

OPTIMIZATION OF HYDROLYSIS IN ETHANOL PRODUCTION FROM BAMBOO

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Abstract. This research involved optimizing acid hydrolysis in the development of ethanol, a promising alternative energy source for restricted crude oil, from lignocellulosic materials (bamboo). The conversion of bamboo to ethanol can mainly be accomplished through three process steps: pretreatment of bamboo wood for the removal of lignin and hemicellulose, acid hydrolysis of pretreated bamboo for the conversion of cellulose into sugar reduction (glucose) and fermentation of sugars into ethanol using anaerobic *Saccharomyces cerevisiae*. The effects of parameters (factors) in the hydrolysis step were investigated and the optimum combination of parameters values (temperature, time and acid concentration) was set by experimentation. Factorial design of three-factors-at-two-level with a replica of two ($2^3 = 8$, $8 \cdot 2 = 16$) was applied to the hydrolysis step to investigate the effect of hydrolysis parameters on the response variable (ethanol yield) using Design-Expert® 7 software.

Keywords: bamboo ethanol, fermentation, hydrolysis, optimization.

1. Introduction

The use of bioethanol can reduce our dependence on fossil fuels, while reducing net emissions of carbon dioxide, the main greenhouse gas.¹ The feedstock used for biofuels has been categorized into three major groups, cellulose biomass, sugar and starchy crops, and oil-producing plants.² Interest is currently focused on the first group also referred to as a biofuel of the second generation. This is because there are conflicts with food production for human and animal consumption in the second and third groups.³

Brazil and the US together accounted for about 60.0 % of the world ethanol production exploiting sugarcane and corn, respectively.⁴ However, using these food crops for ethanol production may raise concerns of food security

environmental degradation debate and other issues. Fortunately, there is a growing interest worldwide to develop new and cheaper carbohydrate sources for production of bioethanol.⁵ The most attractive feedstock source is the lignocellulosic biomass, from which ethanol or other chemical agents can be produced *via* scarification and fermentation.⁶

Lignocellulosic biomass is the primary and most abundant organic material on the earth which makes it the most promising resource for the alternative energy.⁷ Among the available lignocellulosic feedstocks, bamboos are receiving a renewed interest due to their high growth rate and better reduction of carbon footprint compared to an equivalent area of woody plants.⁸

The high overall cost of the cellulosic biofuel supply chain (CBSC) is the principal explanation for this enormous difference between the target and actual output. Researchers and industrial societies have made efforts to reduce the cost of industrialization of cellulosic biofuels using different approaches to tackle this issue, including the supply chain optimization and management.⁹

A well-planned supply chain can help to promote the adoption of cellulosic biofuel since it has the great potential to enhance economic viability.¹⁰ A typical CBSC consists of five entities: biomass collector, biomass inventory, biorefinery, biofuel storage, and end-product distributor.

Many studies have investigated CBSC design and optimization considering multiple aspects, including location selection,^{11,12} feedstock uncertainty,^{13,14} economic performance,^{15,16} transportation,^{17,18} financial risk,¹⁹⁻²¹ and energy consumption.²²

A number of lignocellulose pre-treatment technologies existed in both laboratory scales and as pilot plants, such as dilute acid, flow-through, ammonia fiber explosion, ammonia recycle percolation, lime, steam explosion, and organosolv (OS) pre-treatment which have suffered from relatively low sugar yields, severe reaction conditions, large capital investment, or high processing costs.²³ Recently, a novel fractionating recalcitrant lignocellulose technology under modest reaction conditions was developed. Based on this technology, three components existed in lignocellulosic materials will be separated for further use. The cellulose component will be used for ethanol *via* enzyme scarification and fermentation.²⁴

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Another barrier or challenge is the absence of robust organisms for ethanol production. Currently, different recombinant strains have been engineered to produce ethanol from lignocellulosic biomass, such as genetically engineered *Saccharomyces cerevisiae*, *Escherichia coli*, *Klebsiella oxytoca*, and *Zymomonas mobilis*, which provide a basis for constructing an industrially suitable engineered strain on cellulose ethanol industrialization. Among all these strains, *Z. mobilis* used historically in tropical areas to make alcoholic beverages from plant sap, showed fast growth rates and high specific ethanol production compared with *S. cerevisiae*. The advantages that *Z. mobilis* holds over traditional yeast processes have led to more economical methods of producing ethanol.²⁵⁻²⁷ However, its narrow spectrum of fermentable carbohydrates has limited its use, especially for fuel ethanol production from lignocellulosic materials.²⁸

