Effect of Alkaline Pre-treatment on Fermentable Sugar Yield of Ethiopian Bamboo (Yushania Alpine)

Netsanet Alemayehu[3](✉), Yalew W/Amanuel[2], and Nigus Gabbiye[1]

[1] Faculty of Chemical and Food Engineering, Process-Engineering Stream, Bahir Dar Institute of Technology, Bahir Dar University, Bahir Dar, Ethiopia

[2] College of Biological and Chemical Engineering, Addis Ababa Science and Technology University, Addis Ababa, Ethiopia

[3] Department of Chemical Engineering, Institute of Technology, University of Gondar, Gondar, Ethiopia

Abstract. In the recent years, bioethanol derived from lignocellulosic biomass as an alternative to the conventional fuels is becoming a focus of many researchers. In this study, bamboo is used as a feedstock for the production of bioethanol via alkaline pretreatment followed by, enzymatic and acid hydrolysis process. Specifically, the focus is mainly on the investigation of the effect of alkaline pretreatment on the yield of total fermentable sugar from bamboo (yushania alpine) biomass using sodium hydroxide as a catalyst. Alkali concentration, pretreatment temperature, and reaction time were varied in the alkali pretreatment experiments. Following the pretreatment, enzymatic and dilute sulfuric acid hydrolysis of the pretreated bamboo were carried out separately to measure the success of the alkaline pretreatment in improving the total reducing sugar (fermentable sugar) yields. The composition of raw and pretreated bamboo were characterized using NREL and ASTM protocols. The total reducing sugar concentrations after hydrolysis were determined by DNSA method. It was found that, the raw bamboo has a composition of 42.45% cellulose, 23.6% hemicelluloses, and 32.4% Lignin. The alkali pretreatment resulted in a maximum lignin removal of 35.5% at temperature of 60 °C, contact time of 50 min, and alkali concentration of 4.5% w/v, which leads to an increment of cellulose fraction to 64.93% and reduction of lignin to 20.8%. A maximum total reducing sugar yield of 206.3 mg/g and 351.9 mg/g were obtained for pretreated bamboo by using enzymatic and dilute sulfuric acid hydrolysis, respectively.

Keywords: High land bamboo · Pretreatment process · Delignification · Fermentable sugar

1 Introduction

Lignocelluloses biomass is the primary and most abundant organic material on the earth, which makes it the most promising resource for the alternative energy sources. Among

M. A. Delele et al. (Eds.): ICAST 2020, LNICST 384, pp. 59–67, 2021.
https://doi.org/10.1007/978-3-030-80621-7_4

them, bamboos is receiving a renewed interest due to its high growth rate and better education of carbon footprint compared to an equivalent area of woody plants (Mengistie et al. 2013). Ethiopia shares about 67% of the bamboo resourse in africa (Kindu et al. 2016). Despite its abundance in the country, it has not exploited to use as renewable feedstock for bioethanol production. The most critical step in bioethanol production from lignocellulosic materials is the pretreatment step. Multitudes of pretreatment processes have been developed in the past and each of them has their own advantage and disadvantages. In principle, a pretreatment process has to increase the surface area and pore size, reduce the Crystallinity, remove lignin content, require less chemical dosage and avoid sever process conditions. (Yamashita et al. 2010) have been reported that alkaline pretreatment is generally more effective on agricultural residues and herbaceous crops than on wood materials. However, they cause less cellulose and hemicelluloses hydrolysis and solubilization than acid processes. It has been reported elsewhere (Zhiqiang et al. 2012), (Shangxing et al. 2014), that the dilute alkali treatment was highly effective in delignification of the biomass, at high temperature. There was studied pretreatment of bamboo using at high operating conditions (Megumi et al. 2010). Among the pretreatment processes, alkaline pretreatment can be carried out at mild operating conditions and successful in lignin removal. Thus, the primary aim of the present study is to investigate the effect of mild alkaline pretreatment method on yields of fermentable sugar from bamboo (yushania alpine) biomass.

