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Extraction of xylan from wood sawdust for xylose production using enzymatic hydrolysis method

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Abstract: Wood sawdust xylan was used as source for produce of xylose which is used in the production of xylitol. Extraction of xylan from sawdust was performed under alkaline and alkaline autoclaved conditions as a suitable pretreatment method before enzymatic hydrolysis. The results showed that the highest of xylan yield was 26.13% using (10% NaOH with autoclave 121 C for 15 min) in comparison to non-autoclaved 10% sodium hydroxide at room temperature for 24 hrs was (19.34%). Xylan was hydrolyzed to xylose using xylanase. The effect of concentration, temperature, and incubation time on the yield of xylose production in the enzymatic hydrolysis was studied, the highest productivity of xylose was 64.46%, at 50 ° C for a 45-hour incubation time, while the concentration of xylose produced decreased when the incubation time increased, even more. Alkaline autoclaved pretreatment was best for production of xylan which converted to xylose using enzymatic hydrolysis of xylan.

Keywords: Wood sawdust, hemicelluloses, alkaline extraction, autoclave and enzymatic hydrolysis.

1. Introduction

Lignocelluloses materials such as beech wood composed of cellulose, hemicelluloses and lignin.[1] It is abundant throughout the year and its available around the world. Xylan is distinguished among the components of hemicellulose is its relatively high content of xylose. [2, 3] They constitute a valuable source for the production of high added value products for functional foods (xylo-oligosaccharides and/or xylitol from xylose) according to.[4] An important factor in the success of enzymatic hydrolysis of xylan is the ability of enzyme to access hydrolysable such as hemicellulose to monosaccharides such as glucose or pentose (xylose, arabinose, etc.).[5] The pretreatment enhances to release of monosaccharides and makes xylan more accessible for subsequent processes such as enzymatic hydrolysis.

Xylose commonly called wood sugar is a crystalline, natural five-carbon obtained from the xylan rich portion of hemicelluloses from plants (cell walls).[6] The pretreatment process is very important to broken lignin in the plant cells walls to allow access of enzyme to increase the porosity of the material.[7] Pretreatment of lignocellulosics and enzymatic hydrolysis are important steps to hydrolysis of lignocellulose.[8]

There are several treatments methods which including acid and alkali extractions, enzymes, hot water, and steam explosion, which are used to extraction of hemicelluloses from lignocelluloses. to optimization the economic efficiency of the biorefineries using a mild alkaline extraction process, the purification of the alkaline extract its different components is of importance.[9] The knowledge of the hydrolysis of xylan, temperature, optimum pH and thermal stability of a commercial enzyme preparation is important when considering its use in xylan hydrolysis, to have a good xylose yield. The great characteristic of enzymatic hydrolysis which is give information about hydrolysate compositions which can vary significantly from other chemicals treatment depending on biomass and enzymatic hydrolysis conditions.[10]

The objective of this work is to study chemical pretreatment of xylose production from wood sawdust wastes through enzymatic hydrolysis of xylan using Xylanase.

2. Materials and Methods

Materials

The wood sawdust was collected from Al- Kut industrial area. The samples were washed with distilled water and dried at room temperature and chopped into parts approximately 1.0 - 2.0 mm then stored at room temperature.

Commercial Xylanase, Beachwood xylan, and xylose standard were purchased from Sigma-Aldrich. All other chemicals were of analytical grade.

Extraction of xylan from wood sawdust

The extraction of xylan from wood sawdust was performed as described by,[11] with some modified. The extraction process was carried out using two methods. The first method of extraction of xylan from milled wood sawdust samples were carried out using 10% NaOH solutions (w/w) in 500 mL Erlenmeyer flasks at room temperature and exposure time of 24 hrs. with a solid to liquid ratio of 1:10 w/v on a rotary shaker set at 150 rpm. The second method of extraction of xylan from the milled wood sawdust samples were soaked in 10% NaOH solutions (w/w) with a solid to liquid ratio of 1:10 w/v 500 mL Erlenmeyer flasks and, then autoclaved at 121 °C for 15 minutes, then cooled at room temperature.

