Vol 3 Issue 1 ISSN 2581-3862

ScholarsReport
Open Access Peer Reviewed Journal

RESEARCH ARTICLE

Subject: Chemical and Bio Engineering

Production of Bioethanol from Barley Spent Grains(BSG) by two-stage dilute acid hydrolysis

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ARTICLE INFO

Article History:
Received 24 April 2018
Accepted 24 June 2018
Available online 30 June 2018

Keywords: Bioethanol; Barley spent grains; dilute acid hydrolysis; *Saccharomyces cerevisiae*

Abbreviations: RSM, response surface methodology; BSG, Barley Spent Grain; CCD, Central Composite Design; R^2, regression coefficient; ANOVA, Analysis of variance

ABSTRACT

The objective of this study was to produce bioethanol from barley spent grains (BSG) using two-stage dilute acid hydrolysis and fermentation. During the first stage of the dilute acid hydrolysis, the process variables were fixed at the best optimum condition and for the second stage hydrolysis, 23 full factorial central composite design (CCD) was employed to investigate the effect of temperature (130-150°C), reaction time (20-30 min) and acid concentration (1.5%-2%) using Design expert® 6. Fermentation of the hydrolyzate was performed using *Saccharomyces cerevisiae* at 30°C, pH 5.0 and 72 h. After the fermentation, the specific gravity of the produced alcohol was measured and the alcohol concentration was obtained from the relationship between the specific gravity and the proportion of ethanol in alcohol solution at 20°C. The maximum yield of reducing sugar and bioethanol was 47.56% (w/w) and 8.33mL per 50g dry barley spent grain, respectively at 144.3°C, 26.26 min and 1.68% (w/w) acid concentration.

1. Introduction

The probability of producing large quantities of ethanol for use as a transportation fuel has resulted in the production of bioethanol mainly from lignocellulosic byproducts, but the economic success depends on the competent conversion of cellulosic components to the monomeric sugars, fermentation of the same to ethanol at an optimum costs.[1] Favourable octane number, compression ratio, flammability limits, heat of vaporization, less CO_2 build-up, low NO_x emission, sulfur dioxide-free properties and burning time have increased the attraction towards this strategy adoption.[2] However, the disadvantage is the low energy density, low vapor pressure and high water miscibility which makes its application more tedious.[3,4] It could be source for the production of other chemicals, acetaldehyde, butadiene, ethane, propylene, ethylene, hydrogen, and carbon monoxide and could support hydrogen economy from renewable, clean energy source.[5]

The raw materials for the production of ethanol by fermentation could be grouped into sugars, starches, and lignocellulosics. The first two groups are in great demand by the human population as food, making them expensive feedstocks, hence the cellulosic materials are the only prospective feedstock for the ethanol production. Lignocellulosic materials are most commonly obtained from wood, agricultural residues, energy crops and municipal wastes.[6] All the basic structure of lignocellulosics consists of cellulose, hemicelluloses, and lignin. They are available in abundance and

Citation: Jabasingh SA, Fissha A (2018) Production of Bioethanol from Barley Spent Grains(BSG) by two-stage dilute acid hydrolysis. ScholReps 3(1).

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are relatively cheap. But some disadvantages, including the incompatibility towards the hydrolysis exist.[8] Starch-based materials need to be broken down for obtaining glucose. This incurs a high cost towards the energy and amylase enzymes.[9]

Production of bioethanol from BSG, which is a chief byproduct of the Beer industries, could be beneficial from the fact of lowering the energy input. [10] These by-products of the brewing industry correspond to 87% of the total by-products. Many researchers have carried out investigations to consider it as a low-cost, energy productive raw material. The most important aspect of this discussion is that the chemical composition of BSG varies according to the barley variety, harvesting time, malting, mashing, and quality and type of adjuncts employed in the brewing process. [11] In Ethiopia, around 263,736 kg of BSG is generated every day and this is used as animal feed, and sometimes dumped into the landfills. This number is on the rise, due to the increasing number of breweries in the country with the potential to generate a huge amount of BSG. Conversion of this by-product waste into biofuel helps to reduce environmental pollution and solve the energy crisis. It also overcomes the fight and the debate 'food versus energy' controversy. [12] Therefore this study was intended to convert this low valuable by-product, the barley spent grain into a highly valuable product, the bioethanol. The study evaluates the effect of all the process variables, including the reaction time, H2SO4 concentration and temperature using response surface methodology (RSM). [13] The reducing sugar after the diluted acid hydrolysis and the bioethanol content after the fermentation and distillation are evaluated. A preliminary engineering economic analysis of bioethanol production from Barley spent grain is also performed.