Pure natural bamboo forest in Ethiopia is the largest in Africa, over 1 million ha, and 85 % of this area is covered by lowland bamboo.²⁹ The uses of bamboo as cellulosic feedstocks are very important because of bamboo fast growth; they require marginal and uncultivated lands. This study is very important because bamboo is a widely available plant and the production of ethanol from bamboo will bring about a cheaper feedstock source of ethanol and also ethanol from bamboo will be much better for the environment and human health than other regular energy fuels and reduce fossil fuel dependence on imported oil increase our energy security, reduce our trade deficit, and rural economies can benefit in the form of increased incomes and jobs. It should be regarded as an attractive feedstock for ethanol production. Recently, some studies also indicated bamboo culms and bamboo residues could be exploited as the feedstock of biomass energy, such as ethanol and methanol for its high productivity. Bamboo is one of the potential non-timber species to be commercially planted in Ethiopia.²⁵

Lignocellulosic biomass is the primary and most abundant organic material on the earth which makes it the most promising resource for the alternative energy.¹ Among the available lignocellulosic feedstock, bamboos are receiving a renewed interest due to their high growth rate and better reduction of carbon footprint compared to an equivalent area of woody plants.² Bamboos are a group of perennial evergreens belonging to the true grass family and enjoying wide distribution in India, especially in the north eastern region, where it is an important resource with multiple applications.³

Therefore, this study investigates bamboo as the source of carbon and the optimization of the medium for ethanol production. The design expert was employed to screen the effects of different medium ingredients on ethanol yield in order to perform this study and further optimization of the medium was carried out using the response surface methodology.

2. Experimental

2.1. Equipment and Chemicals

Polyethylene plastic bags were used to store the sample, a grinding machine was used for grinding and homogenizing of the samples. Measuring cylinders (Duran, Germany) and deionizer (type 04/05, Italy) were used to remove ions from water. Vessels, hydrometer, pH-meter, vertical autoclave, cutting mill, autoclavable bioreactor, shaker, funnel, sieves, digital balances, vacuum filter, rotary evaporator and sulfuric acid (70 % Spectrosol, BDH, England), sodium hydroxide (30 %, Riedel de Haen), yeast extract, urea, dextrose sugar, Mg SO₄·7H₂O and yeast (*Saccharomyces cerevisiae*) were used for the experiments.

2.2. Sample Preparation

Bamboo was collected from Bamboo selling center in Gondar; it was collected in plastic bags and dispatched to the laboratory for further work. 3 kg of bamboo was taken and it was cut by knife into pieces of about 3–5 cm length for ease drying and grinding. Sample drying was carried out in the oven (SMO5CR-2 clean room oven includes a HEPA filter, a Watlow Controller and three adjustable air intake) at 378 K. After drying, the samples were weighed and crushed in the cutting mill (D51820007W). The maximum particle size of the ground mixed sample was 2 mm. The sample with particle size larger than 2 mm was ground over and over again until all particle size was 2 mm (Clean the sieves of sieve shaker using a cleaning brush if any particles are struck in the openings, record the weight of each sieve and receiving pan, dry the specimen in the oven for 3–4 min to get the dried specimen (ignore, if the specimen is already dried), weigh the specimen and record its weight. Finally, the sieves were arranged in the following order: the smaller openings sieve in the bottom and larger openings sieve on the top using BS410 Standard sieves (0.1 g accuracy balance). The sample was then kept at a temperature 298 K until the next stage of experiment.

2.2.1. Steam pretreatment

The powder bamboos were treated at a temperature of 393 K. First, the bamboo powder was treated and it was fed as batches, every batch contains 100 g of screened bamboo powder with 5:1 (v/w) ratio of water to the sample with 0.75 % of sulfuric acid, the pressure 202.65 kPa was constant during the treatment process for all the batches. The retention time for every batch was half an hour similar to the other batches.¹¹ Finally, the samples were kept in a Vertical autoclave (“Tempo”) for the given pretreatment time and pretreatment temperature and were allowed to cool.