After alkali pretreatment, the supernatant was centrifugated at (8,000 rpm for 20 minutes) and was acidify with 12N of HCL to pH 5.0. Then 1.5 v/v of ethanol (95%)was added to precipitate of xylan. Then, xylan was dried in air dry before drying in oven dry for 6 hours at 60°C. Xylan was weighed and stored at room temperature for further analyses. Xylan recovery was calculated by the following formula:

xylan recovery (%) = dry weight of extracted xylan (g) / weight of the sample (g) × 100.

Enzymatic hydrolysis of xylan

Xylan was reached to final xylan concentration of 100 g/l in 250 mL Erlenmeyer flasks containing 50 mL citrate buffer solution (50 mM) with pH (5) and used as substrates in enzymatic hydrolysis with commercial xylanase (500 IU/ g dry substrates) at 50 °C on a rotary shaker set at 250 rpm for 48 hrs. The enzyme was added to the reaction environment at a rate of 5 ml. withdrawn the aliquots of reaction media at desired times.[12] To determine the effectiveness of different factors, the effect of temperature (35, 40, 45, 50, 55, and 60 C) and incubation time (12, 24, 36, and 48 hours) on enzymatic hydrolysis of xylan was studied.

Xylanase activity

Xylanase activity was assayed according to.[13] Commercial xylanase (0.1 ml) was incubated with 1.9 ml of a solution of 1% oat spelt xylan (Sigma, USA) in acetate buffer (50mM, pH 4.8) for 30 minutes. The reducing sugars liberated from this substrate are estimated using the DNS method. One unit from enzyme was defined as the amount of enzyme required produced of 1 µmol of xylose per minute under standard assay condition.

Analytical methods: Cellulose, hemicelluloses, and lignin were determined using methods of.[14]

Xylose concentration in the liquid hydrolyzate was performed using the DNS method M. Kresnowati et al 2015 (Abs. was read at 515 nm).

Total Sugar Concentration: Analysis of total sugar concentration in the hydrolysates was done using Phenol-H_2SO_4 method.

Statistical Analysis: Data analysis for statistical significance was conducted by a one-way analysis of variance (ANOVA) at a significance level of 95%.

3. Results

3.1. Compositional Analysis of wood sawdust.

3.2. Xylan extraction from wood sawdust

3.2.1. Enzymatic hydrolysis of xylan

The chemical analyses of wood sawdust were done in order to determine the principal structural components. Table (1) shows the chemical composition of the wood sawdust used in the study as a percentage. The percentages of sawdust chemical compounds were 48.06, 29.64, and 22.22 for cellulose, hemicelluloses, and lignin respectively. The observed results match those data reported by other authors for the same wood species.[15, 16] Previous studies reported that lignocelluloses typically consists of 35-50% cellulose, 20-35% hemicelluloses, and 5-30% lignin.[17] Sawdust is hardwood chips and is higher in cellulose and hemicelluloses than others such as softwood.[6] The results showed that sawdust contained a high amount of hemicelluloses (> 27%) making this biomass a rich source for xylose production

Table 1. Main constituents of wood sawdust based (o.d.b.).

Constituents Content (%w/w)	Study	Zuriana et al. 2016	Guida 2019
Cellulose	48.06	41.58	40.1
Hemicelluloses	29.64	32.81	26.7
Lignin	22.22	33.56	33.2
Ash	0.43	3.08	11.95

[1] Tables may have a footer.

The wood sawdust are hardwood categories' and its has high cellulose and hemicellulose content compared to the softwood.[6] From the result in Table 1, the composition of hemicellulose is (29.64) % in wood sawdust makes this biomass adequate for further processing of production.

