Sci Forschen
Open HUB for Scientific Research

Journal of Environmental and Toxicological Studies
ISSN 2576-6430 | Open Access

RESEARCH ARTICLE

Volume 4 - Issue 1

Statistical Modeling and Optimization of Reducing Sugar Production for Enzymatic Digestibility of Bamboo Grass by Box-Behnken Design Methodology

Bulcha Belay Etana[1,2,*], Benny Malengier[1], Yasin Ahmed[2], Hundessa Daba Nemomssa[2] and Lieva Van Langenhove[1]

[1]Department of Materials, Textiles and Chemical Engineering, Ghent University, Ghent, Belgium

[2]Jimma Institute of Technology, Jimma University, Jimma, Ethiopia

***Corresponding author:** Bulcha Belay Etana, Department of Materials, Textiles and Chemical Engineering, Ghent University, Ghent, Belgium, E-mail: BulchaBelay.Etana@ugent.be ; getbulcha@gmail.com

Received: 04 Dec, 2020 | **Accepted:** 05 Jan, 2021 | **Published:** 13 Jan, 2021

Citation: Etana BB, Malengier B, Ahmed Y, Nemomssa HD, Langenhove LV (2021) Statistical Modeling and Optimization of Reduc-ing Sugar Production for Enzymatic Digestibility of Bamboo Grass by Box-Behnken Design Methodology. J Envi Toxicol Stud 4(1): dx.doi.org/10.16966/2576-6430.129

Abstract

Bamboo plants are plants that can grow quickly and easily, and is an abundant and renewable resource plants analogous to deciduous tree and conifer. In this study, bamboo species of *Yushania alpina* was pretreated with sodium hydroxide (NaOH) and hydrolyzed by dilute sulfuric acid to produce reducing sugar. Phenol-sulphur acid method was used to determine the response. Seventeen experimental runs were carried out at temperature range (110-128oC), concentration of H2SO4 range of (1-7%) and residence time range of (30-60 min) respectively. The effect of the parameters was measured by concentration of reducing sugar produced for each, and the optimization of glucose production was done by using Box-Behnken design methodology. The results reveal that, the temperature, concentration and time significantly affected the glucose production. At range of low temperature, concentration and time, the yield was decreased while at high value of factors the yield of reducing sugar increased. At 113.17oC, 3.43wt%, 33.65min and desirability of 0.71 the optimum reducing sugar of 19.9 found.

Keywords: Bamboo, Yushania alpine, Pretreatment, Acid hydrolysis, Reducing sugar, Phenol-sulphur acid method

Introduction

Production processes based on renewable resources has become one of the most important concerns of today, especially due to the depletion of non-renewable resources such as petroleum. In the past eras, the significant reduction in fossil-based resources containing petroleum, natural gas as well as coal, and conservational anxieties linked to the consumption of these resources, has led to attention more and more on renewable raw materials. It is known that plant-based raw materials and chiefly lingo cellulosic biomass have the prospective to exchange a major part of fossil-based resources for the manufacturing production of energy, chemicals, and materials. Lignocelluloses represent the most copious and lowest-cost biomass in the biosphere and, thus, can be used as substitute raw materials for the invention of fuel ethanol [1].

Bamboo, which goes to the Gramineae family (grasses), with a growth period of 3-8years, develops more quickly than any extra woods on the earth [2]. According to, Bamboo is a hopeful class for use as a feedstock in a biorefinery for the fabrication of pulp, bioethanol, and other chemicals because of numerous reasons. For instance, it not single matures profligate but also has a dissimilar chemical composition, short renovation, and easy propagation.

Bamboo is hypothetically anauspicious feedstock for unconventional bioethanol production due to geographies such as its speedy growth, constant wildlife, acceptance to risky climatic circumstances, and low controlling supplies. Internationally, Asia has the unlikeliest bamboo capitals, secretarial for 65% of worldwide bamboo resources. The foremost bamboo producing nations comprise India (11.4 million ha bamboo woods), China (5.4 million ha), and Indonesia (2 million ha) [3,4].

The major steps in ethanol production from lignocellulose biomass are treatment, hydrolyses, fermentation, and separation of products from spent. Because of the differentiation ability of hemicellulose and lignin from cellulose and, also surge the absorbency of supplies which recovers enzymatic scarification, pretreatment is an important step in ethanol production. There are more than a few rumors on the pretreatment of bamboo utilizing for example dilute sulfuric acid [5-10], solvent [11-13], hydrothermal treatment [14-15], steam [16-18], alkali [19-20]. All pretreatment techniques might have diverse properties both on the lignocellulosic assembly and on the development of inhibitory mixtures for microbial procedures.

Acid pretreatment chemicals are more caustic than alkaline chemicals such as sodium hydroxide. Pretreatments of biomass utilizing alkaline are conceded out at milder situations, specific of them even at ambient temperature, as demonstrated by wringing in NaOH, or NH_4OH. Such methods can eradicate the requirement for expensive materials and unusual designs to handle with deterioration and Spartan feedback conditions. Alkaline pretreatments are more effective in grass species than wood species in delignification [21].