2. Material and methods

2.1. Feedstock

Barley Spent Grain (BSG), the remains of the mashing process, was obtained from BGI brewery industry, Addis Ababa, Ethiopia and its composition were measured. [14,15] 3 kg of BSG was washed to remove the unwanted matter and dried at 70°C for 24h until 10% moisture content remained in the samples. The dried samples were sieved and milled to appropriately 2mm, particle size. The milled sample was sterilized at 121°C for 20 min and stored at less than 4°C, until the execution of the hydrolysis. The moisture content of the BSG samples was determined.

2.2. Chemicals

98% H_2SO_4 was used for pretreatment and hydrolysis of BSG, NaOH was used to adjust the pH before fermentation, dextrose, yeast extracts, urea, and $MgSO_4.7H_2O$ were used in the media preparation. Fermentation was carried out using *Saccharomyces cerevisiae* obtained from the Ethiopian Biodiversity Institute, Addis Ababa.

2.3. Dilute acid hydrolysis

2.3.1. The First stage dilute acid hydrolysis

The process parameters were fixed at the best process conditions at the liquid/solid ratio of 8 g/g, acid concentration of 100mg H_2SO_4/g of dry matter, the reaction time of 17min and temperature of 121oC during the first stage hydrolysis or the pretreatment. The pretreated feedstock was treated with 1.25%(w/w) or 100 mg H_2SO_4/g of dry matter sulfuric acid solutions in 500 mL Erlenmeyer flasks with a liquid to solid ratio of 8:1. The mixture was allowed to stand for 10 min at room temperature. Hydrolysis was performed in an autoclave at 121°C for 17 min.[2]

2.3.2. Th Second stage dilute acid hydrolysis

The slurry generated during the first stage dilute acid hydrolysis was introduced into the second stage hydrolysis unit. In the second stage dilute acid hydrolysis, the process conditions were optimized with the full factorial central composite design (CCD) using RSM. The conditions investigated were the temperature (130°C-150°C), reaction time (20 min-30 min) and acid concentration(1.5%-2.0 % equivalent to 120-160 mg H_2SO_4 per g dry mass). The hydrolyzate obtained after the hydrolysis was neutralized and then introduced into the fermentation process. Data analysis was carried out by design expert version 6.0 software. A 2^3 full factorial experimental design with 20 experiments were employed, which includes 8 trails for factorial design, 6 trails for axial points and 6 trails for replication of the central points to estimate error based on the pattern generated through software.[16] The response variable was sugar content after hydrolysis and ethanol yield after fermentation.

2.4. Fermentation and distillation

Fermentation was carried out in shaker incubator, at 30°C, at 200 rpm, for 72h with 10% (v/v) of *Saccharomyces cerevisiae* inoculum. The prepared hydrolyzates were adjusted to pH of (4.5-5.0), the optimum for *Saccharomyces cerevisiae*. The culture medium was composed in (g/L), Yeast extract, 10; Dextrose, 20; Urea, 5; $MgSO_4.7H_2O$, 5; and Peptone, 20. The media were sterilized at 121°C for 15 min and 0.5 mL of the 10% (v/v) of *Saccharomyces cerevisiae* was inoculated into 100 mL prepared media. The culture was proliferated in the shaker incubator for 24h, at 30°C and 200rpm.[17] After the BSG hydrolysis, the liquid fraction was analyzed for their reducing sugar content using the phenol-sulfuric acid method. The absorption values of the samples were recorded at 490nm on a UV-vis spectrophotometer. Following the 72h fermentation, the samples were taken out and subjected to distillation. Distillation is the final step in the production of ethanol, whereby ethanol is separated and purified from water, based on their different boiling points. Separation was carried out by simple distillation at 85°C for 2h.

