Enhanced cellulose efficiency of pressurized hot water pretreated highland Ethiopian bamboo (*Yushania alpina*): A potential feedstock for ethanol production

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ABSTRACT

For the production of ethanol from pretreated lignocellulose biomass play a paramount role in facilitating the conversion of cellulose into glucose in the hydrolysis step. Therefore, this study is focused on the effect of hot water pretreatment on the chemical composition (cellulose and lignin) of highland bamboo of Ethiopia. The chemical composition of highland bamboo showed 46.76% (w/w) cellulose, 25.27% (w/w) lignin, 12.18% (w/w) hemicellulose, 3.77% (w/w) ash, 12.23% (w/w) hot-water extractive and 3.93% (w/w) ethanol-toluene extractives. The effect of hot water pretreatment was observed after the biomass was treated in the autoclave at 121, 128 and 135 °C with 5, 10, and 15 min pretreatment time with distilled water. The best pretreatment method was selected based on the pretreatment method which maximized the cellulose content and minimized the lignin content. Based on the selected pretreatment method a higher cellulose content of 52.44% and lower lignin content of 27.85% was achieved at 128 °C temperature and 10 min pretreatment time.

Capsule Summary: This study is focused on the effect of hot water pretreatment on the chemical composition (cellulose and lignin contents) of highland bamboo for potential feedstock to produce ethanol.

INTRODUCTION

The production of biofuel from renewable materials is increasing throughout the world due to different environmental and economic reasons. From various types of biofuels produced from lignocellulosic materials, bioethanol is one of the major and most important renewable liquid biofuel (Sun and Cheng, 2002; Niphadkar et al., 2018). Ethanol can be used in automobiles by mixing with gasoline with a different percentage. The most common ones are E5

(E5, a fuel mixture of 5% anhydrous ethanol and 95% gasoline) and E10 (E10, a fuel mixture of 10% anhydrous ethanol and 90% gasoline) (Sarris and Papanikolaou, 2016). Even though ethanol has a lot of advantages over fossil fuel; as an energy source, it has a high cost relative to fossil fuel. This is due to the cost of raw material and the need for the sophisticated instrument in second generation ethanol (Sun and Cheng, 2002).

Second generation biofuel can be produced from agricultural lignocellulose biomass, which is either residues of food crop production or non-edible plant biomass (e.g. grasses or dedicated trees). Biological, biochemical or thermochemical processing of lignocelluloses materials are the fundamental methods to produce second-generation bioethanol. In order to minimize the cost of the process, effective pretreatment methods play a great role besides using non-edible biomass (to reduce direct fuel versus food competitions), by facilitating the hydrolysis step, increasing the sugar yield and ethanol, and by minimizing the production cost.

Just like sugar cane and other perennial crops bamboos belong to the family of grasses (*Poaceae*). Bamboo is a fast-growing and short rotation crop, matures in a short time (3–5 years) compared to other trees of softwood and hardwood. Bamboo is widely distributed around the world (Krzesinska et al., 2009). Bamboo spread naturally in a range between 40 degrees southern and northern latitude in all continents, except Europe. An annual mean temperature of 20 to 30 ºC and precipitation levels from 1000 to 2000 mm is preferred by most bamboo species. But some species, such as *Dendrocalamus strictus*, survive under drier conditions in India, with 750 to 1000 mm of annual precipitation. Looking at the global bamboo cover, Asia has the richest bamboo species and resources, accounting for 65% of global bamboo resources. Sub-Sahara Africa is home to approximately 3 million hectares of bamboo forest of *Yushania alpina* (synonyms *Arundinaria alpina*) (a highland bamboo) and *Oxytenanthera abyssinica* (a lowland bamboo) growing on arid or very poor soils. Ethiopia has the largest area of natural bamboo in Africa: about 67% of all African bamboos and 7% of all global bamboo resources are found in the country (Kassahun, 2000).