2 Material and Methods

2.1 Raw Material Preparation

High land bamboo (yushania alpine) sample having an age of 2.5 years was collected from Injibara town, in the district of Awi zone. The raw material was then washed with tap water, dried in sunlight for a week, cut into piece with cutter machines, milled with disc mill, sieved to get a particle size passing through a (0.5–0.71) mm sieve, and stored in a plastic bag at room temperature until used (Fig. 1).

2.2 Method

Alkali Pre-treatment Experiments

Alkaline pretreatment experiments on bamboo (yushania alpine) biomass were performed over a wide range of NaOH solutions (0.5–4.5% (w/v)), reactor temperature (30–80 °C) and contact time of (10–50 min) with SLR a ratio of 1:40 (solid: liquid) respectively (see the experimental design in Table 1).

All experiments were performed in a glass reactor (Atlas syrris reactor) at varying process operating conditions as indicate in Table 1 at constant stirrer speed of 400 rpm and particle sizes of 0.5–0.71 mm. For this 5 g, dried bamboo was immersed in 200 ml distill water with specific amount of NaOH solution. The reaction was allowed to proceed the specified time. The residue was separated from black liquor using vacuum filtration. The solid residue was washed thoroughly until the pH of the washing water becomes neutral.

Fig. 1. Size reduction by manual and Cutter milling setup

Table 1. Experiment design for the

Parameter	Range of values for the parameter		
Temperature (°C)	30	60	80
Alkali Concentration (%w/v)	0.5	2.5	4.5
Time (min)			

The washed residue was dried at 105°C for 12h and milled with ultrafine centrifugal grinder to the required size. NREL and ASTM protocols were used to determine the compositions (Lignin, hemicelluloses, and cellulose) of pretreated and raw bamboo (Sluiter et al. 2012).

Hydrolysis experiments: Pretreated bamboos were hydrolysis by both enzymatic and acid hydrolysis. In enzymatic hydrolysis, the pretreated bamboo was performed using endoglucanase and β-glucosidase enzymes at a pH of 4.9 in a shaking water bath set at 50 °C and 150 rpm for 24 h. Acid hydrolysis was also carried out using 1% sulfuric acid in autoclave reactor set at 121 °C. Then, the lignin removal and the reducing sugar content are determined by using NREL and DNS methods respectively (Sluiter et al. 2012) (Fig. 2).

3 Results and Discussions

The compositional analyses of cellulose, hemicellulose, and lignin were performed for the raw and treated bamboo. The Ethiopian native bamboo (yushania alpine) used in this research was found to be, 42.45% cellulose, 23.6% hemicelluloses, and 32.4% lignin by mass. Moreover, the lignin contents have separated into acid-soluble (3.8% wt/wt of dry biomass) and acid insoluble (28.6% wt/wt of dry biomass) components. However, after pretreatment with 4.5% w/v alkaline solution, at a temperature of 60 °C

Fig. 2. Alkaline pretreatment and hydrolysis equipment setup

for 50 min, the cellulose content of alkaline pretreated bamboo increased particularly (64.93%) but the lignin and hemicelluloses content decreased to 20.8% and 13.56%, respectively. Therefore, in the specified mild alkaline pretreatment, hemicelluloses and lignin contents were partially dissolved and removed which suggest the requirement of further treatment.