The enzymatic activities of the yeast can be enhanced by applying the best hydrolysis process to convert cellulose to simple sugar for a high yield of bioethanol. A lot of literature is written about chemical and enzymatic hydrolysis approaches to enhance the ferment-ability of lignocellulosic material. Almost all researchers' focus on the enzymatic hydrolysis of bamboo [22-28]. A few studies have been done on chemical hydrolysis [29-33, 2].

More than 7% of the biosphere`s bamboo enclosed area is found in Ethiopia above 1ha [34], and which shelters 67% of African bamboo capitals. Currently, the bamboo (highland bamboo or lowland bamboo) found in Ethiopia is not utilized and there is no conversion of this resource into glucose and other chemicals. That is maybe due to a lack of awareness about the multiple uses of bamboo and scientific knowledge about their derived products and main properties [35]. Moreover, limited researches, especially in Ethiopia, was done on acid hydrolysis of bamboo and optimization of glucose production.

In the present study, pretreatment and hydrolysis of bamboo were done using sodium hydroxide and dilute sulfuric acid respectively. The hydrolysis process was improved by recycling the water and sulfuric acid, which could reduce the environmental impact of the whole process. The production of glucose was optimized using a design expert version 11.1.2.0 and results were analyzed by ANOVA.

Materials and methods

Raw material collection and preparation

Highland bamboo (*Yushania alpina*) stems, were harvested from Jimma (Ethiopia). The collected bamboo culm was permitted to dehydrate in the open air for a week. The dehydrated bamboo Culm was then expurgated into minor fragments and, desiccated in an oven (Labquip Ltd., UK) for 1day at 50°C. The desiccated bamboo culm was crushed into powder in a hammer mill (Glen Creston Ltd., UK) and filtered to 2mm particle dimensions [31] then store at room temperature for further work.

Pretreatment of Bamboo

The pretreatment process was done according to [31,32]. Accordingly, bamboo powder (dry weight) was pretreated with prepared reagents of NaOH, at a temperature of 860C for 2h at a 1:10wt ratio of solid and alkaline solution loading. After pretreatment, the solids (alkaline insoluble) were centrifuged and the solid remainder was desiccated in the open environment to removing moister content and preparing for the hydrolysis process.

Dilute acid hydrolysis of Bamboo

Pretreated bamboo residues were hydrolyzed with different concentrations sulfuric acid of (1, 4, and 7 wt %)to convert the cellulose to glucose. Hydrolysis lasted for a time of 30, 45, and 60 minutes, and at different temperatures of 110, 118, and 126°C.The liquid and remainder later hydrolysis were parted using a vacuum screen [31].

Total reducing sugar determination using the phenol-sulfuric acid method

The total reducing sugar after hydrolysis was resolved by phenol sulphur acid methods and the absorbance of the reduced was dignified with a UV-visible spectrophotometer (6505, Jenway Ltd. UK) at 540 nm wavelength of maximum glucose absorbance. Quantification was made from a standardization curve of maximum absorption against concentration of glucose as standard (Figure 1) and calculation was achieved by equation of the linear regression obtained from the standardization curve. The standard system was prepared as follows. 2 mL aliquot of a sample solution was mixed with 0.4 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 2 mL of concentrated sulfuric acid was added rapidly to the mixture. The test tubes were allowed to keep for 10 min at room temperature, and placed in a water bath for 20 min for color development. Absolute solutions were arranged in the same way as above, excluding that the 2 mL aliquot of a sample solution was substituted by distilled water [36].

Standard and reagent solution preparation

3.6 g of glucose in 100 mL of distilled water was dissolved to prepare a glucose stock solution. By pipetting a known volume of the stock solution (1, 2, 3, 4 and 5 mL) into a 100 mL volumetric flask, several thinning the stock glucose solutions were made and filling the capacity with distilled water as far as the mark. The concentrations completed for this study were: 0.036, 0.072, 0.108, 0.144 and 0.181 g/mL. To resolve the standardization curve for typical glucose, 2 mL of each of the typical solutions were pipetted out and taken into a distinct test tube. Then 0.4 mL of 5% aqueous solution of phenol chemical and 2 mL of 96% sulfuric acid were added. The combination was reserved for 10 min at room temperature and sited in a water wash for 20 min. Then the absorbance was read at 540 nm using a UV-visible spectrophotometer (6505, Jenway Ltd. UK). Blank solutions were ready in the same way as above, except that the 2 mL of the standard solution was replaced by distilled water. Then, the quantity of total reduced sugar content existing in the sample solution was calculated using the standard graph and expressed as gram glucose equivalents (GE) per 10 g of sample [36].