Bamboo has been used in different applications, such as construction, flooring, paper, furniture, and direct combustion for energy (Scurlock, 2000). Due to its high holocellulose content, rapid growth, perennial nature, availability of species growing in extreme climatic conditions and low management requirements it is considered as a potential feedstock for bioethanol production in recent years. Bamboo's cell wall like other lignocellulosic material is comprised of the polymeric constituents of cellulose, hemicelluloses, and lignin. However, the complex physical and chemical interactions that exist between cellulose, hemicelluloses and lignin require a pretreatment stage to maximize the hydrolysis of cell wall components into monomer sugars prior to fermentation into ethanol (Li et al., 2015; Yuan and Wen, 2017). Even though different

pretreatment technologies such as mechanical, chemical, and physical have been studied, so far no effective universal method to disrupt the biomass cell wall structure, increase the accessibility of the cellulose surface area by solubilizing lignin and/or hemicellulose and reduce cellulose crystallinity found.

In recent years, a number of different methods were investigated for the pretreatment of bamboo for cellulosic bioethanol production. Various pre-treatments, chemical (alkaline and acidic) and enzymatic (e.g. cellulase, xylanase), were applied to liberate the sugars for fermentation (NL Agency, 2013). Many researchers have focused on bamboo pretreatment for bioethanol production by using acid or alkali pretreatment to enhance the digestibility of bamboo. Among the pretreatment methods, dilute sulfuric acid pretreatment was found to be relatively inexpensive and it produces high hemicellulose recoveries and cellulose digestibility (Lee et al., 2008; Li et al., 2014). Li et al. (2014) reported that dilute acid pretreatment leaving only celluloses and lignin in the dilute acid pretreated substrates by dissolving the hemicellulose in bamboo fractions. Sindhu et al. (2014) also reported that dilute acid pretreatment of bamboo increased the enzymatic saccharification rate by removing hemicelluloses.

Dilute phosphoric acid pretreatment of Moso bamboo was studied at optimum pretreatment conditions of 170 ºC and 45 min (Hong et al., 2012). Pretreatment was carried out by immersing the bamboo in KOH (12% and 8% w/w bamboo) solution and exposing the slurry to microwave radiation power of 400 W for 30 min, the result shows high sugar yield (Zhiqiang et al., 2012). Ethanosolv with NaOH pretreatment of Moso bamboo for efficient enzymatic saccharification was reported by Li et al. (2012). Ethanol organosolv pretreatment with dilute sulfuric acid as a catalyst was studied in order to enhance enzymatic saccharification of bamboo (Li et al., 2012). The work showed that organosolv pretreatment with dilute sulfuric acid as a catalyst significantly accelerated hemicellulose and lignin removal and increased the enzymatic digestibility of bamboo substrates.

The pretreatment methods play a great role in increasing efficiency since it disrupts the cell wall structure and makes ease the hydrolysis treatment. The main purpose of the hot water pretreatment is to reduce lignin and hemicellulose contents, reduce cellulose crystallinity, and increase the porosity of the materials to maximize the efficiency of hydrolysis. A good pretreatment method must meet the following requirements: (1) improve the formation of sugars by hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of the inhibitory compound that affects hydrolysis and fermentation processes; and (4) should be cost-effective (Sun and Cheng, 2002).

Since the primary components of lignocellulosic feed stocks are cellulose, hemicellulose, and lignin, information on the chemical composition of the feed stocks is very important for chemical and biochemical-based products. The real-time

monitoring of the components is of great importance for the optimization of biomass process (Xiaoli et al., 2015). This study was conducted in order to evaluate the effect of hot water pretreatment on the cellulose and lignin content of Ethiopian highland bamboo (*Yushania alpina*) and to investigate the pretreatment conditions that increase the lignin removal (delignification) and minimize cellulose solubilization of highland bamboo of Ethiopia.