2.5. Characterization of Ethanol

The specific gravity of the ethanol was determined using 20°C pycnometer. The bioethanol volume of each fermented sample was determined as follows,

$$\text{Ethanol volume}(V_{ae}) = \frac{V_{he} \times \rho_{he} \times x_e}{\rho_{ae}} \qquad \text{.........................(1)}$$

Where, V_{he}, V_{ae}, ρ_{ae}, ρ_{he} and x_e are the volume of hydrous ethanol, volume of anhydrous ethanol, density of anhydrous ethanol(0.789g/mL), density of hydrous ethanol (sample) and mass fraction of ethanol, respectively.The functional groups of BSG Bioethanol were determined by using FT-IR (PerkinElmer, Model Spectrum 65, FT-IR Spectrometer). [7]

3. Results and Discussion

3.1. Reducing sugar yield and Ethanol concentration

The moisture content of the BSG sample with 100, 125, and 150g was 49.13, 48.18 and 47.90%, respectively. Thus, the average moisture content of BSG samples was 48.40%. The concentration of total reducing sugar in the sample was expressed in % (w/w). The volume of hydrolyzate per sample was 400mL. The highest yield of total reducing sugar 47.52% (w/w) at 140°C temperature, 25 min at 1.75% acid concentration and the minimum yield of 25.44% (w/w) was obtained at 123.18°C.[11] The dry mass of BSG composed of cellulose and hemicelluloses is about 60% and from this total carbohydrate (cellulose and hemicellulose) 79.27% was converted into monomeric sugar in this investigation. **Table 1** shows the yield of total reducing sugar of samples.

Table 1. Reducing sugar yield and Ethanol concentration of BSG samples

Run No.	Factor1 Temp (°C)	Time (min)	Factor 3 Acid conc. (%)	Reducing sugar yield %(w/w)	Ethanol conc. %(w/w)	Density of hydrous ethanol(g/mL)
1	140.00	25.00	1.75	47.89	48.86	0.9163
2	150.00	30.00	2.00	34.56	35.33	0.9443
3	150.00	20.00	1.50	43.04	39.56	0.9360
4	140.00	25.00	1.75	47.28	38.00	0.9391
5	130.00	30.00	1.50	32.00	33.18	0.9482
6	140.00	16.59	1.75	36.40	39.53	0.9361
7	140.00	25.00	1.75	47.28	35.40	0.9441
8	140.00	25.00	1.75	47.89	47.12	0.9201
9	123.18	25.00	1.75	25.44	48.96	0.9161
10	150.00	20.00	2.00	36.96	36.50	0.9421
11	150.00	30.00	1.50	44.80	33.27	0.9481
12	140.00	25.00	1.75	47.28	38.00	0.9391
13	140.00	25.00	2.17	38.80	28.60	0.9561
14	130.00	30.00	2.00	43.68	32.08	0.9502
15	140.00	33.41	1.75	42.40	28.68	0.9560
16	140.00	25.00	1.75	47.92	47.10	0.9201
17	130.00	20.00	1.50	27.28	28.60	0.9561
18	156.82	25.00	1.75	38.16	31.04	0.9520
19	140.00	25.00	1.33	38.16	41.50	0.9321
20	130.00	20.00	2.00	41.20	35.49	0.9440

The specific gravity of ethanol was determined using pycnometer and was related with concentration of ethanol. The volume of hydrous ethanol and the yield of ethanol was also determined. The yield of total reducing sugar and ethanol were observed to have a direct relationship. The maximum sugar content of 47.52% (w/w) and yield of ethanol 8.29 mL/50g dry BSG were obtained at run 1, 4, 7, 8, 12 and 16 (25min, 140°C and 1.75% acid concentration). After the fermentation, 53% sugar conversion was found to be achieved. This indicates the conversion of sugar into ethanol is small. [20]

