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Bioethanol Production from Brewery Spent Grain using Cellulases Enzyme Hydrolysis

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Abstract

Large amounts of brewery spent grain are generated from beer factories in Ethiopia. Its high source of cellulose, but discharge into environments cause environmental and health problems, This study was aimed to quantify reducing sugar and bio-ethanol production from brewery spent grain, using cellulases enzyme hydrolysis. The sugar content of the hydrolysate quantified using a spectrophotometer measuring its absorbance. The best yield of reducing sugar was found at 40 ^{0}C temperature, 4.5 pH, and 48 hrs time and 1.5 ml enzyme loading. 2.5 % Saccharomyces cerevisiae and at 30 ºC temperature, 5.0 pH and 72 hrs time Fermentation was performed Hydrolysate. Then 96.55, %w/w and 53.68 % per 6.58 g barley spent grain of reducing sugar and bio-ethanol respectively obtained.

GRAPHICAL ABSTRACT

Keywords: Bio-ethanol, Barley spent grains, Cellulases, enzyme hydrolysis, fermentation, and Saccharomyces cerevisiae

1. Introduction

At this time, energy applications investigation have caused increasing awareness and greater than ever and continuously growing especially for bio-ethanol, due to diminution of fossil feedstock and climate changes, like global warming, due to greenhouse gas emissions (GHGs), set in motioning the worldwide affinity to produce bio-fuels in replacement of the fossil-based ones, **(1)**. The most plentiful renewable resource generated all just about the world are Lignocelluloses materials (**2**), along with them, the higher alarm is the center of concentration on brewers' spent grain (BSG). Solid deposit of breweries acquiring following mashing and lautering incorporating of exhaust grain husks is called BSG. Based on the working conditions take on throughout

harvest, malting and mashing time, the composition of brewery spent grain changes (**3**). The total by-products In beer processing factories about 85 % produced is BSG (**4**). On a dry weight center, the quantity of BSG contains concerning 40 - 50 % polysaccharides (24 - 31 % hemicelluloses, 15 - 18 % cellulose and 2 - 3 % starch) and 30 % or more proteins (**5**). Brewery spent grain negligible quarrel with food of human used for bioethanol production as it occurs with the first generation bioethanol produced from agricultural crops, such as corn and sugarcane (**6**).

The four-unit functions entail in Biochemical process methodology are i.e. pretreatment for the disintegration of cellulose, hemicelluloses and lignin, enzyme hydrolysis, fermentation distillation (**7; 8**). Hydrolysis is indispensable earlier than fermentation to liberate the fermentable sugars. In the course of action, simple sugars like glucose, pentoses, and hexoses are cleaved from cellulose and hemicelluloses respectively (**9**).

$$cellulose \xrightarrow{\square ydrolysis} glucose \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

$$\text{Hemicelluloses} \xrightarrow{\text{hydrolysis}} \text{pentose and hexoses} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

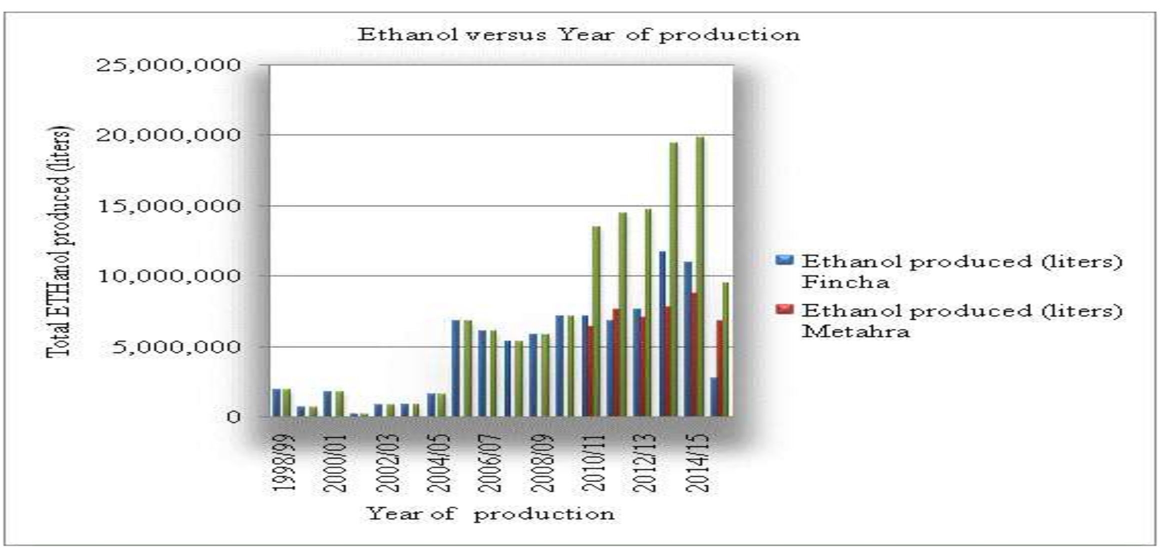

Figure 1.1 In Ethiopia since 1998/99 Ethanol produced sugar factory (**10**)

Conversion of Brewers' Spent Grain to bioethanol has been reported **(11; 12)**, enzymatic hydrolysis of brewer's spent grain for cellulose production, Effect of hemicelluloses and lignin have been examined **(13)**.