MATERIAL AND METHODS

Chemicals and reagents

Anhydrous ethanol (Carloerba Reagents, France), ethanol absolute (Carloerba Reagents, France), nitric acid (Carloerba Reagents, France), sulfuric acid (H_2SO_4) (Carloerba Reagents, France), sodium hydroxide (Lobalo Chemicals Laboratory reagents and Fine Chemicals, India), toluene (Indenta Chemicals Pvt. Ltd., India), and sodium sulfite (Central Drug House (p) Ltd., India) were the chemical used in this study.
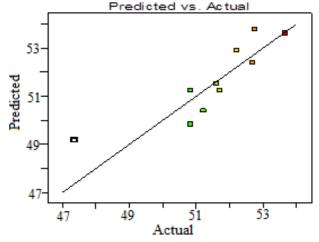
Sample collection and preparation

Three years old Ethiopian highland bamboo stems were harvested from Enjibara (10°57'N36°56'E, 2560 meters above sea level), Ethiopia. The harvested bamboo culm was allowed to dry in the open air for a week. The dried bamboo culm was cut into small pieces and dried in an oven for 24 h at 50 °C. The dried culms were placed in a hammer mill (550110A, Glen Creston Ltd., UK) and Willey mill (SR-200, UK) to reduce small pieces of appropriate size of 2 mm particle size. The prepared sample was sealed into plastic bags and labeled for further analysis.

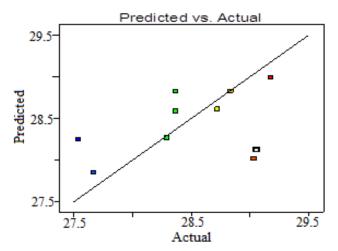### Characterization of the highland bamboo chemical composition

The chemical composition of highland bamboo was determined by using the reported methods. All the chemical composition (ash, moisture, hot water soluble, ethanol-toluene extractives, and hemicellulose content) was determined according to the ASTM (ASTM, 2011; ASTM, 2013a; ASTM, 2013b), Kurschner-Hoffer method (Kurschner and Hoffer, 1931) for cellulose and TAPPI T222 (TAPPI, 2006) for Klason lignin determination. The experiment was performed in triplicate and the result was taken as average.

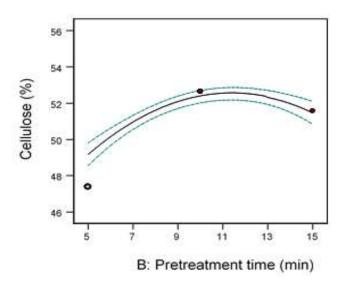### Effects of steam pretreatment on the highland bamboo culm

In this experiment, the effects of pretreatment temperature and time were considered as the independent variable on the amount of cellulose. Three different pretreatment temperature and three different pretreatment time; a total of 9 different pretreatment combination was applied. Ten grams of 2 mm particle size of the highland bamboo sample was transferred into 250 mL conical flask and 100 mL

Fig. 1: Predicted vs. actual value for cellulose content of *Yushania alpina*

Fig. 2: Predicted vs. actual value for lignin content of *Yushania alpina*

Fig. 3: Effect of pretreatment time on the cellulose content after hot water pretreatment

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distilled water was added and allowed to stand for different pre-treatment time (5, 10 and 15 min) at 121, 128 and 135 °C, respectively, in autoclave (SA-300H, Gemmy Industrial Corp. Co. Ltd., Taiwan). The sample was filtered by using Whatman filter paper No. 3 and the cellulose and Klason-lignin content of the residue were determined by using Kurschner and Hoffer (1931) and TAPPI (2006) method, respectively. The experimental conditions for the pretreatments of highland bamboo culms are given in Table 1. In this experiment, a total of 42 experimental observations were carried out for a total of 9 combinations of factors.
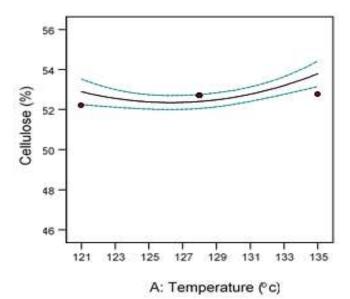
Statistical analysis