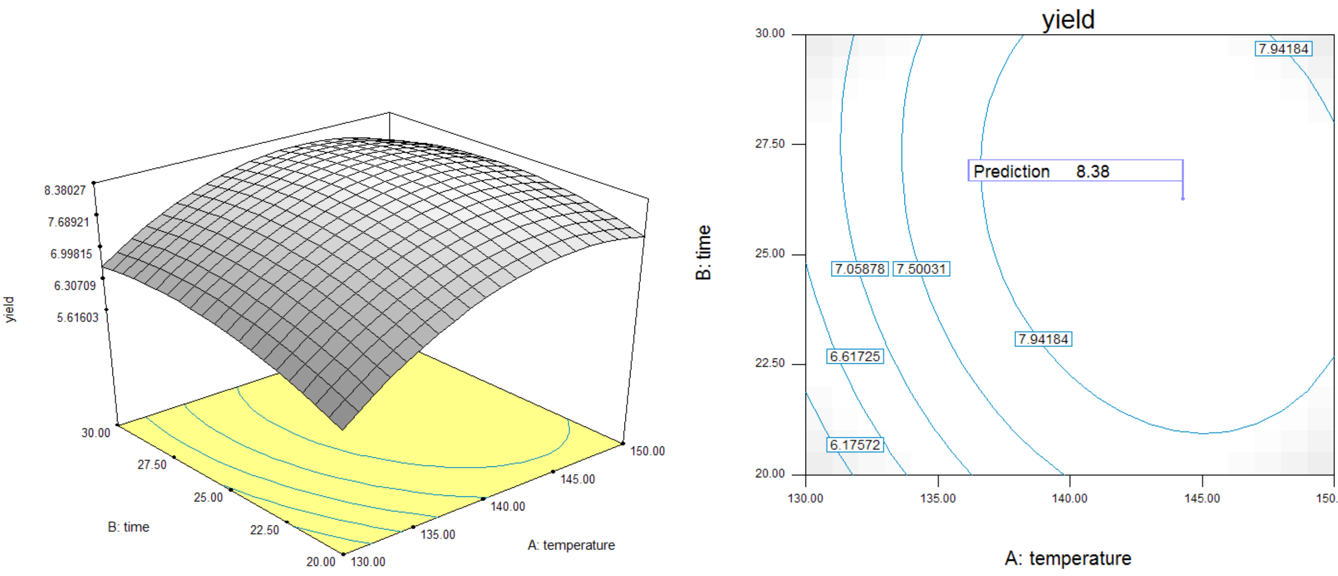
3.2. Analysis of variance (ANOVA)

The analysis of variance of the quadratic regression model was a significant model, from evident of Fisher's F test with a very low probability value [(P-model > F) =0.0001]. It was observed that the Values of "Prob > F" less than 0.05 indicate model terms are significant. In this case, A, B, A^2, B^2, C^2, AC are the significant model terms. Values greater than 0.1 indicate the model terms are not significant. The coefficient for the linear effect of temperature and time was highly significant, and that of acid concentration was less significant. It was also observed that there is an interaction effect between temperature and acid concentration. From the 95% CI High and Low values of each model term, it could be concluded that the regression coefficients of temperature, time and the interaction terms of temperature and acid concentration have highly significant effects in ethanol production. The regression coefficient (R^2) quantitatively evaluates the correlation between the experimental data and the predicted responses. Results of R^2=0.9637 and Adj-R^2=0.9309 obtained explicates that the predicted values were found to be in good agreement with experimental values. Since the R^2 value is closer to 1.0 it indicates that the regression line perfectly fits the data.[16] Similar to that in this investigation, R2 obtained was 0.9637, which was close to 1. The results imply that the predicted values were found to be in good agreement with experimental values (R^2=0.9637and Adj-R^2=0.9309), indicating the achievement of the RSM. The model's goodness of fit was checked by regression coefficient (R^2). In this case, the value of the coefficient (R^2 =0.9637) indicated that only 3.63% of the total variance was not explained by the developed regression model. The obtained R2 values suggest good adjustments to the experimental results. The adjusted determination coefficient (Adj-R^2=0.9309) was also satisfactory for confirming the significance of the model. Pred R-Squared indicating that the model will probably explain a high percentage (about 82.46%) of the variability in new data. "Adeq precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. In this study 17.677 indicates an adequate signal.