Hence, the aim of this research was to study the cellulases enzyme hydrolysis and ascertaining the top achievable bioethanol production from brewery spent grains.

2. Methods

2.1 Sample collection

Barley spent grain (BSG) which remains after the mashing and lautoring process was obtained from the Heineken brewery industry, Addis Ababa, Ethiopia. This Sample transported to Addis Ababa institute technology (AAiT), School of chemical and bioengineering laboratory and it was packed in polyethylene bags. The Samples used for this study were prepared in the biochemical engineering laboratory. 180 g of BSG was washed in order to remove unwanted matter and dried at 70 ºC for 24hrs until 10.8 % of moisture content remains. Followed by dried sample was milled and sieved into appropriate particle size which is less than 0.5 mm. The milled sample was sterilized at 120 ºC for 16 min and stored at less than 4 º C refrigerators.

2.1.1 Chemicals

Sulfuric acid (98%, sd fine-chem. limited, Mumbai, India),peptone, Dextrose sugar, sodium hydroxide (Abron Chemicals, India), yeast extracts, hydrochloric acid (Abron Chemicals, India), Urea, $MgSO_4.7\ H_2O$, quantitative Benedict reagent solution, and Whatman No. 1 filter papers were used in this research work.

2.2 Material and Equipment's

Oven (fuse 10, code DAS4200 Made of Italy), muffle furnace, UV-Vis spectrophotometer (serial No-8UVD 11200 lambed, Inc, U.S.A country of origin U.S.A), and Electric Thermostatic Heated Dry Box (Model. 202-0A, Made in Sweden), autoclave (Electrical Heated Vertical Steam Sterilizer Model LX-B50 LC Digital, Made in China), thermostatic Incubator shaker (Model. DH-500A, Bio base Biodustry (Shandong) Co, Ltd, N0.51. South Gengye and Road. Jinan City, China), centrifuge (model REF 1406, Andreas Hettich GmbH and CO.KG 78532.TUttingey, Germany) and water-bath (Type: TXF200 model, serNo.T41611001, supply 200-240 v, Grant instrument (Cambridge) Ltd Shepreth SG8 6GB made in England) and Fourier transform infrared spectroscopy (prinks Elmer spectrum, 65 FTlR).

2.3 Optimization of parameters

The process parameters for enzyme hydrolysis in brief expressed below, so optimization of parameters of enzyme hydrolysis (temperature, pH, hydrolysis time, and enzyme concentration). Brewery spent grain: was collected from the Heineken brewery industry, Addis Ababa, and then dried out in the oven at 75°C for 24 hours. The dried BSG was ground in the grinder, for function of mounting the surface area with proper particle size which is 0.5 mm. The sample that was acquired had to be prepared and conditioned for pretreatment, hydrolysis, fermentation, and distillation. The ground feedstock was mixed with 1.5% (v/v) sulfuric acid solutions in 1000-ml closed universal flasks with a liquid-to-solid ratio of 20 % (w/w). Erlenmeyer Flasks were placed contained by an autoclave for intention of pretreatment at 121 ºC, the mixture with a pH of 1.65, and 16 min. Ground brewery spent grain Measured 180 g using electronic balance then put in 2000 ml flasks. The Pretreatment aim is to: demolish lignin part for the enzyme to access

substrate by Increase porosity, get better the creation of sugar, diminish crystalline of cellulose. Earlier than put into the enzyme hydrolysis, the mixture alienated from the pretreatment was washed and dried.

a. Strain Specification

From Ethiopian Biodiversity Institute Culture Collection, Addis Ababa, Ethiopia isolated from soil and water Bacillus subtilus was obtained. Nutrient agar is the main medium for Bacillus subtilus cultivation.

b. Liquid State Fermentation

Solid substrate (BSG) is taken in Erlenmeyer flasks. Production medium containing (g/l); KH_2PO_4 (1); $MgSO_4$ (0.5); K_2HPO_4 (1); $FeSO_4.7H_2O$ (0.03); KCl (0.5); Yeast extract (2); and bacillus subtilus (1 ml) was introduced in to the liquid medium. Liquid medium containing mineral salt medium is stir well, sterilize in an autoclave at 121 ºC at 15 min and cool. Then 20 g BSG as a source of cellulose with sterile medium under aseptic condition and incubate at room temperature, but the fermentation carried out with pH 5.0 for both cellulase produced using BSG cellulose and refined cellulose powder, and 40 ºC temperature of cellulase produced by Bacillus subtilis (**14**). Enzyme Extraction from liquid State Fermentation Samples obtaining from the liquid fermentation is centrifuged at 10000 rpm at 4 ºC for 15 min and the supernatant collect is taken as a crude enzyme solution.