Experimental design and data analysis were done by Design Expert® 10 software using a randomized central composite design (CCD).
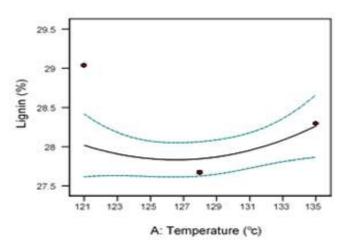
RESULTS AND DISCUSSION

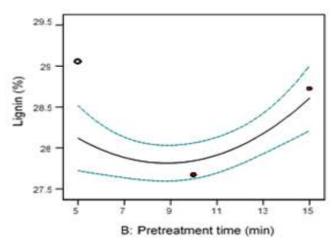**Chemical composition of *Yushania alpina* before pretreatment**

Ash is the inorganic residue remaining after ignition at high temperature (Song, 2013). The ash content of bamboo is mostly silica, along with metal oxides such as calcium and potassium oxides (Li et al., 2007). The moisture and ash content of *Yushania alpina* before pretreatment is 3.92% and 3.77% on average, respectively, the ash content is lower than of *Oxytenanthera abyssinica* grown in Ethiopia (Amsalu et al., 2017) and highly agreed with that reported by Kapu and Trajano (2014). The extraneous components in wood and bamboo are the substances other than the structural components (the cellulose, hemicelluloses, and lignin), which are extractable with organic solvents. They do not contribute to the cell wall structure, and most of these are soluble in neutral organic solvents (Song et al., 2008). The ethanol-toluene extractive content of *Yushania alpina* is 3.93% (Table 2). This result is less than that of *Oxytenanthera abyssinica* (Amsalu et al., 2017) which is 5.60% and also 6-13% of other bamboo species (Kapu and Trajano, 2014). It is also less than that reported by Li et al. (2015) for *Bambusa blumeana* and *Bambusa pervariabilis*. This shows that *Yushania alpina* has fewer substances which can be extracted by using alcohol-toluene mixture. The low content of neutral organic soluble extractive substance reduces the risk of fermentation by inhibiting the growth of yeast. The hot water extractives of *Yushania alpina* is 12.23% which is very high when compared to *Oxytenanthera abyssinica* reported by Amsalu et al. (2017) and in a range reported for softwood and hardwood by Kapu and Trajano (2014). This variation on extractives content of bamboo is caused by the factors such as; the type of the species, environmental conditions, harvesting time, geographical locations, and also ecological factors as well (Song, 2013). The high amount of hot water soluble components indicates that it is rich with low molecular

Fig. 4: Effect of temperature on cellulose content after hot water pretreatment

Fig. 5: Effect of temperature on lignin after hot water pretreatment

Fig. 6: Effect of pretreatment time on lignin after hot water pretreatment
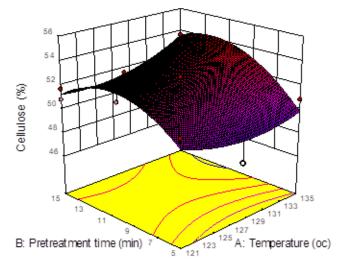
weight oligosaccharide sugar components which could be fermented to ethanol.