Experimental design

For experimental design, Response surface methodology (RSM) was implemented in this study. Mainly RSM is employed to reduce the number of investigational rounds needed to deliver adequate information for statistically satisfactory results. Box-Behnken Design (BBD) of response surface methodology is based on the construction of balanced incomplete block designs and requires at least three levels for each factor. The level of one of the factors is fixed at the center level while combinations of all levels of the other factors are applied in the Box-Behnken experimental design [37].This strategy requires an

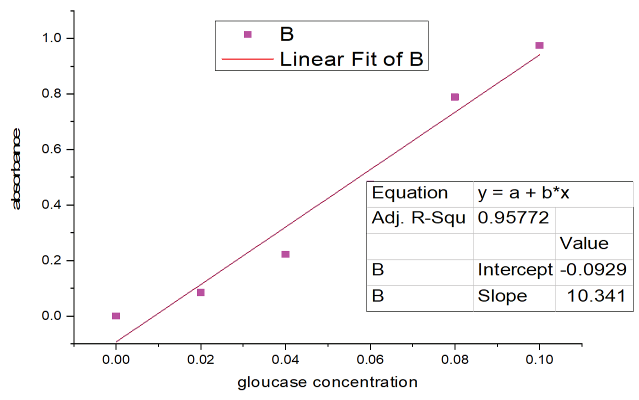

Figure 1: Standard calibration curve for glucose

experimental quantity of runs according to N=K^2+K+C_P. Kis the factor number (3) and C_P is the number of replications at the center point (5).

The effects of unexplained variability in the observed responses due to extraneous factors were maximized by randomized experimental work. The levels of the independent variables as shown in table 1 were selected based on preliminary experiments.

The three main factors selected for hydrolysis Bamboo were acid concentration, exposure time, and temperature with three levels for each factor. The factors among their levels were described in Table 1. The experiments were performed as a completely randomized design. The responses that were considered during the optimization of the Processing variables were reducing sugar.

Analysis of variance

The competence of the model was patterned by analysis of variance (ANOVA) and diagnostic plots. Analysis of variance (ANOVA) was engaged to test the implication of the developed models. The significance of the result was performed using analysis of variance (ANOVA) which was developed using the software (Stat-ease, Inc. Minneapolis, USA). A 2nd-degree polynomial was snug to the experimental data to approximate the response of the dependent variable and predict the optimal point.

Results and discussion

Measurement of Reducing Sugar

In this work, the total reduced sugar content was produced by the phenol sulfuric acid method was investigated (Figure 1). The effect of acid concentration, hydrolysis time, and temperature on the hydrolysis of bamboo was determined.

Statistical analysis

Usually, it is critical to approve first whether the fitted model delivers a satisfactory estimation of the real values or not. Table 2 shows the summary of the analysis of variance (ANOVA) of the reducing sugar. Values of p-values less than 0.05 indicate model terms are significant. In this study, A, B, C, A2, B2, C2, AC, BC are significant model terms, while AB was not significant. The Lack of Fit is not significant relative to the pure error since its value is 1.95. There is a 26.29% casual that a "Lack of Fit F-value" this large might happen due to noise. A non-significant lack of fit is good -- we need the model to fit.

For a respectable arithmetical model, the R^2 value (correlation coefficient) should be near to one. The result is given in (Table 3) and shows a reasonable modification of the quadratic model to the investigational data. It was indicated that approximately 99.79 % of the variability in the dependent variable reducing sugar could be explained by this model. The value of R^2 was very high and near to the one which shows a good agreement among experimental and forecast values. The "Pred R-Squared" of 0.9789 is in realistic agreement with the "Adj R-Squared" of 0.9953.

Table 1: Independent variables and levels used in the BBD for optimization for acid hydrolysis of bamboo (Yushaniaalpine).

Variable	Unit	Coded symbol	Coded levels		
			-1	0	1
Temperature	°C	A	110°C	119°C	128°C
Hydrolysis time	Minute	B	30	45	60
Concentration of H_2SO_4	wt %	C	1	4	7

Table 2: Analysis of variance for quadratic model acid hydrolysis of bamboo.

Source	Sum of Squares	D_f	Mean Squares	F value	p-value prob>F
Model	648.18	9	72.02	373.97	< 0.0001
A	11.12	1	11.12	57.72	0.0001
B	2.41	1	2.41	12.51	0.0095
C	387.53	1	387.53	2012.3	< 0.0001
A^2	66.34	1	66.34	344.5	< 0.0001
B^2	46.54	1	46.54	241.64	< 0.0001
C^2	102	1	102	529.66	< 0.0001
AB	0.036	1	0.036	0.19	0.6781
AC	5.83	1	5.83	30.28	0.0009
BC	2	1	2	10.4	0.0146
Residual	1.35	7	0.19		
Lack of Fit	0.8	3	0.27	1.95	0.2629

Table 3: Model adequacy measures for reducing sugar.

Std. Dev.	0.44	R-Squared	0.998
Mean	20.46	Adj R-Squared	0.995
C.V.	2.15	Pred R-Squared	0.979
PRESS	13.68	Adeq Precision	50.26

Effect of Process Parameters on the reducing sugar

Here, direct effect, interaction effect or a comparison between any two input parameters have been discussed and the third parameter would be on its central level. 3D surface plots were drawn by using BBD methods to examine the effect of all the independent variables on the out puts. The individual effect of factors was shown in figures 2-4.

Figure 2 demonstrates the effect of temperature at a fixed time on the glucose concentration. As it is shown (Figure 2), the total reduced sugar from bamboo was increase as temperature increases from 110°C to 119°C. The maximum reduced sugar was achieved at 119°C with 26.44g/ml. Nevertheless, when temperature increases beyond 119°C decrease in concentration was observed which is due to sugar being degraded into not fermentable products such as hydroxymethyl furfural (HMF) and furfural that are toxic to the cell of microorganisms [10].