2.3.1 Enzyme hydrolysis

A series of experiments were performed for Brewery spent grain hydrolyzed with different hydrolysis temperature, pH of the solution, times, and cellulases enzyme with concentration, in

500 ml separately in Erlenmeyer flask using incubator shaker. The main purpose of this process was too degraded cellulose into its monomer in the optimal condition of temperature, pH, Enzyme concentration and reaction time. The process conditions in enzyme hydrolysis were optimized: Temperature, pH, time of hydrolysis and enzyme concentration. Data analysis was carried response surface method (central composite design) to evaluate the effects of the process variables; temperature (40 ºC, 45 ºC, and 50 ºC), pH (4.0, 4.5, 5.0) reaction time (24 hrs, 48 hrs, and 72 hrs) and enzyme loading (1, 1.5 and 2 ml). The temperature of 45 ºC, 4.5 pH of the solution, 48 hrs and 1.5ml enzyme concentration, was used center point. An experimental design (Central Composite Design) with 26 experiments was employed, which includes 24 non-center points and 2 trails for replication of the central points to estimate error based on the pattern generated through the software. Response Surface Methodology (RSM) helps us to optimize process parameters for this design of the experiment. The response variable was the yield of reducing sugar amount after enzyme hydrolysis. The analysis of variance (ANOVA) was used to situate the significance of the result.

The spectrophotometer was used for determining their reducing sugar by measuring its absorbance with Benedict reagent for all the hydrolyzed experimental samples. Only the one experiment with maximum reducing sugar (glucose) was taken for fermentation.

The sugar (glucose) content yield of enzyme hydrolysis is defined as

$$\text{Sugar yield } (\%) = \left(\frac{\text{M sugar}}{\text{M polysaccharides} \ * \ \text{f}}\right) * \ 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3$$

Where

M= sugar is the amount of glucose released by enzyme hydrolysis

M= polysaccharide is the amount of cellulose in the raw material

f= is the conversion factor from polysaccharides to monomeric sugars (180/162) for glucose and (150/132) for Xylan to xylose (**15**).

2.3.2 Fermentation

500 ml of Erlenmeyer flask in incubator shaker was used for carried out the prepared hydrolysate sample from enzymatic hydrolysis with maximum reducing sugar, the fermentation process operated at temperature of 30 ºC, pH with 5.0 which is optimum for Saccharomyces cerevisiae using sodium hydroxide solution, stirring at 160 rpm, for 72 hrs of fermentation time. The assay was done with 2.5% (v/v) of inoculums.

a. Media Preparation for Saccharomyces cerevisiae

The culture medium was prepared in a 250 ml test tube composed of (g/l): Yeast extract (10); Dextrose (20); Urea (5); Mg SO_4.7 H_2O (5); Peptone (20). The media was sterilized at 121 ºC for 15 min and 0.50 g of Saccharomyces cerevisiae were added into 100 ml prepared media at 250 ml conical flask and The conical flasks were properly covered with aluminum foil and placed to a shaker incubator for 24 hrs, at 30 ºC and 200 rpm.

3. Result and Discussion

According Fig. 3.1 the minimum(20.69 %w/w) and maximum (96.55%w/w) reducing sugar were obtained temperature of 45 °C, a pH of 4.5, time 24 hrs, enzyme loading of 1.5 ml, and temperature of 40 °C, a pH of 4.5, time of hydrolysis 48 hrs, enzyme loading of 1.5 ml respectively.