Lignin is a phenolic substance consisting of an irregular array of variously bonded hydroxyl- and methoxy-substituted phenylpropane units. The lignin content of *Yushania alpina* is 25.27%. This result is higher than that of *Oxytenanthera abyssinica* reported by Amsalu et al. (2017) which is 22.47%. Whereas it is less than *Bambusa blumeana* (27.1%) and *Bambusa pervariabilis* (28.7%) as reported by Li et al. (2015). But it is in the range with the lignin content reported by Marriot et al. (2016) for softwood and bamboo, and by Kapu and Trajano (2014) which is 21-31% for bamboo species. Cellulose is a polymer of D-glucose consisting of linear chains bonded 1,4-β-anhydrogycosidic linkage. The cellulose content of *Yushania alpina* is 46.76%. This result is less than that reported for *Oxytenanthera abyssinica* by Amsalu et al. (2017) and within the range 40-45% which is reported by Marriot et al. (2016) and to that of 38-51% reported by Kapu and Trajano (2014) for bamboo. The cellulose content of *Yushania alpina* is higher than that reported by Li et al. (2015) for *Bambusa blumeana* (41.9%) and *Bambusa pervariabilis* (39.5%). The high cellulose content of *Yushania alpina* indicates as a potential resource for cellulosic ethanol production. Hemicelluloses are structural components of the cell wall with a low degree of polymerization and branched in structures which make it susceptible to hydrolysis. The hemicellulose content of *Yushania alpina* is 12.18% which is less than 16.90% of *Oxytenanthera abyssinica* by Amsalu et al. (2017) and 20-30 and 20-35% for softwood and hardwood, respectively, as reported by Marriot et al. (2016). It is also relatively lower than that reported by Li et al. (2015) for *Bambusa blumeana* (22.9%) and *Bambusa pervariabilis* (24.6%).
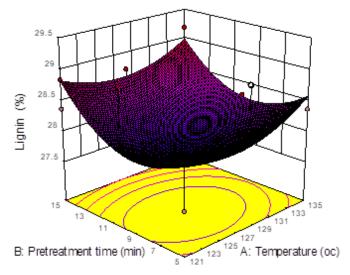
Effect of pressurized hot water pretreatment on the cellulose and lignin content of *Yushania alpina*

During hot water treatment of the biomass, an autohydrolysis of hemicelluloses occurred due to the weak acid which is catalyzed by hydronium ions (H_3O^+). At this stage, hydronium ions, the catalysts of hydrolysis, are coming from water auto-ionization at high temperature. The presence of hydronium ions leads to depolymerization of non-cellulosic carbohydrates by hydrolysis of both acetyl groups and glycosidic linkages (Song, 2013).

The cell wall structural components are essentially un-attacked at room temperature by neutral organic solvents and by cold water. The structural components (cellulose, hemicellulose (non-cellulosic polysaccharides) and lignin) of biomass are insoluble in water at mild conditions, except for some low molecular mass of the hemicelluloses, and pectins with low molar mass. However, cellulose, hemicelluloses, and lignin are all sensitive to thermal and chemical degradation, and hemicelluloses are the most sensitive (Levan et al.,1990; Winandy, 1995).

Fig. 7: 3D for the interaction effect of temperature and pretreatment time on cellulose content after hot water pretreatment

Fig. 8: 3D for the interaction effect of temperature and pretreatment time on lignin content after hot water pretreatment

During pretreatment of wood, the acetyl and side groups of hemicellulose are usually cleaved first followed by cleavage of the hemicellulose backbones (Sweet and Winandy, 1999). The amount of material dissolved by water increases significantly as the temperature is increased. The effect of hot water at a higher temperature and prolonged time dissociate both polysaccharides and lignin. The action of water at 150-175 °C leads to the formation of hydrolysis or degradation products such as sugars, uronic acids, and nonvolatile organic acids from carbohydrates. At higher temperature and in the presence of water the lignin became softened and partially plasticized (Song et al., 2008). Therefore, auto-hydrolysis of the amorphous
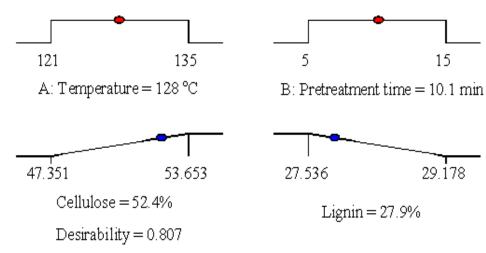
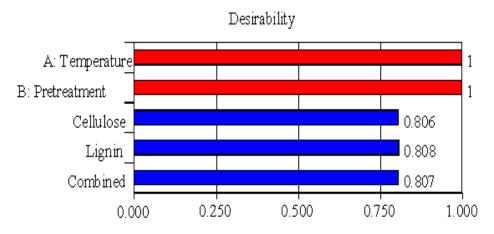**Fig. 9:** Ramp plot of optimization solution for the responses