3.1 Effect of Alkaline Concentration on Delignification and Total Reducing Sugar

The effect of NaOH concentration on the composition of pretreated bamboo is indicated in Fig. 3. As it can be seen in the figure, delignification rate increases with increasing alkali concentration and less pronounce effect is seen in lower alkali concentration ranges. Such higher rate of delignification with an increase in alkali concentration can be attributed to the fact that hydroxyl ions catalyze the cleavage of ether linkages in the lignin and thus liberate the soluble sodium phenolates in the liquid (Ribeiro et al. 2019). This is because the accessible and soluble lignin type dissolved and removed by alkali pretreatment at 2.5% concentration. But, the highly intact and stable lignin type cannot be dissolved easily, needs high alkalinity of the solution to cause solvation of hydroxyl groups in carbohydrates, and creates the swelling effect in the matrix. Investigation of alkaline pretreatment of lignocellulosic biomass has been found to cause swelling, disruption of the lignin structure and increase in internal surface area by breaking the bond between lignin monomers or between lignin and hemicelluloses components (Cao et al. 2012). This further increases the diffusivity of the NaOH reagent through the capillaries to the biomass structure. In such cases, more hydroxyl ions are in the solution to participate in ether bond breakage and the dissolution of the lignin from the matrix. Furthermore, at higher concentration of alkaline catalyst, there will be more number of hydroxide ions, which can attack the cellulosic bond (glycosidic bond) to release sugar molecules through subsequently protonation of the bond to form carbonium cation (Yan et al. 2014).

The reducing sugar yield of the hydrolysis experiment is also presented in Fig. 4. As the enzymatic hydrolysis yield is also dependent on lignin and hemicelluloses removals,

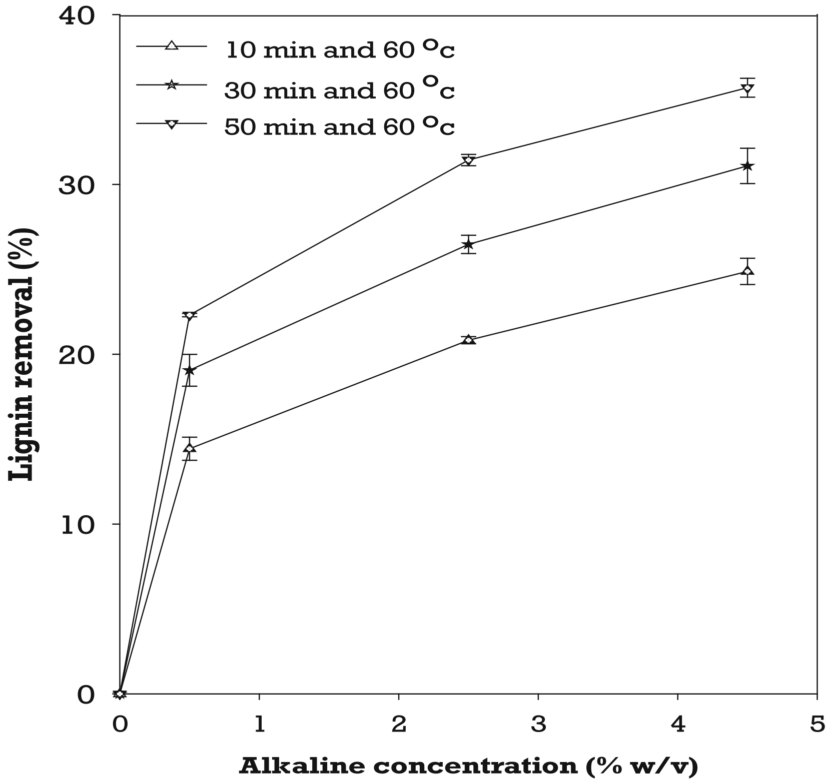

Fig. 3. Effect of alkaline concentration on delignification

higher reducing sugar yield was obtained at high alkali concentration (Fig. 4) for both enzymatic and acid hydrolysis. The presence of less lignin and hemicelluloses components in the biomass facilitated disruption to the biomass matrix, which facilitates enzymatic/acid hydrolysis of the holocellulose (i.e. cellulose and hemicelluloses).