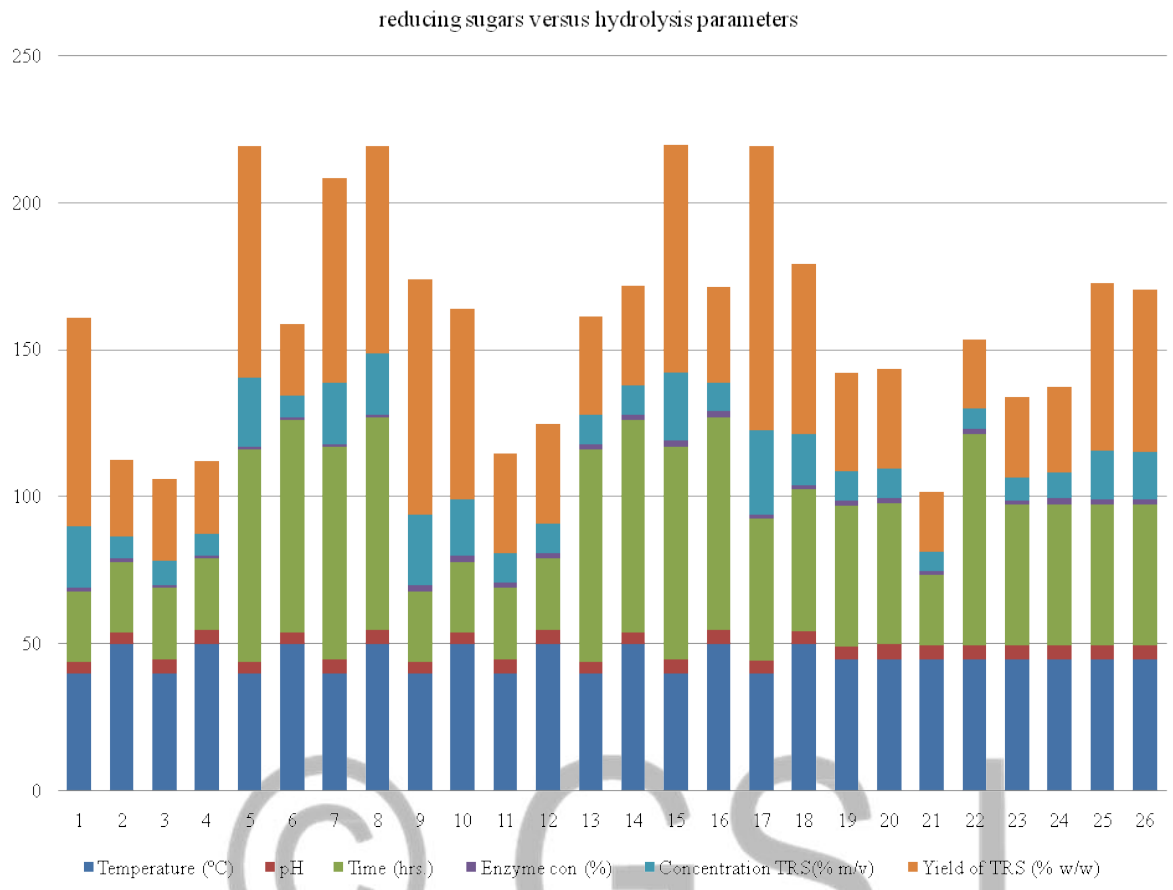

Figure 3.1 Effect of hydrolysis process variables in the yield of reducing sugar

3.1 Statistical Analysis of the experimental results

3.1.1 Analysis of variance (ANOVA)

The analysis of variance of the cubic regression model was a significant model, from evident of Fisher‟s F test with a very low probability value [(P-model > F) =0.0001]. From Table 3.1 it was observed that the Model F-value of 3.63 implies the model is significant, and the Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, A^2, BC, and CD are significant model terms,but B, C, D, B^2, C^2, D^2, AB, AC, BD, and AD have not significant effect on the yield of reducing sugar due to values of "Prob > F" greater than 0.1000. The coefficient

for the linear upshot of temperature was highly significant, and that of pH, time and enzyme loading were not significant. The " (16)Lack of Fit F-value" of 156.84 implies there is a 6.21 % probability that a "Lack of Fit F-value" huge could come about due to noise. It was also examined that there is an interaction effect between pH with time and time with enzyme loading.

Table 3.1 Analysis of variance (ANOVA) for Response Surface Quadratic Model

Source	Sum of squares	DF	Mean Square	F value	Prob > F	
Model	10472.87	14	748.06	3.63	<0.0189	Significant
A	2236.02	1	2236.02	10.85	<0.0072	
B	95.68	1	95.68	0.46	0.5098	
C	209.99	1	209.99	1.02	0.3345	
D	0.12	1	0.12	5.902E-004	0.9811	
A^2	3727.56	1	3727.56	18.08	<0.0014	
B^2	71.90	1	71.90	0.35	0.5668	
C^2	744.37	1	744.37	3.61	0.0839	
D^2	295.33	1	295.33	1.43	0.2565	
AB	285.27	1	285.27	1.38	0.2643	
AC	73.96	1	73.96	0.36	0.5613	
AD	104.55	1	104.55	0.51	0.4912	
BC	2515.52	1	2515.52	12.20	<0.0050	
BD	43.03	1	43.03	0.21	0.6567	
CD	1038.77	1	1038.77	5.04	<0.0463	

Residual	2267.82	11	206.17			
Lack of Fit	2266.37	10	226.64	156.84	0.0621	not significant
Pure Error	1.45	1	1.45			
Cor Total	12740.68	25				

The regression coefficients and the corresponding 95 % CI (Confidence Interval) High and Low were presented in table 3.2 below. If zero was in the range High and Low 95 % Confidence interval, the factors have no effect. From the 95 % CI High and Low values of each model term, it could be concluded that the regression coefficients of temperature and the interaction conditions of temperature and time in addition to time and enzyme loading have an extremely significant effect in the yield of reducing sugar production.

Table 3.2 Regression coefficients and the corresponding 95% CI High and Low

Factor	Coefficient Estimate	DF	Standard Error	95% CI Low	95% CI High	VIF
Intercept	43.28	1	5.69	30.76	55.81	
A-temperature	-11.15	1	3.38	-18.59	-3.70	1.00
B-pH	-2.31	1	3.38	-9.75	5.14	1.00
C-time	3.42	1	3.38	-4.03	10.86	1.00
D-enzyme loading	-0.082	1	3.38	-7.53	7.37	1.00
A^2	38.15	1	8.97	18.40	57.90	2.16

B^2	-5.30	1	8.97	-25.05	14.45	2.16
C^2	-17.05	1	8.97	-36.80	2.70	2.16
D^2	-10.74	1	8.97	-30.49	9.01	2.16
AB	4.22	1	3.59	-3.68	12.12	1.00
AC	-2.15	1	3.59	-10.05	5.75	1.00
AD	2.56	1	3.59	-5.34	10.46	1.00
BC	12.54	1	3.59	4.64	20.44	1.00
BD	-1.64	1	3.59	-9.54	6.26	1.00
CD	-8.06	1	3.59	-15.96	-0.16	1.00