2.2.2. Hydrolysis

The three-parameter-two-level ($2^3 = 8$, $8 \cdot 2 = 16$) factorial design was applied to the hydrolysis step of the experimentation. The hydrolysis experiments for ethanol production and optimization were conducted in a

completely randomized design using Design-Expert® 7 software. 100 g of grinding bamboo chips used for each experiment and the factors for hydrolysis time were (15 and 30 min), hydrolysis temperature (363 and 383 K), and acid concentration (1 and 3 %) each at two level and two replicas (see Table 1)

Table 1. Ethanol yield in the experiments

Run No	Time, min	Temperature, K	Acid concentration, %, v/v	Yield, %	* Yield, %
1	15	363	1	37.6	38.6
2	15	363	3	43.2	39.5
3	15	383	1	37.0	38.6
4	15	383	3	42.3	39.5
5	30	363	1	41.4	43.3
6	30	363	3	38.0	38.6
7	30	383	1	43.3	41.4
8	30	383	3	37.7	35.0

Note: *replica

2.2.3. Fermentation

Before fermentation there was pH adjustment and sterilization of the reactor then the clear solution went to fermentation. The fermentation was carried out under anaerobic condition at a temperature of 303 K and at 150 rpm for 3 days. Before conducting fermentation, we have to prepare the media for the yeast. We needed the favorable condition for yeast growth or required amount of nutrients. For preparing 200 mL of media, we mixed sugar (dextrose) in amount of 20 g, yeast extract (0.4 g), urea (2.0 g), prepared water (200 mL) and $Mg\ SO_4 \cdot 7H_2O$ (2.0 g).

2.2.4. Distillation

In this experiment, the separation was done by rotary evaporator at a temperature of 358 K. Finally, the data was analyzed by Design-Expert® software. Significance of the result was set from analysis of variance (ANOVA).

3. Results and Discussion

Statistical analysis of the experimental results temperature, acid concentration and time hydrolysis are the key variables in this process (Table 1) and the resulting data using Design-Expert® software (Table 2). As well as the design summary for three variables and 2-level factorial design (Table 3).

In order to determine whether or not the quadratic polynomial model is significant for the experiments, it was necessary to conduct analysis of variance. The probability (p-values) values were used as a tool to check the significance of each coefficient, which also indicated

the interaction strength of each parameter. The smaller the p-values are, the bigger the significance of the corresponding coefficient.

The results of statistical analysis including the estimated values of factors' coefficients, interactive terms, F-value, and p-values are shown in Table 4. The larger magnitude of the F-value and the smaller magnitude of the p-value indicate more significance of the corresponding coefficient. Acid concentrations in linear and quadratic polynomial model are highly significant for the yield of ethanol ($p < 0.05$). Among the interactive terms, only the interaction between time and acid concentration was highly significant.

Using the designed experimental data (Table 3) the quadratic polynomial model for ethanol production from bamboo by the dilute acid hydrolysis was retreated and shown as below:

Final equation in terms of coded factors:

$$\text{Ethanol yield} = +39.65 + 0.11A - 0.30B - 0.50C - 0.11AB - 2.09AC - 0.23BC - 0.19ABC \quad (1)$$

Final equation in terms of actual factors:

$$\begin{aligned} \text{Ethanol yield} = & +34.16250 + 0.22167 \cdot \text{time} - \\ & - 0.063750 \cdot \text{temperature} + 2.38750 \cdot \text{acid concentration} + \\ & + 3.50000E^{-003} \cdot \text{time} \cdot \text{temperature} - \\ & - 0.028333 \cdot \text{time} \cdot \text{acid concentration} + \\ & + 0.033750 \cdot \text{temperature} \cdot \text{acid concentration} - \\ & - 2.50000E^{-003} \cdot \text{time} \cdot \text{temperature} \cdot \text{acid concentration} \quad (2) \end{aligned}$$

The actual *versus* predicted values using the model in the above equation are tabulated in Table 5. Regression analysis based on the coded variables on the experimental data was performed, and coefficients of the second-order models were calculated. Substitution of coefficients calculated and response variables in the above equation resulted in the empirical equations for ethanol yields.

To see how the quadratic polynomial model satisfies the assumptions of the analysis of variance (ANOVA) in the experiments, plots of Table 4 were analyzed in Fig. 1 in terms of normal plots of residuals and residual versus predicted values. The normal

probability plot (Fig. 1) indicates the residuals following a normal distribution, in the case of this experiment the points in the plots show fit to a straight line indicating that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA).