3.3. Effects of the experimental variables on the ethanol yield

3.3.1. Response surface and contour plot on the experimental variables

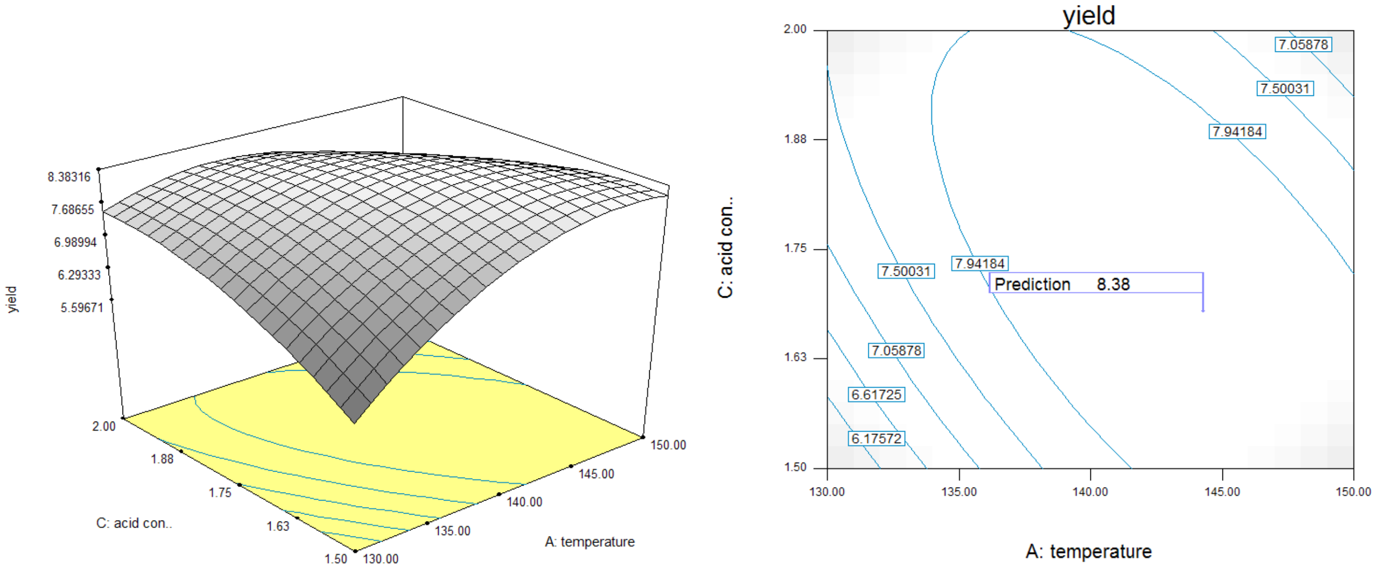
The current investigation, deals with the effect of hydrolysis temperature, time and acid concentration on the yield of ethanol. In order to analyze the regression equation of the model, three-dimensional surface and 2D contour plots were obtained by plotting the response (yield) against any two variables, while keeping the other variable at zero level. [13]These plots are created to analyze the changes in the response surface. Response surface and Contour plots showing the effect of the independent variables and their mutual interaction on the yield of ethanol are shown in the Fig. 1, Fig. 2, and Fig. 3.



(a) (b)

Figure 1. (a) Response surface plot and (b) Contour plot showing the effect of temperature and time on the yield of ethanol at fixed acid concentrations.

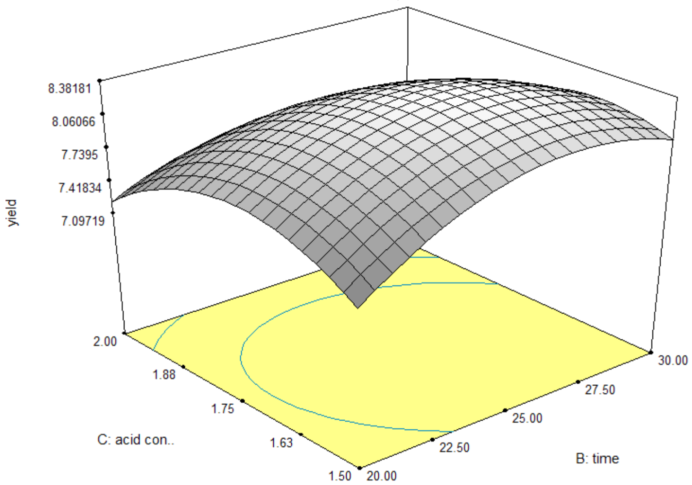
Fig.1. shows the response surface and contour plots, respectively developed as a function of temperature and time, while the acid concentration was kept constant at 1.75%. It was observed that the yield of ethanol was more sensitive to temperature changes, when the temperature was changed from 130-142.5°C, wherein the yield of ethanol reaches the maximum and beyond 142.5°C, the yield slightly decreases. The reason for this observation may be because, when the cellulose is exposed to beyond 142.5°C, the reducing sugar obtained from the cellulose degrades giving rise to a low yield of ethanol. As observed, there is no specific interaction between time and temperature at any given interval and positive yield was only observed at 30 min and around 142.5°C. The yield of ethanol at 20 min is less than the yield at 30 min, when the fermentation was carried out at the same temperature.