Figure 3 shows the consequence of hydrolysis time on the concentration of reducing sugar at constant temperature and acid concentration in the center point. As observed in figure 3 the concentration was first positively affected by hydrolysis time, as the hydrolysis time increased from 30min to 45min. However, beyond 45min the concentration of sugars lightly decreased and reached 23.43g/ml in agreement with previously reported work [5, 38,33].

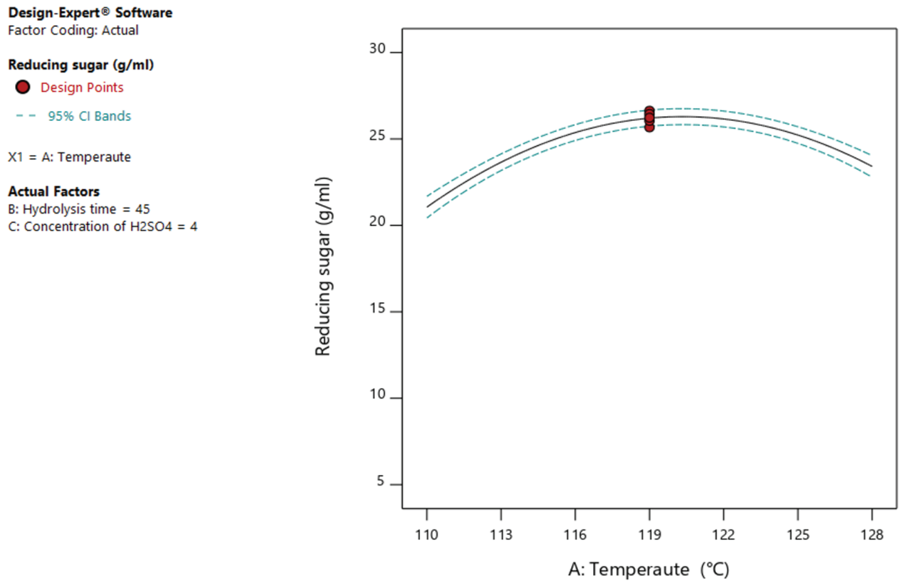

Figure 2: Effect of temperature on the reducing sugar concentration

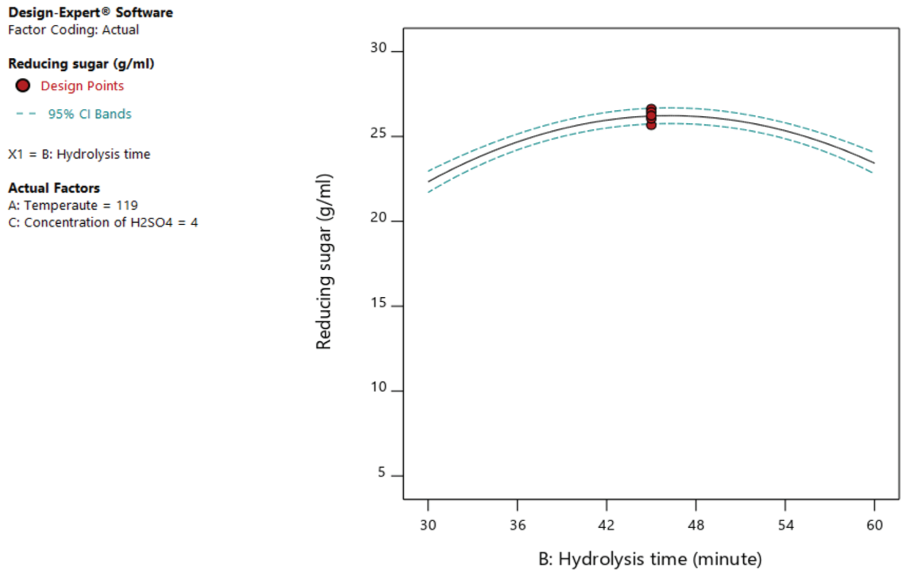

Figure 3: Effect of hydrolysis time on the reducing sugar concentration

At constant temperature and hydrolysis time, an increase in acid concentration increased the concentration of reducing sugar, as shown in figure 4. The curve (Figure 4) shows a sharp increase of reducing sugar up to 5%, there after the increase steadily falls and at 7% it's almost no further increase of reducing sugar. The decrement of reduced sugar content with increasing acid concentration may be due to the decomposition of the sugars and the development of some inhibitor such as hydroxymethylfurfural (HMF) and methyl furfural.

Not only single factors but also interaction factors affected reducing sugar concentration, as was clear from table 2. The collaboration effect of factors on the reducing sugar concentration was illustrated in figures 5-7.

Figure 5 shows the 3D plots developed as a function of temperature and time, while the acid concentration was kept at the center point on reducing sugar concentration. At lower hydrolysis time with increasing temperature, a slight decrease in reducing sugar was observed. However, at lower temperatures with increasing time the reducing sugar increased which higher lower than at lower exposure time with increased temperature. This is observed when the cellulose is exposed to high temperature, the sugar obtained from cellulose degraded into not fermentable products.

