate, enzyme inhibitor and enzyme deactivation [14].



**Figure 4:** Reducing sugar concentration for different reaction time at pH 5.5 and 50 °C with 0.5% (w/v) of endo-1,4-xylanase

## 4. Conclusion

In conclusion, the highest reducing sugar concentration obtained from the enzymatic hydrolysis of lignocellulosic pineapple leaves by using endo-1,4-xylanase was 72 mg/L, which were recorded at 50 °C, pH 5.5 with enzyme loading 0.5% for 45 minute of reaction time. Such reducing sugar consists of xylo-oligosaccharides that includes xylo-biose, triose, tetraose, heptose and so on. As for the future perspective, multiple enzymatic hydrolysis such as  $\beta$ -xylosidase and acetyl xylan esterase can be

incorporated as the action of both enzymes could further degrade reducing sugar i.e. xylo-oligosaccharides to monosaccharides i.e. xylose which can be used a precursor many other applications such as pharmaceutical, food and beverages as well as biofuel.

## 5. Acknowledgement

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