Fig. 10: Desirability plot for the optimization solution for the responses

hemicelluloses is the dominating reaction taking place during the pretreatment of the bamboo sample. Hemicelluloses are the major components in the extracts, appearing mainly in oligo- and mono-form (Garrote et al., 1999; Carvalheiro et al., 2004).

The data were analyzed to determine the effect of temperature and pretreatment time on the decreasing amount of the lignin and increasing the cellulose content of Ethiopian highland bamboo. The dependent variables were lignin and cellulose content of the pretreated highland bamboo. All the experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors. The results are given in Table 3.

To see how well the model satisfies the assumptions of the analysis, the plots of residuals versus predicted were analyzed. Normal probability plot of the raw data used to check the assumption of normality when using t-test which shows the distribution of treatments. The plot in Figure 1 and 2 shows predicted vs. actual result of

cellulose and lignin, respectively, as shown in the plot the predicted and the actual value shows variation for both. But some data are distributed around the normal probability plot.

Effect of individual factors

The pretreatment of the lignocellulosic sample can be affected due to different factors starting from sample preparation, particle size, liquid to solid ratio, temperature, pressure and pretreatment time. The pretreatment step has a very high connection with each variable. The best way to observe the effects of these parameters on the pretreatment is to plot each factor vs. response with the assumption that other factors are constant.

Figure 3 and 4 show the effect of pretreatment time and temperature respectively on the amount of cellulose content after pretreatment. The effect of pretreatment time on the cellulose content shows that as the time increases the cellulose content increase in the first

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121 min and then started to decrease after it reaches the maximum time; the maximum cellulose content was observed around the 11th min pretreatment time.

As the pretreatment time increase, high cellulose content was found until it reaches its maximum of around 53% and then the cellulose content starts to decline as shown in Figure 3. The decline of cellulose content with pretreatment time is attributed to the hydrolytic effects in the amorphous region of the cellulose structure.

Slight decreasing effects on cellulose content observed at the temperature of 127 °C but this reduction slightly curved up until 135 °C (Figure 4). Whereas lignin content showed a decreasing trend until it reaches maximum delignification and the lignin content start to increase as the temperature increased as shown in Figure 5. The lignin amount in the sample started to decline sharply

Table 1: Experimental conditions for the pretreatments of Ethiopian highland bamboo culms

Factors	Unit	Level one	Level two	Level three
Temperature	°C	121	128	135
Hydrolysis time	Min	5	10	15

Table 2: The chemical composition of different lignocellulose material in dry based including the present study

Species	Moisture (%)	Ash (%)	Extractives (%)		Lignin (%)	Cellulose (%)	Hemi-cellulose (%)	Reference
			Hot-water	Ethanol-toluene				
Y. alpina	3.92±0.46	3.77±0.86	12.23±1.2	3.93±3.34	25.27±1.52	46.76±1.09	12.18±1.01	This study
O. abyssinica	-	5.30	6.80	5.60	22.47	52.06	16.90	Amsalu et al. (2017)
B. blumeana	-	-		8.0	27.1	41.9	22.9	Li et al.
B. pervariabilis	-	-		5.6	28.7	39.5	24.6	(2015)
Bamboo	-	0.9-4		6-13	21-31	38-51	-	Kapu and
Softwood	-	0.1-1		1-24	25-39	40-45	-	Trajano (2014)
Softwoods	-	-	-	-	25-35	40-45	20-30	Marriott et
Hardwood					18-25	40-55	20-35	al. (2016)