The following second order polynomial model was derived to explain the yield TRS after enzyme hydrolysis from brewery spent grain

Final Equation in Terms of coded Factors:

Y_h = +3159.39664-147.84816 A+69.82089 B

+0.095166C+144.43706D+1.52605A^2-21.19467 B^2-0.029598C^2-42.95467D^2+1.68900AB-0.017917AC+1.02250AD+1.04490BC-6.56000BD-0.67146CD

From the above quadratic formula Yield of TRS after hydrolysis(Yh) positively affected by pH,time,enzyme loading, square of temperature with interaction effect of temperature and pH, temperature and time, temperature and enzyme loading , and pH and time, but negatively affected by temperature, square of pH, square of time, square of enzyme loading in addition to interaction temperature and time, pH and enzyme loading.

Table 3.3 Model adequacy measures

Std. Dev.	14.36
R-Squared	0.9220
Mean	46.79
Adj R-Squared	0.855
C.V.	30.69
Pred R-Squared	-0.2494
PRESS	15918.36
Adeq Precision	6.983

The regression coefficient (R^2) quantitatively estimates the link between the experimental data and the predicted responses, used for verifyed Goodness of fit of the model‟s. Results of R^2= 0.9220 and Adj-R^2= 0.855 acquired spell outs that the predicted values were found to be in good consistency with experimental values. Since the R^2 value is closer to 1.0 it points out that the regression line absolutely fits the data. Results entail that the predicted values were found to be in good conformity with experimental values indicating the achievement of the RSM. In this case, the value of the coefficient (R^2=0.9220) from Table 3.3 indicated that only 5.47 % of the total variance was not explained by the developed regression model. The adjusted determination coefficient (Adj-R^2= 0.855) was also agreeable for proving the significance of the model. "Adeq Precision" computes the signal to noise ratio. A ratio larger than 4 is enviable. Your ratio of 6.983 indicates enough signal. This model can be used to steer the design space.

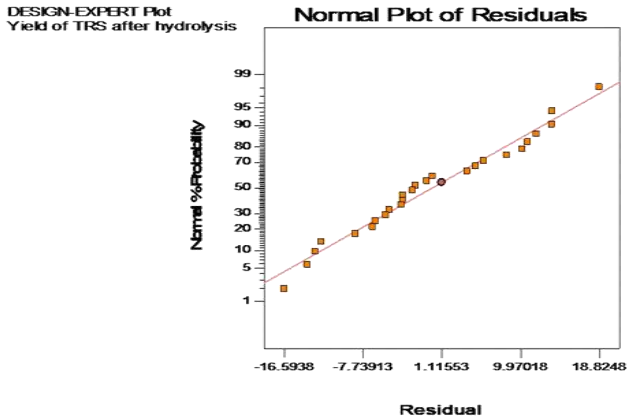

Figure 3.2 Normal plots of residuals

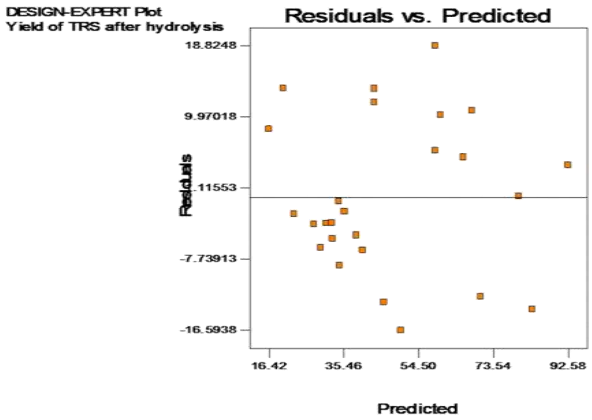

figure 3.3 Residual versus predicted values

From the plot Figure 3.2, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure, this shows that the quadratic polynomial model satisfies the analysis of the assumptions of variance (ANOVA) i.e. the error distribution is approximately normal. The residuals should be structureless, If the model is accurate and the assumptions are satisfied; in particular, they should be unrelated to any other variable including the predicted response. A simple check is to plot the residuals versus the fitted (predicted) values based on figure 3.3. A plot of the residuals versus the rising predicted response values test the assumption of constant variance. The plot shows random scatter which justifying no need for an alteration to minimize personal error.