The interaction effect of acid concentration and temperature at constant time on reducing sugar was drawn (Figure 6). At a definite acid concentration, the sugar yield first increased slightly with

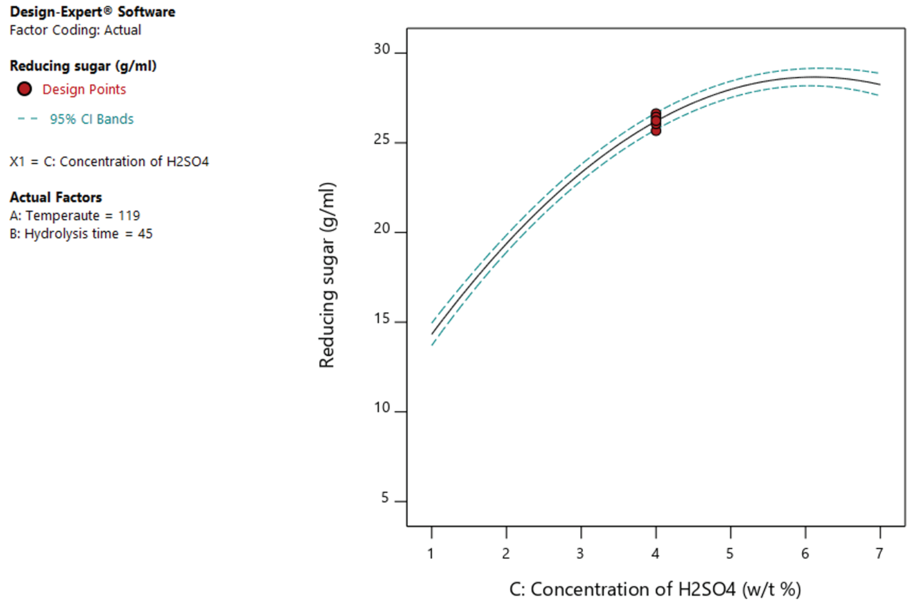

Figure 4: Effect of acid concentration on the reducing sugar concentration

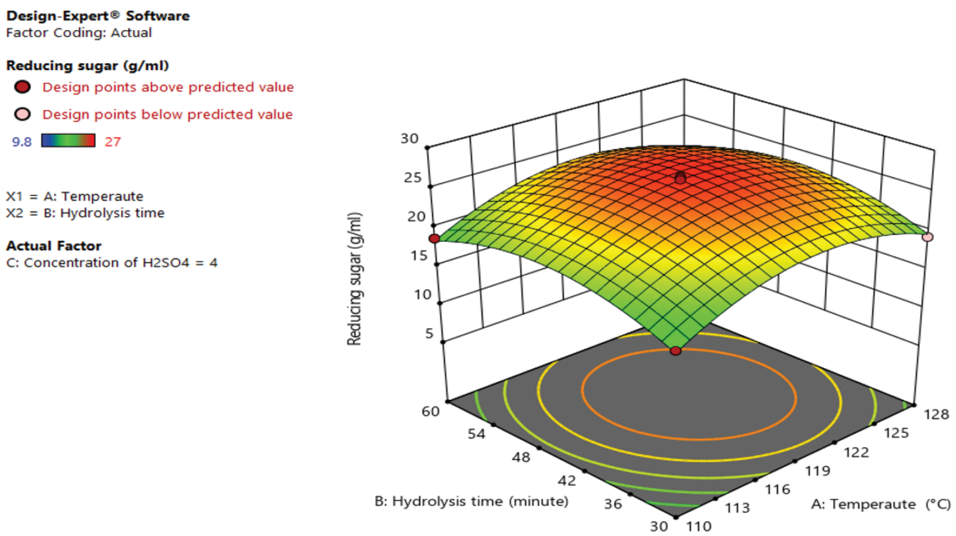

Figure 5: Interaction effects of hydrolysis time and temperature on the reducing sugar concentration

hydrolysis temperature up to a peak. However, upon further increasing the hydrolysis temperature there was a gradual decline in the yield. This means that, as the acid concentration increased the reducing sugar yield increased as the temperature decreased; for the reason, that sugar degrade to the toxic hydroxymethyl-furfural (HMF) which leads to decreased glucose yield might be occurred [26].

Figure 7 demonstrate the effect of acid concentration and hydrolysis time when the temperature was designated at the middle point. The extreme yield of sugar was observed at high acid concentration and hydrolysis time. At increasing acid concentration, and time the yield of reducing sugar became increases since the possible development of additional particles instead of glucose formation or the conversion sugars such as glucose and xylose in to other fermentation inhibitors.

Optimizations

Optimization of reducing sugar concentration was carried out by a numerous output method called desirability (D) function to improved is similar groupings of process parameters. The objective of optimization was to exploit economic benefit or increasing sugar concentration by reducing process costs, so a lower temperature, a short hydrolysis time, and low acid use. The conditions to investigate the optimum values of sugar from bamboo through dilute acid hydrolysis were summarized in table 4.