Table 2. The resulting data using Design-Expert® software

Run	Time, min	Temperature, K	Acid concentration, %, v/v	Yield, %
1	15.00	363	3.00	43.2
2	15.00	363	1.00	37.6
3	30.00	383	3.00	37.7
4*	15.00	363	3.00	39.5
5	30.00	363	1.00	41.4
6	15.00	383	3.00	42.3
7	30.00	383	1.00	43.3
8	15.00	383	1.00	37.0
9*	15.00	363	1.00	38.6
10*	30.00	363	1.00	43.3
11*	15.00	383	1.00	38.6
12	30.00	363	3.00	38.0
13*	15.00	383	3.00	39.5
14*	30.00	363	3.00	38.0
15*	30.00	383	3.00	35.0
16*	30.00	383	1.00	41.4

Note: *replica

Table 3. Design summary for three variables and 2-level factorial design

Design Summary	
Study Type	Factorial
Initial design	2 Level Factorial
Center Points	0
Design Model	Quadratic Polynomial
Runs	16
Blocks	No Blocks

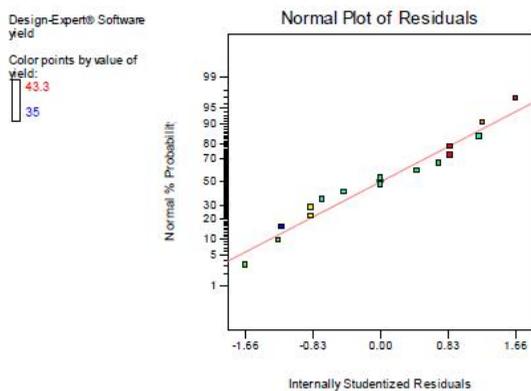
Table 4. Analysis of variance for quadratic polynomial model

Source	Sum of squares	df	Mean squares	F Value	p-value p > F
Model	76.94	7	10.99	4.44	0.0164*
A-time	0.20	1	0.20	0.082	0.0821
B-Temperature	1.44	1	1.44	0.58	0.0675
C-acid concentration	4.00	1	4.00	7.62	0.0393
AB	0.20	1	0.20	0.082	0.7821
AC	69.72	1	69.72	28.17	0.0007
BC	0.81	1	0.81	0.33	0.5830
ABC	0.56	1	0.56	0.23	0.6463
Pure Error	19.80	8	2.48		
Cor Total	96.74	15			

Note: *significant

Table 5. Actual *versus* model predicted for ethanol yields

Diagnostics case statistics				Internal	External studentized residual	Run order
Standard value	Actual value	Predicted value	Residual			
1	37.60	38.10	-0.50	-0.449	-0.426	2
2	38.60	38.10	0.50	0.449	0.426	9
3	41.40	41.40	-0.95	0.854	-0.838	5
4	43.30	43.35	0.95	0.854	0.838	10
5	37.00	37.80	-0.80	-0.719	-0.696	8
6	38.00	37.80	0.80	0.719	0.696	11
7	43.30	42.35	0.95	0.854	0.838	7
8	41.40	42.35	-0.95	-0.854	-0.838	16
9	43.2	41.35	1.85	1.663	1.923	1
10	39.50	41.35	-1.85	-1.663	-1.923	4
11	38.00	38.00	0.000	0.000	0.000	12
12	38.00	38.00	0.000	0.000	0.000	14
13	39.50	40.90	-1.40	-1.259	-1.315	13
14	42.30	40.90	1.40	1.259	1.315	6
15	37.70	36.35	1.35	1.214	1.257	3
16	35.00	36.35	-1.35	-1.214	-1.257	15

**Fig. 1.** Normal plots of residual

3.1. Optimization

The optimization hydrolysis criteria for ethanol production from bamboo dilute acid are summarized as follows (Table 6). The optimum possible solutions for acid hydrolysis are determined by various factors and the ethanol yield has a complex relationship with independent variables that include first, second and third-order polynomials and may have more than one limit point. The best way of expressing the effect of any parameter on the yield within the experimental space under investigation was to produce response surface plots of the equation. The three-dimensional response surfaces, contours and interactions were plotted in Figs. 2 and 3.