Table 3: Average cellulose and lignin content of *Yushania alpina* after pretreatment present in the pretreated substrate

Temperature (°C)	Pretreatment time (min)	Cellulose (%)	Lignin (%)
121	5	51.2±0	27.5±0
121	10	52.2±0	29.1±0
121	15	50.8±0.6	28.4±0.2
128	5	47.4±0	29.1±0
128	10	52.7±0	27.7±0
128	15	51.6±0	28.7±0
135	5	50.8±0	28.4±0
135	10	52.8±0	28.3±0
135	15	53.7±0	29.2±0

Table 4: Constraints applied for optimization of pressurized hot water pretreatment

Factors and response	Goal	Lower limit	Upper limit
A: Temperature (°C)	Is in range	121	135
B: Pretreatment time (min)	Is in range	5	15
Cellulose (% w/w)	Maximize	47.4	53.7
Lignin (% w/w)	Minimize	27.5	29.2

at the temperature of 128 ᵒC but this reduction curved up and the maximum amount was observed 135 ᵒC such condition can happen when either the extractive substances or the lignin monomers start to condense and form a pseudolignin. The lignin amount showed a decline as the pretreatment time increase and reached its lowest level but further increase in pretreatment time the result showed a reverse increasing trend (Figure 6). The increasing trend of the lignin in the second phase attributed to the condensation of the lignin monomers forming pseudo-lignin.

Interactive effect of factors

An interaction between process variables can affect the cellulose crystallinity and increase delignification. The interaction effect of pretreatment time and temperature presented in Figure 7 and 8 show the 3-D plot for cellulose and lignin, respectively. From the effect of the interaction of temperature and pretreatment time, a general increase in cellulose content was observed around a temperature of 128 ᵒC and 11ᵗʰ min pretreatment time. The reverse situation was also observed for lignin as the pretreatment time and temperature increased to a certain level the lignin content decreased but further increase in temperature and time resulted in an increase of the lignin level. Thus, indicating reverse condensation reactions within the lignin monomers and possibly with the sugar monomers.

Optimization

The process parameters; temperature and pretreatment time have been optimized in order to get a specific pretreatment time and temperature at which higher cellulose and lower lignin content can be achieved after pressurized hot water pretreatment. In optimizing the pressurized hot water pretreatment temperature and pretreatment time held to be "in range'' while the cellulose and lignin content after pretreatment are the two responses that need to "maximized'' and "minimized'', respectively. Table 4 shows the constraints applied for optimization of the pretreatment.

The predicted result for the maximum cellulose content and the minimum lignin content shows that the optimum temperature was 128 ᵒC and 10.1 min pretreatment time. Under this optimum condition, the predicted cellulose content and lignin contents were 52.4% and 27.9%, respectively. The desirability for the optimum parameters were 0.807. The ramp and the desirability plots are shown in Figure 9 and 10.

CONCLUSIONS

The chemical composition of Ethiopian highland bamboo (*Yushania alpina*) was found to contain 46.76±1.09% (w/w) cellulose, 25.27±1.52% (w/w) lignin and 12.18±1.01% (w/w) hemicellulose. From different pretreatment methods, the pressurized hot water pretreatment method was used due to its less environmental impact and low cost. Depending on the experimental result and the parameters used, the maximum cellulose content (52.4%) and the minimum lignin content (27.85%) were achieved under the optimum condition of 128 ᵒC and 10 min pretreatment time while before pretreatment the lignin and cellulose content were 25.27±1.52 and 46.76±1.09%, respectively. After the pretreatment of the highland bamboo the cellulose content increase significantly and this indicates that the highland bamboo (*Yushania alpina*) can be a potential feedstock for lignocellulose ethanol production.

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