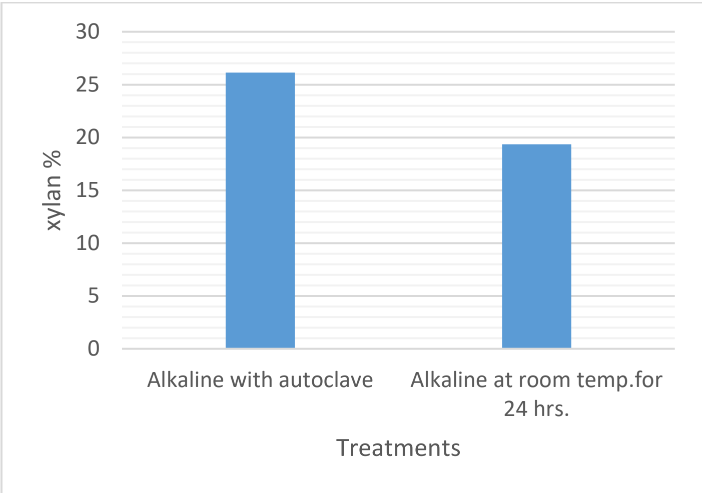
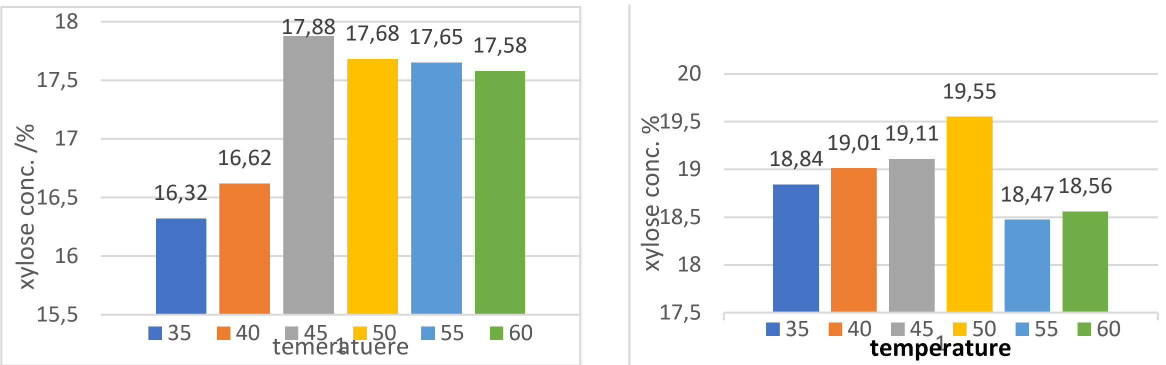
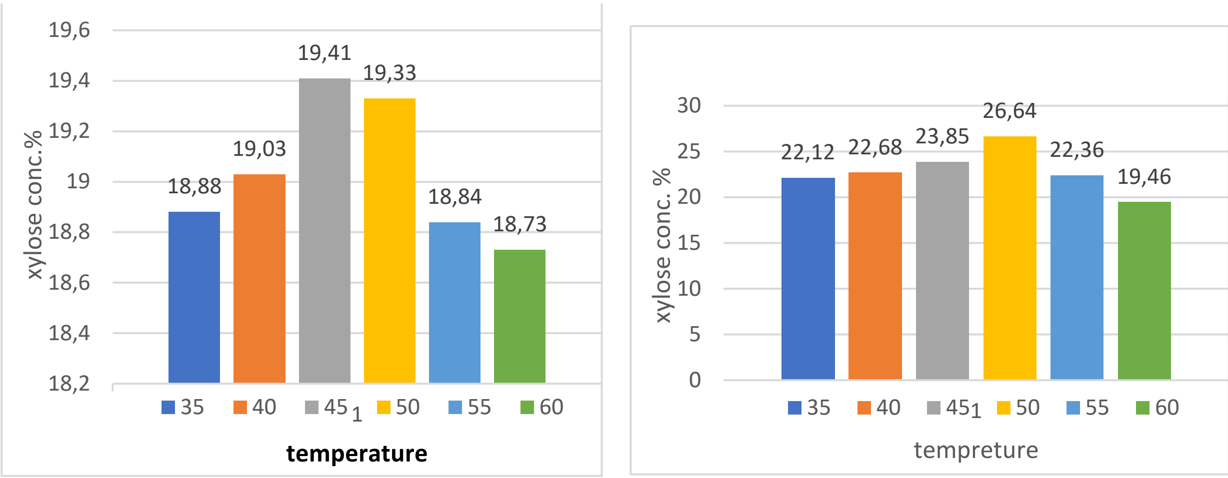


Figure 1. comparison of alkaline with autoclave with alkaline at room temperature treatment for the production of xylan from wood sawdust

In report of,[18] that more than 50% of the original hemicelluloses in the lignocelluloses materials could be extracted using a 10% sodium hydroxide solution whereas without treatment only 3.4% of hemicelluloses could be extracted from pine. In a previous study, the autoclave pretreatment of lignocelluloses materials resulted in a 22% higher yield of reducing sugars compared to the non-treated samples. Also,[18] reported that the maximum yield of xylan with 10% and 5% NaOH were as high as 90% and 60% for 2 h extraction, respectively.