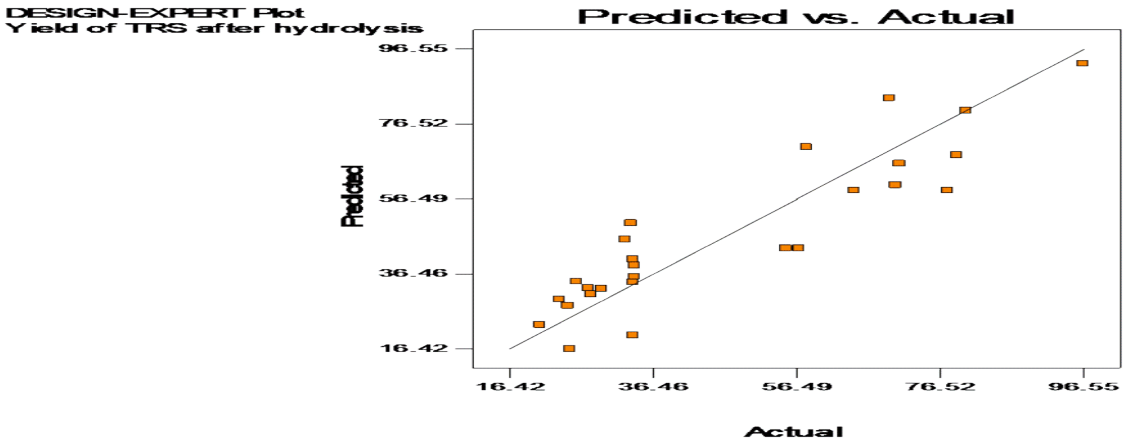

Figure 3.4 predictor and actual value

From graph 3.4 the predictor and actual value of individual experiments were not equal, but the medium actual value versus the predicted value was lined at temperature 40 ºC, pH 4.0, time 24 hrs and enzyme loading 2 %, both had a value of 80.12 % w/w and the maximum has occurred 96.55 % and 92.51 % respectively.

3.1.2 Response surface on the experimental variables

In order to analyze the regression equation of the model, the three-dimensional surface was obtained by plotting the response (yield of reducing sugar) on the Z-axis against any two variables while keeping the other two variables at zero levels are presented in Fig. (a-f). A total of six response surfaces were obtained by considering all the possible combinations for each parameter, but the only valid interactions were two of them, which pointed out below. These plots show the type of interaction between the tested variables, thereby permitting optimum conditions. The maximum predicted value of the response under the optimum experimental condition is represented by the surface-confined in the smallest ellipse in the contour diagram. Conical shape response surface plot indicates optimum operating conditions. The response optimized value for the production of bioethanol was based on the two process variables

described in the response surface plot. From fig (a) Represents the response surface developed as a function of pH and time on the yield of reducing sugar, which indicates a good deal of great interaction between the factors (B and C) on the yield of reducing sugar. Fig. (b) Response surface plots of the effect of time and enzyme loading on the yield of reducing sugar at fixed pH and temperature, which indicates a good deal of highest interaction between the factors (C and D) on the yield of reducing sugar. Fig. (a) depicts the three-dimensional response surface graphical representation showing the effect of pH and time on the yield of reducing sugar. The maximum yield of reducing sugar of 43.53%w/w was achieved at a pH value of 4.5 and 48 hr hydrolysis time. The maximum yield of reducing sugar of 40.45%w/w was seen at 48 hr and 1.5 ml (Fig. b). In the response, plots were almost elliptical and tilted (Fig. a), and full elliptical and tilted (Fig. b) indicating significant cross-product interaction between the factors, B and C and C and D respectively. Since, Response surface plots of the effect of temperature and pH (Fig.(c)), temperature and time(Fig.(d)), temperature and enzyme loading(Fig.(e)), not elliptical and not tilted, pH and enzyme(Fig.(f)) elliptical and not tilted which indicating a good deal of negligible interaction between the factors (A and B, A and C, A and D, B and D) on the yield of reducing sugar respectively.

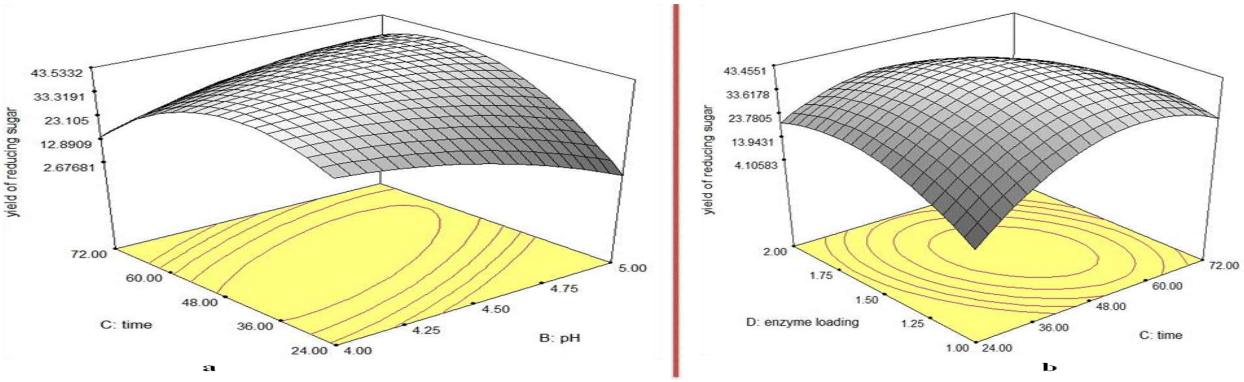

Figure.3.5 -a) Response surface plots of the effect of pH and time; b) time and enzyme loading on the yield of reducing sugar