With respect to hydrolysis agents, it appears that dilute sulfuric acid hydrolysis yields higher fermentable sugar than enzymatic hydrolysis with the same bamboo samples pretreated at the same conditions. So, dilute sulfuric acid hydrolysis has highly catalyzed the β - glycosidase bond of the hemicellulose and cellulose to convert them into total reducing sugars. However, the enzymatic (endoglucase and β-glucase) hydrolysis is limited in mass transfer (due to a slow adsorption and desorption processes) as compared to dilute sulfuric acid hydrolysis process. Additional, increase in the NaOH concentration and reaction time in the pre-treatment condition resulted in an increase in reducing sugar due to the removal of lignin.

3.2 Effect of Time on Delignification and Total Reducing Sugar

Previous reports show that delignification occurs in three distinct phases: initial, bulk, and residual lignin reactions (Monica et al. 2009). The bulk phase is normally the reaction

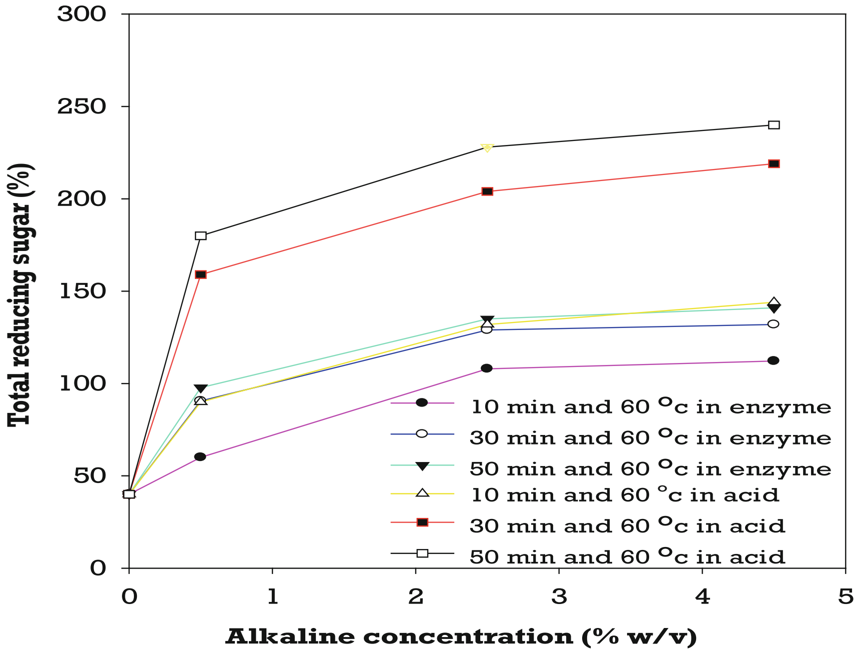

Fig. 4. Effect of alkaline concentration on total reducing sugar

stage in which the largest portion of lignin is removed while the smaller fraction of residual lignin is removed in the residual reaction phase, which is also slower reaction and observable in the later stages of delignification (Macfarlane et al. (2009)).

As shown on Fig. 3, most of the lignin was removed in 10 min and this phase is the bulk delignification phase. Increasing contact time from 10 to 50 min slightly increased the lignin removal, which shows slower reaction phase and can easily be recognized as the residual delignification phase. The residual lignin, which is not accessible by NaOH reagent, was gradually removed over a longer time. However, for higher alkali concentration, solvation of the hydroxyl ions in the biomass swells and opened up the structure, which makes the lignin and hemicelluloses easily dissolved by the reagent. Higher alkali concentration and time increases the internal surface area and porosity by swelling and hemicelluloses degradation. Hence, for longer pretreatment time, the internal surface area, and pores of the biomass increase. These improve the enzyme (endoglucase and β-glucase) and dilute sulfuric acid digestibility of the cellulose.

The effect of reaction time on the total reducing sugar yield is shown in Fig. 4. It shows that, the yield of total reducing sugar increases with an increase in the length of pretreatment time. So, the maximum total reducing sugar yield was obtained by dilute sulfuric acid hydrolysis as compared to enzymatic (endoglucase and β-glucase) hydrolysis.