The optimum possible solutions in the hydrolysis of bamboo for the production of total reducing sugar were presented in figure 8. The predicted optimum yield of TRS was 19.97g/ml observed at the process variables 113.17°C, 33.65min, and 3.43wt% at 0.71 desirability.

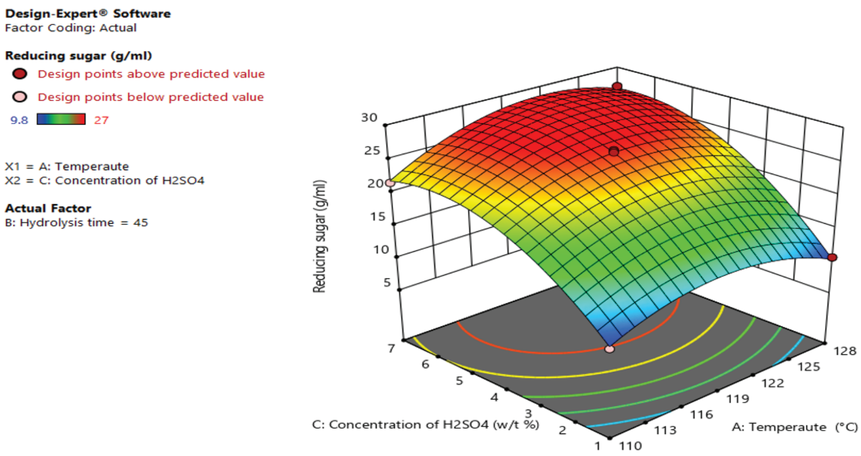

Figure 6: Interaction effects of temperature and acid concentration on the reducing sugar concentration

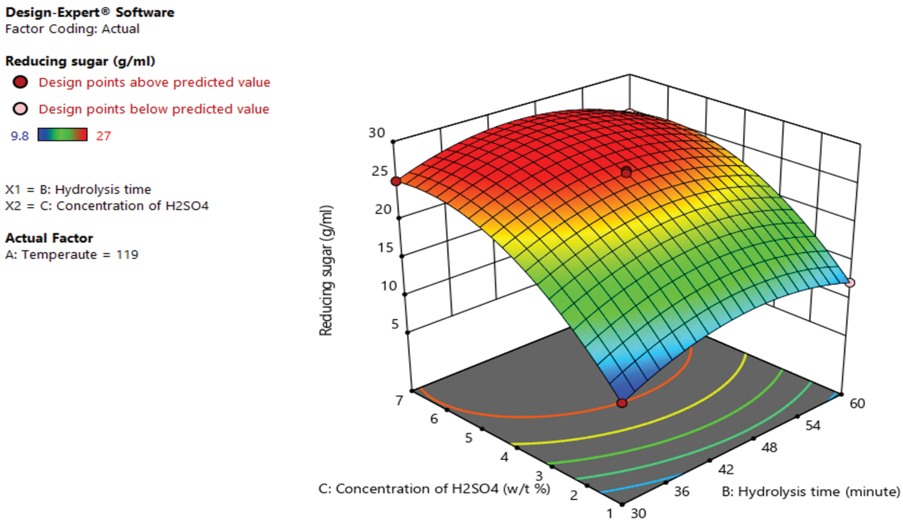

Figure 7: Interaction effects of hydrolysis time and acid concentration on the reducing sugar concentration

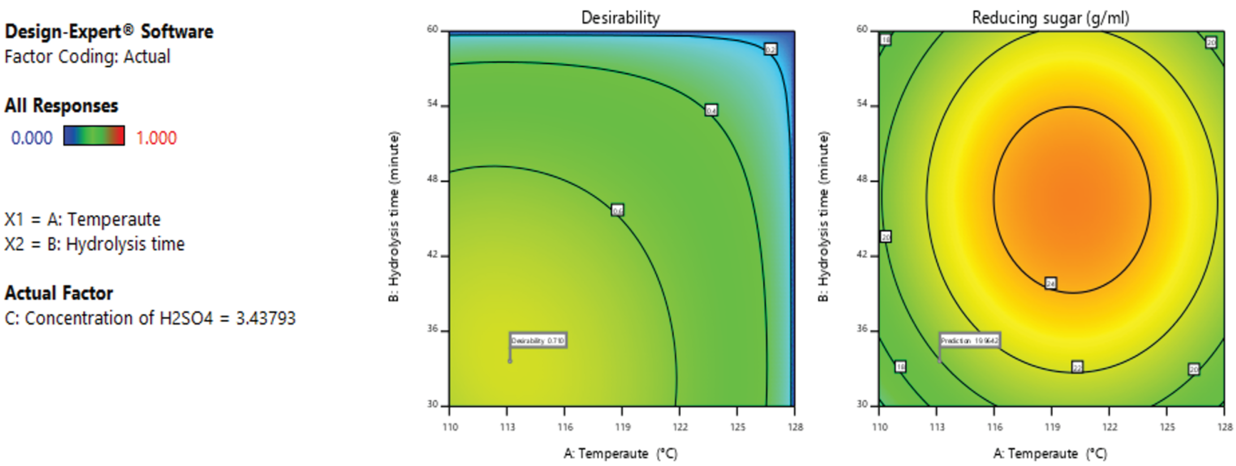

Figure 8: Contours plot of predicted reducing sugar concentration at fixed acid concentration

Table 4: Optimization criteria for optimum concentration of reducing sugar.