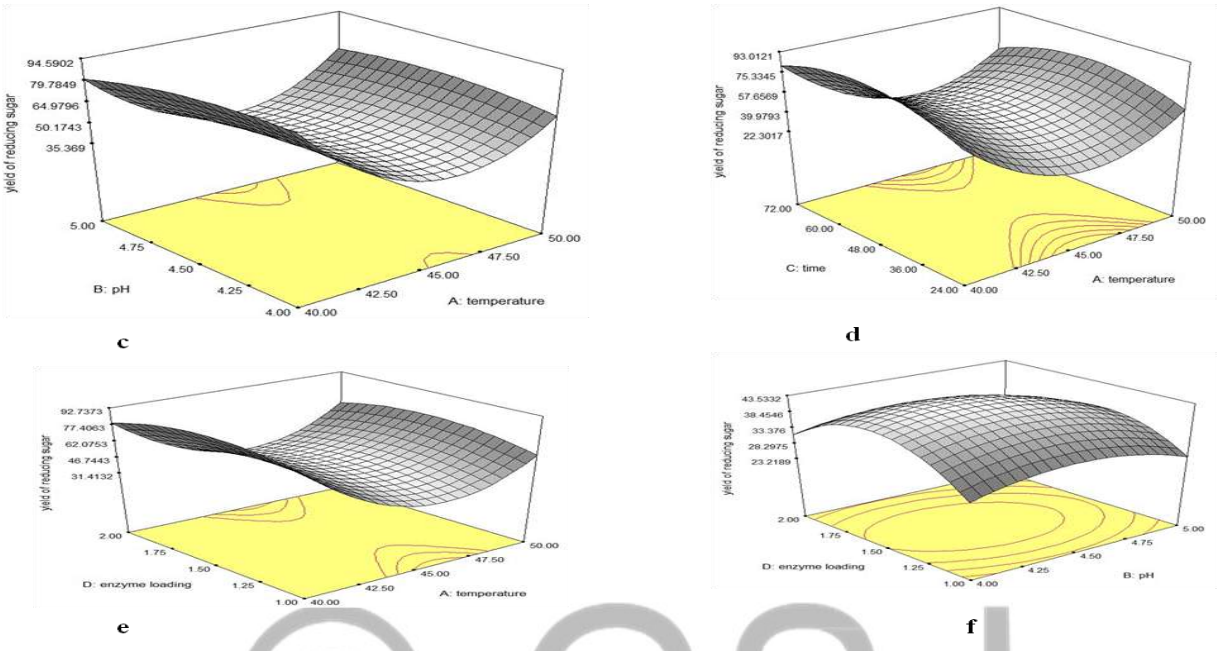

Figure.3.7 –c) Response surface plots of the effect of temperature and pH; d) temperature and time; e) temperature and enzyme loading; f) pH and enzyme loading on the yield of reducing sugar

3.2 FT-IR Characterization of the Produced Bioethanol

Prinks Elmer spectrum 65 FT-lR found in Addis Ababa University, 4kilo campus was used to determine the functional groups of BSG Bioethanol with the help of lR correlation charts. The IR spectrum was reported by % transmittance. The examination form in region wave number displayed the average-infrared array was 4000-400 cm^{-1}. The O-H, C-H, C-O stretching vibrations associated have characteristic IR absorptions of Alcohols. Broadband specified the O-H stretch of alcohol in the region 3500-3200 cm^{-1} with a incredibly concentrated when it runs like a liquid layer, while the region 1260-1050 cm^{-1} proves the C–O stretch. The groups

at about 2880 were allocated as the symmetric elongating modes of the $–CH_2$ and 2930 cm^{-1} were assigned as the symmetric elongating modes of the $–CH_3$ groups (**16; 17**). This makes certain that the product gained from Barley spent grain (BSG) is absolutely ethanol due to the verification of these regions (Figure 3.8).

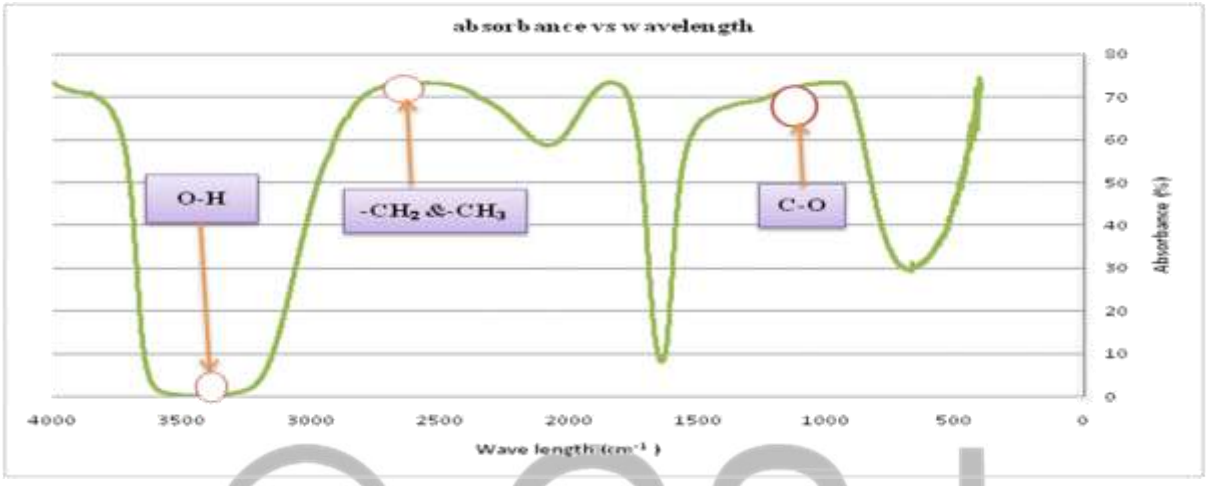

Figure 3.8 Fourier transform Infrared spectra of the produced bioethanol from BSG