3.3 Effect of Temperature on Delignification and Total Reducing Sugar

The result presented in Fig. 5 shows that increasing reaction temperature favors lignin and hemicelluloses removals. Sodium hydroxide used in this case as a catalyst is more active at higher temperature and it catalyzes the ether and ester bonds in the lignin matrix and between the hemicelluloses chains, effectively. The more soluble portions of lignin accessible by the alkali can be removed easily by raising the temperature from 30 to 60 °C. Further increment to 80 °C facilitates the removals marginally. On the other hand, hemicelluloses removal continuously increases, as its solubility is more dependent on temperature.

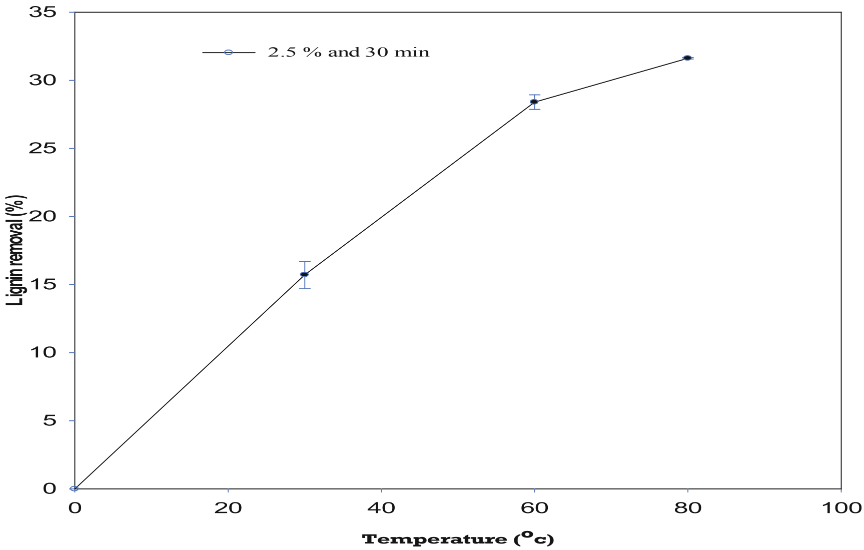

Fig. 5. Effect of temperature on delignification

Eventually, lignin and hemicelluloses removals and creation of more surface areas (porosities) in the biomass are responsible for increasing the acid and enzymatic hydrolysis yield, depicted below in Fig. 6.

As it can be seen in Fig. 6, the total reducing sugar yield of dilute sulfuric acid hydrolysis was higher than in the yield from enzymatic hydrolysis for all range of temperature. Therefore, this indicates that the dilute sulfuric acid hydrolysis has preferable than enzyme (endoglucase and β-glucase) hydrolysis in order to produce high total reducing sugar in alkaline pretreatment at specific condition.