Name	Goal	Lower limit	Upper limit
Temperature (°C)	Minimize	110	128
Hydrolysis time (minute)	Minimize	30	60
Acid concentration (wt%)	Minimize	1	7
Concentration of reducing sugar (g/ml)	Maximize	9.8	27

Conclusion

The production process of monosaccharaides from lignocellulosic biomass was effective through optimization of independent variables. The current study indicates that RSM with engaging BBD has provided a consistent and precise procedure in optimizing the reducing sugar production from the bamboo species *Yushania alpina*. The independents variables: temperature, hydrolysis time and acid concentration were affecting the concentration of reducing sugar individual and interaction each other. In the order of single effect: acid concentration temperature and hydrolysis time were affect mainly the production process respectively. The optimum condition of reducing sugar was obtained at temperature, acid concentration and hydrolysis time of 13.179°C, 3.43wt% and 33.65 min respectively with the desirability of 0.71.

Acknowledgements

The authors would like to express appreciation for the support of the NASCERE. The NASCERE project was sponsored with funds from the Ethiopian Government Bulcha Belay Etana.

References

1. Z. Hu and Z. Wen (2008) Enhancing enzymatic digestibility of switchgrass by microwave-assisted alkali pretreatment. Biochem Eng J 38: 369-378.
2. J. Zhuang and X. Li (2012) Hydrolysis of bamboo fiber for production of fermentable sugars in saturated formic acid. Adv Mater Res 535-537: 2442-2445.
3. Z. Yuan, Y. Wen, N. S. Kapu, R. Beatson, and D. Mark Martinez (2017) A biorefinery scheme to fractionate bamboo into high-grade dissolving pulp and ethanol. Biotechnol. Biofuels 10: 1-16.
4. R. J. Murphy. L. Wanga, J. Littlewoodb (2012) An economic and environmental evaluation for bamboo-derived bioethanol. RSC Adv 1-8.
5. J. song, Shangxing Chen, Zongde Wang, Kai Fang, Guorong Fan, et al. (2014) Impact of Dilute Sulfuric Acid Pretreatment on Fermentable Sugars and Structure of Bamboo for Bioethanol Production. BioResources 9: 7636–7652.
6. N. Tippayawong and N. Chanhom (2011) Conversion of Bamboo to Sugars by Dilute Acid and Enzymatic Hydrolysis. Int J Renew Energy Res 1: 240-244.
7. X. Luo, Z. Gong, J. Shi, L. Chen, W. Zhu, et al. (2020) Integrating Benzenesulfonic Acid Pretreatment and Bio-Based Lignin-Shielding Agent for Robust Enzymatic Conversion of Cellulose in Bamboo. Polymers (Basel) 12.
8. X. Guo. Bo Hong, Guoxin Xue, Liqing Weng (2012) Pretreatment of Moso Bamboo with Dilute Phosphoric Acid. BioResources 7: 4902-4913.
9. W. Leenakul and N. Tippayawong (2010) Dilute Acid Pretreatment of Bamboo for Fermentable Sugar Production. J Sustain Energy Environ 1: 117-120.
10. R. Sindhu, M. Kuttiraja, P. Binod, R. K. Sukumaran, and A. Pandey (2014) Bioethanol production from dilute acid pretreated Indian bamboo variety (*Dendrocalamus sp*.) by separate hydrolysis and fermentation. Ind Crops Prod 52: 169-176.
11. Yan Y. Zhiqian Li, Zehui jaing, Benhua Fei, Xuejun Pan, et al. (2012) Ethanol organosolv pretreatment of bamboo for efficient enzymatic saccharification. Bioresources 7: 3452–3462.
12. J. Liu, Z. Gong, G. Yang, L. Chen, L. Huang, et al. (2018) Novel Kinetic Models of Xylan Dissolution and Degradation during Ethanol Based Auto-Catalyzed Organosolv Pretreatment of Bamboo. Polymers (Basel) 10.
13. N. Sathitsuksanoh, Z. Zhu, T. Ho, M. Bai, and Y. P. Zhang (2010) Bamboo saccharification through cellulose solvent-based biomass pretreatment followed by enzymatic hydrolysis at ultra-low cellulase loadings. Bioresour. Technol 101: 4926-4929.
14. X.J.Ma, S.L.Cao, L.Lin, X.L.Luo, H.C.Hu, et al. (2013) Hydrothermal Pretreatment of Bamboo and Cellulose Degradation. Bioresour Technol 148: 408-413.
15. C. Q. Lingzhi Huang, Zeguang Yang, Mei Li, Zhaomeng Liu, S. N. et al. (2020) Effect of Pre-Corrected pH on the Carbohydrate Hydrolysis of Bamboo during Hydrothermal Pretreatment. Polymers (Basel) 12: 1–16.
16. Z. Yuan, G. Li, W. Wei, J. Wang, and Z. Fang (2020) A comparison of different pre-extraction methods followed by steam pretreatment of bamboo to improve the enzymatic digestibility and ethanol production. Energy 196: 117156.
17. Z. jiang, Zhiqiang Li, Benhua fei (2015) Effect of Steam Explosion Pretreatment on Bamboo for Enzymatic Hydrolysis and Ethanol Fermentation. Bioresources 10: 1037-1047.
18. Y. Xing, H. Yu, L. Zhu, and J. Jiang (2013) Efficient Enzymatic Hydrolysis of Bamboo by Pretreatment with Steam Explosion and Alkaline Peroxide. Bioresources 8: 5392-5408.
19. N. Ali, A. S. Giwa, M. Abdalla, X. Liu (2020) Alkaline hydrogen peroxide pretreatment of bamboo culm for improved enzymatic release of reducing sugars using recombinant cellulases. Cellulose 27: 769-779.
20. S. Qian, H. Mao, K. Sheng, J. Lu, Y. Luo, and C. Hou (2013) Effect of Low-Concentration Alkali Solution Pretreatment on the Properties of Bamboo Particles Reinforced Poly (lactic acid) Composites. Applied Polymer 1667-1674.
21. J. Seok, Y. Y. Lee, and T. Hyun (2016) A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. Bioresour Technol 199: 42-48.
22. X. Z. Ã, H. Yu, H. Huang, and Y. Liu (2007) Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms. 60: 159-164.
23. Z. Li, Z. Jiang, B. Fei, Z. Cai, and X. Pan (2014) Comparison of bamboo green , timber and yellow in sulfite, sulfuric acid and sodium hydroxide pretreatments for enzymatic saccharification," Bioresour Technol 151: 91-99.
24. W. Lin, S. Xing, Y. Jin, X. Lu, C. Huang, et al. (2020) Insight into understanding the performance of deep eutectic solvent pretreatment on improving enzymatic digestibility of bamboo residues. Bioresour Technol 306: 1231-1263.