4. Conclusions

The optimistic yield of ethanol was acquired at a average pH and temperature which was 40 ºC as well as at the average time and enzyme concentration. As a result of RSM optimization at 40 ºC,4.5 pH, 48 hrs and 1.5 % enzyme loading were the hydrolysis temperature, pH, time and acid concentration, respectively afforded 96.55 % w/w yield of reducing sugar and 53.68 % ethanol respectively. The design was all points central, it is clear that the preferred technique of optimization was competent and consistent. From this result, it can be completed that elevated potential and inexpensive raw material of Barley spent grain for bioethanol production and the enzyme hydrolysis process was incredibly successful; moreover, the yield of ethanol was highest with the own yield of reducing sugar.

Acknowledgments

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References

1. “Waste valorization by biotechnological conversion into added-value products". **A. Amore, R. Liguori, and V. Faraco.** no.14, 2013, Applied Microbiology and Biotechnology, Vol. vol.97, pp. pp.6129– 6147.

2. Ethanol as an alternative fuel from agricultural, industrial and urban residues. **Prasad, S.; Singh, A.; Joshi, H.C.** 2007, Conserv. Recycle, Vol. 50, pp. 1–39.

3. Variability of brewers’ spent grain within a brewery. . **Santos, M.; Jiménez, J.J.; Bartolome, B.; Gómez-Cordovés, C.; del Nozal, M.J.** 2003, Food Chem, Vol. 80, pp. 17–21.

4. Brewers ‘spent grain: generation, characteristics, and potential applications. **Mussatto, S.I.; Dragone, G.; Roberto, I.C.** 2006, J. Cereal Sci., Vol. 43, pp. 1–14.

5. Pretreatment and hydrolysis of brewers’ spent grains,. **Macheiner D., Adamitsh B.F., Kawer F., Hampel W.A.** 2003, Eng. Life Sci., Vol. 3, pp. 401-405.

6. Life cycle assessment of soybean biodiesel and LPG as automotive fuels in Portugal. **Morais S., Mata T.M., Ferreira E.** 2010, Chemical Engineering Transactions, Vol. 19, pp. 267-272.

7. Trends in biotechnological production of fuel ethanol from different feedstocks. **Sánchez OJ, Cardona CA.** 99, 2008, Bioresour Technol, Vol. 2008, p. 527095.

8. Life cycle evaluation of emerging lignocellulosic ethanol conversion technologies. **Spatari S, Bagley DM, MacLean HL.** 2010, Bioresour Technol, Vol. 101, pp. 654–67.

9. Acid-Based Hydrolysis Processes for Ethanol from Lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. **Taherzadeh, M J., and Karimi, K.** 2007, Biomass and Bioenergy , pp. p 384–410.

10. **Ethiopia sugar development agency.** Ethiopia : sugar corporation, 2015.

11. Second Generation Ethanol Production from. **Rossana Liguori, Carlos Ricardo Soccol , Luciana Porto de Souza Vandenberghe,Adenise Lorenci Woiciechowski and Vincenza Faraco.** 1996-1073, 2015, energies, Vol. 8, pp. 2575-2586.

12. Bioconversion of brewer's spent grains to bioethanol. **Jane S. White, Biju K. Yohannan, Graeme M. Walker.** 7, November 2008, FEMS Yeast Research, Vol. 8, pp. 1175–1184.

13. Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain. **Solange I.Mussatto, MarcelaFernandes,Adriane M.F.Milagres, Inês C.Roberto.** 2,5, August 2008, Enzyme and Microbial Technology, Vol. 43 , pp. 124-129.

14. Application of Spent Grains to increase Porosity in Bricks. **Russ, W., Mo¨rtel, H., and Meyer-Pittroff, R.** 2005, Construction and Building Materials, Vol. 19, pp. 117–126.

15. **Zeng, M., Mosier, N. S., Huang, C. P., Sherman, D. M., & Ladisch, M. R.** 2007, Biotechnology and Bioengineering, Vol. 97, pp. 265–278.

16. Interpretation of Infrared Spectra, A Practical Approach, Encyclopedia of Analytical Chemistry. **Coates J, and Meyers R. A.** 2000, John Wiley & Sons Ltd: Chichester, pp. pp. 10815-10837.

17. New C-H Stretching Vibrational Spectral Features in the Raman Spectra of Gaseous and Liquid Ethanol. **Yu Y, Lin K, Zhou X, Wang H, Liu S, Ma X,.** 2007, J. Phys. Chem. C, Vol. 111, pp. 8971-8978.