(a) (b)



(c) (d)

Figure 2. Effect of temperature and incubation time. (a) incubation time 12 hrs on xylose release from xylan using Xylanase.(b) incubation time 24 hrs on xylose release from xylan using Xylanase. (c) incubation time 36 hrs on xylose release from xylan using Xylanase. (d) incubation time 45 hrs on xylose release from xylan using Xylanase

Figure 2, the results showed high percentages of xylose produced from sawdust treated by autoclave, that the pretreatment process plays an important role in the conversion of lignin because it helps break down the lignin structure and change the crystal structure of hemicellulose and cellulose, thus improving access to the enzyme during the hydrolysis process. [19]recorded that, pretreatment is necessary to degrade lignin in the cell wall, before starting the enzymatic hydrolysis so that the access of enzyme to the substrates is increased. [20]stated that xylans could be released by alkaline extraction since it is known that alkaline pre-treatment can completely or partially cleave ester bonds between lignin and hemicellulose in the plant cell walls. The further optimization experiments were carried out with autoclave treatment in order to improve the xylan release.

4. Discussion

Figures 2. (A, B, C and D) shows the effect of temperatures and incubation time on the release of xylose from xylan. The research included the use of temperatures ranging from (35 to 65 °C) to study their effects on the release of xylose. The results indicate that the rate of hydrolysis has increased with increasing temperatures, and the maximum level of hydrolysis was at (45°C) and then decreased when the temperature became (50°C). This result can be attributed to thermal inhibition of Xylanase at high temperatures.

[20]found that the temperature of (50°C) was ideal for enzymatic hydrolysis of various materials of lignocelluloses.

Also, results from (Fig. 2) showed that incubation temperature for enzyme substrate reaction plays a critical role in xylose productivity. The xylose yield at 50 ºC was significantly higher than at other temperatures. However, a comparison of time hydrolysis of xylan at different temperatures showed that the enzyme was more stable at 50ºC than at other). The increment of hemicelluloses hydrolysis through Thermal conditions was due to the hydronium ions (in the buffer system) at high temperatures that interfered with the β-1,4 linkages in the xylan structure and therefore resulting in depolymerization efficient into xylose.[21]

Considering the stability of the enzyme and the economic cost a more specific product of xylose, a temperature of (50) °C was chosen as the temperature at which hydrolysis would be performed for subsequent experiments.

Back to Figure 2. The enzymatic hydrolysis of xylan was carried out at an incubation time ranging from (12 to 45) hours. The results also showed that the highest productivity of xylose was 64.46%, at 50 ° C for a 45-hour incubation time, while the concentration of xylose produced decreased when the incubation time increased, even more. This behaviour may be due to inhibition of enzyme activity by the accumulated hydrolysis products.

It is difficult to set a single ideal condition for enzymatic degradation, because the optimum condition is influenced by several factors such as temperature, pH, incubation time and enzyme activity. The enzyme is inhibited by the final products, as the accumulation of these products negatively effects on the enzyme activity.[22]

The degree of polymerization (DP), besides the release of xylose in the hydrolysate, is influenced by the xylanolytic activities of xylanase preparation and biochemical properties of xylanases. Enzymatic hydrolysis of wood xylan using xylanase from Thermomyces lanuginosus produced more than 90% (w/v) of xylose in the hydrolysate, equivalent to 9.28 mg/mL in 24 h of reaction time.[16]

5. Conclusions

The results revealed that the pretreatment of wood sawdust with the alkaline was more efficient in extracting of xylan. The results showed that treatment was effective to increase the accessibility of enzymes. Autoclave treatment with sodium hydroxide treatment was efficient processes to produce xylose with the enzymatic hydrolysis after 36 hours, at 50°C, and 120 rpm speed rotation in a shaker incubator. Wood sawdust biomass contains above 29.64% hemicelluloses, which is a promising source for xylose production.

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