25. T. Shimokawa, M. Ishida, S. Yoshida, and M. Nojiri (2009) Effects of growth stage on enzymatic saccharification and simultaneous saccharification and fermentation of bamboo shoots for bioethanol production. Bioresour Technol 100: 6651-6654.

26. X. Xiao, J. Bian, M. Li, H. Xu, B. Xiao, and R. Sun (2014) Enhanced enzymatic hydrolysis of bamboo (*Dendrocalamus giganteus Munro*) culm by hydrothermal pretreatment. Bioresour Technol 159: 41-47.

27. D. Xin, Z. Yang, F. Liu, X. Xu, and J. Zhang (2015) Comparison of aqueous ammonia and dilute acid pretreatment of bamboo fractions : Structure properties and enzymatic hydrolysis. Bioresour Technol 175: 529-536.

28. Z. Yang, M. Zhang, D. Xin, J. Wang, and J. Zhang (2014) Evaluation of aqueous ammonia pretreatment for enzymatic hydrolysis of different fractions of bamboo shoot and mature bamboo. Bioresour Technol 173: 198-206.

29. Z. Sun, Y. Tang, S. Morimura, and K. Kida (2013) Reduction in environmental impact of sulfuric acid hydrolysis of bamboo for production of fuel ethanol. Bioresour Technol 128: 87-93.

30. Y. S. and L. LIN (2010) Hydrolysis Behavior of Bamboo Fiber in Formic Acid Reaction System. J Agric Food Chem 58: 2253-2259.

31. T. Mahelete, C. B. Singh, F. Sisay, and R. Mesfin (2019) Cellulosic ethanol production from highland bamboo (*Yushania alpina*) grown in Ethiopia. Biofuels.

32. S. Kassaye, K. K. Pant, and S. Jain (2017) Hydrolysis of cellulosic bamboo biomass into reducing sugars via a combined alkaline solution and ionic liquid pretreament steps. Renew Energy 104: 177-184.

33. L. T. Zhao-Yong Sun, Ting Wang and K. K. Kida, Yue-Qin Tang (2015) Development of a more efficient process for production of fuel ethanol from bamboo. Bioprocess Biosyst Eng 38: 1033-1043.

34. E. G. Aklilu (2020) Optimization and modeling of ethanol–alkali pulping process of bamboo (*Yushania alpina*) by response surface methodology. Wood Sci 54: 1319-1347.

35. K. Embaye (2000) The Indigenous Bamboo Forests of Ethiopia : An Overview. A J Hum Environ 29: 518-521.

36. A. A. Albalasmeh, A. A. Berhe, and T. A. Ghezzehei (2013) A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. Carbohydr Polym 97: 253-261.

37. M. A. Tekindal, I. Katip, C. Universitesi, and Y. Yavuz (2012) Box-behnken experimental design in factorial experiments: The importance of bread for nutrition and health. Turkish J F Crop 17: 115-123.

38. A. R. Woldu and Y. A. Tsigie (2015) Optimization of Hydrolysis for Reduced Sugar Determination from Avocado Seed Wastes. Am J Environ Energy Power Res 3: 1-10.