Table 6. Optimization criteria for optimum ethanol yield

Solutions number	Time, min	Temperature, K	Acid concentration, %	Yield, %	Desirability
1	17.03	363.00	1.00	38.6757	0.887 Selected
2	17.18	363.00	1.00	38.7165	0.887
3	16.82	363.00	1.00	38.6171	0.887
4	16.88	363.00	1.01	38.6435	0.886
5	16.35	363.00	1.00	38.4833	0.886
6	16.39	363.00	1.01	38.5087	0.886
7	16.15	363.00	1.00	38.4266	0.886
8	18.05	363.00	1.00	38.9652	0.885
9	17.41	363.11	1.00	38.7808	0.885
10	15.82	363.00	1.04	38.8841	0.884
11	15.90	363.00	1.09	38.4786	0.883
12	18.63	363.13	1.00	39.1261	0.882
13	15.00	363.00	1.05	38.1764	0.882
14	19.64	363.16	1.00	39.3818	0.844

Name	Goal	Lower limit	Upper limit
Time	Minimize	15	30
Temperature	Minimize	90	110
Acid Concen.	Minimize	1	3
Yield	Maximize	35	43.3

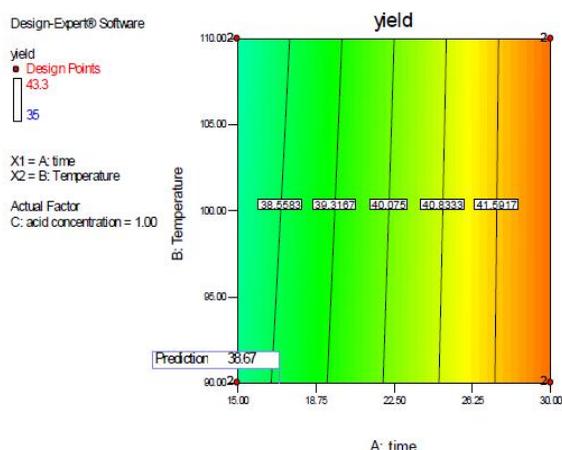


Fig. 2. Optimization of contours in ethanol yield

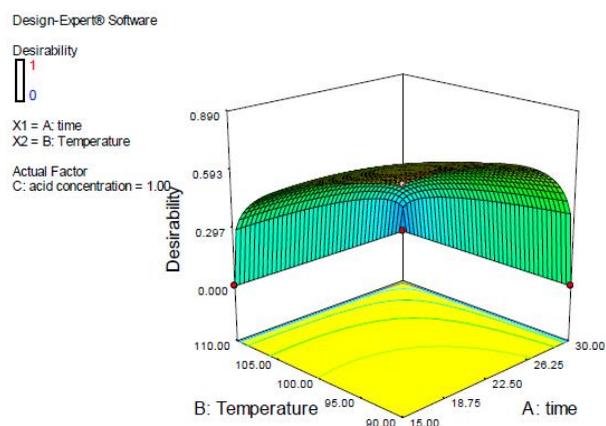


Fig. 3. Surface of possible optimum solution

4. Conclusions

The probability (p-values) values and normal probability plot indicate the quadratic polynomial model satisfying ANOVA assumptions and the model was considered to be accurate and reliable for predicting the yield of ethanol from bamboo using dilute acid hydrolysis. The resulting data were analyzed using Design-Expert® 7 to determine the effects of hydrolysis parameters and optimization in ethanol production. Optimization hydrolysis of ethanol production from bamboo using dilute acid hydrolysis was carried out. A 2-level factorial design method was used to optimize the production of ethanol from bamboo. The probability (p-value) of 0.0164 demonstrates a high significance for the regression model. Yield of ethanol 38.76 % was obtained when optimum conditions were: hydrolysis time of 17.03 min, hydrolysis temperature of 363 K and acidic concentration of 1 %. Validation experiments verified the availability and the accuracy of the model with desirability of 88.7 %.

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ОПТИМІЗАЦІЯ ГІДРОЛІЗУ У ВИРОБНИЦТВІ ЕТАНОЛУ З БАМБУКУ

Анотація. Досліджено оптимізацію кислотного гідролізу при виробництві етанолу, перспективного альтернативного палива, з лігноцелюлозних матеріалів (бамбука). Показано, що перетворення бамбука в етанол може бути здійснено трьома етапами: попереднє оброблення деревини бамбука для видалення лігніну та геміцелюлози, кислотного гідролізу попередньо обробленого бамбука для перетворення целюлози у відновлений цукор (глюкозу) та бродіння цукру з утворенням етанолу з використанням анаеробних бактерій *Saccharomyces cerevisiae*. Досліджено вплив параметрів на стадії гідролізу та експериментально встановлено оптимальні параметри (температура, час та концентрація кислоти). Вплив параметрів гідролізу на вихід етанолу встановлено за допомогою 3x2 факторного експерименту з використанням програмного забезпечення Design-Expert® 7.

Ключові слова: бамбуковий етанол, ферментація, гідроліз, оптимізація.