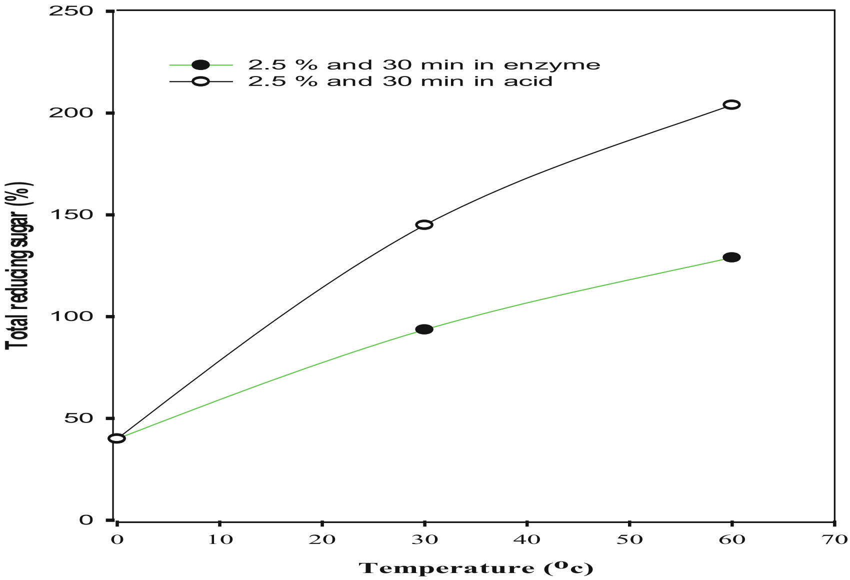

Fig. 6. Effect of temperature on total reducing sugar

4 Conclusions

In this research, the effects of different alkaline pretreatment conditions on lignin removal and their impacts on the subsequent hydrolysis yield have been investigated. Varying alkaline concentration, pretreatment time and temperature were applied to pretreated bamboo biomass. The maximum lignin removal achieved was 35.5% at a temperature of 60 °C, alkali concentration of 4.5% w/v and pretreatment times of 50 min. At these similar pretreatment conditions, the maximum fermentable sugars of 206.3 mg/g raw bamboo from enzymatic hydrolysis and 351.9 mg/g raw bamboo from dilute sulfuric acid hydrolysis were obtained. From the result, acid hydrolysis released higher quantity of reducing sugars than the enzymatic pathways. Therefore, it can be concluded that alkaline pretreatment at such mild operating conditions followed by dilute sulfuric acid hydrolysis is the primary option for production of fermentable sugar from Ethiopian bamboo (yushania alpine). Nevertheless, further experiments is required to improve the fermentable sugar yield to the required level via emerging less energy intensive technologies such ultrasound and micro-wave assisted pretreatments.

Acknowledgment. I would like to thank Bahir Dar University, Bahir Dar Institute of Technology for giving me the scholarship to study my postgraduate study.

References

Cao, L., Bala, G., Caldeira, K.: Climate response to changes in atmospheric carbon dioxide and solar irradiance on the time scale of days to weeks. Environ. Res. Lett. **7**(3), 034015 (2012). https://doi.org/10.1088/1748-9326/7/3/034015

Kindu, et al.: Status of bamboo resource development, utilisation and research in Ethiopia: a review. Ethiopian J. Nat. Resour. 79–98 (2016)

Macfarlane, et al.: Dissolved air flotation: a novel approach to recovery of organosolv lignin. Sciencedirect **5** (2009)

Megumi, et al.: Alkaline Peroxide Pretreatment for Efficient Enzymatic Saccharification of Bamboo. Elsevier.Com/Locate/Carbpol (2010)

Mengistie, et al.: Bioethanol production from bamboo (Dendrocalamus Sp.) process waste. Sciencedirect 142–150 (2013)

Monica, et al.: Pulping chemistry and technology. Sciencedirect (2009)

Ribeiro, et al.: Chemical study of kraft lignin during alkaline delignification of E. Urophylla X E. Grandis hybrid in low and high residual effective alkali. Am. Chem. Soc. Sustain. Chem. Eng. **9** (2019)

Shangxing, et al.: Effects of dilute alkali pretreatment on chemical components and fermentable sugars and structure of bamboo. In: Advanced Materials Research (2014)

Sluiter, et al.: Determination of structural carbohydrates and lignin in biomass. NREL **18** (2012)

Yamashita, et al.: Alkaline peroxide pretreatment for efficient enzymatic saccharification of bamboo. Carbohydrate Polymers 914–920 (2010)

Yan et al.: A comprehensive mechanistic kinetic model for dilute acid hydrolysis of switchgrass cellulose to glucose, 5-HMF and levulinic acid. RSC Adv. **15** (2014)

Zhiqiang et al.: Bioconversion of bamboo to bioethanol using the two-stage organosolv and alkali pretreatment. Bioresources 5691–5699 (2012)