(a) (b)

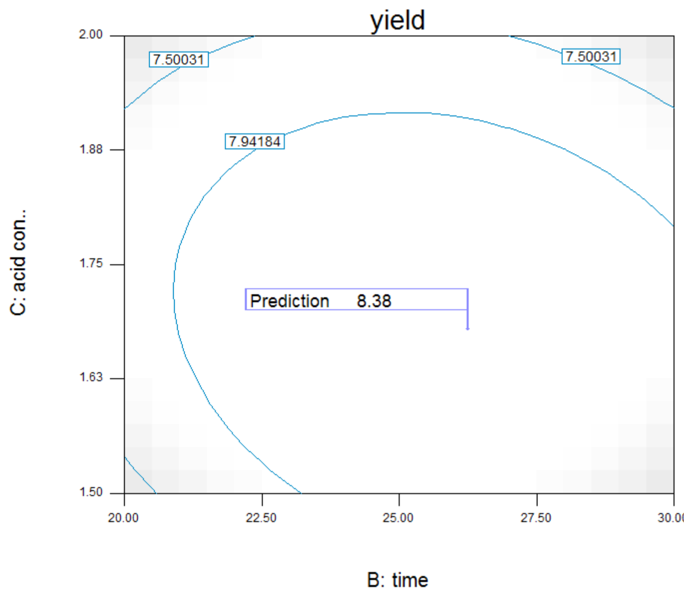
Figure 2. (a) Response surface plot and (b) Contour plot showing the effect of temperature and acid concentration at the constant time

Fig.2 represents the response surface and contour plots developed as a function of acid concentration and temperature, respectively, while, time was kept constant at 25 min. At a fixed acid concentration, the yield of ethanol increased slightly with the increase in the hydrolysis temperature from 130°C to 145°C, and nearly reached the maximum. However, on increasing the hydrolysis temperature beyond 145°C, there was a gradual decline in the yield.



(a)

Figure 3. Response surface (a) and (b) Contour plot of showing the effect of time and acid concentration on the yield of ethanol at constant temperature



(b)

As shown from the contour plot, the maximum yield occurred at 145°C and 1.64% acid concentration. As observed, positive yields were observed at low temperature and high acid concentrations and also at high temperatures and low acid concentrations. Hence, both temperature and acid concentration have strong interaction effect in the hydrolysis process. The highest yield of ethanol was observed at 147°C and 1.5% acid concentration. Fig.3 shows the response surface and contour plots developed as a function of time and acid concentration, respectively, while the temperature was kept constant at 140°C. Upon increasing the acid concentration from 1.5% to 1.75% with an increase of hydrolysis time from 20 min to 26 min, the yield of ethanol increased, but very slightly. Beyond this time, the yield of ethanol gradually decreased. The highest yield was obtained at 26 min and 1.75% acid concentration. The decrement in the ethanol yield with an increasing acid concentration from 1.75-2.00 % is due to the decomposition of sugar and the formation of furfural and 5-Methylhydroxy furfural. These substances, toxic to the yeast, inhibit the yeast growth. As observed, the time and acid concentration have a small interaction beyond 27.5 min and it has a positive effect on the yield of ethanol, at a high acid concentration until the 26th minute. Beyond 26 min, at fixed acid concentration, the yield of ethanol, slightly decreases due to the decomposition of BSG into unwanted products or non-fermentable molecules as they are exposed in high temperature for a longer period of time.[17,18]

3.3.2. Model validation and Optimization

The optimization of hydrolysis criteria for ethanol production from Barley spent grain using dilute acid hydrolysis shows the predicted optimum yield of reducing sugars and ethanol was 48.02 % (w/w) and 8.38 mL/50g dry BSG respectively, at the process variables 144.29°C, 26.26 min and 1.68%. In order to confirm the validity of the RSM model, a confirmatory experiment in triplicate was conducted at the above-specified optimum process conditions predicted by the model. Under these conditions the yield of ethanol was found 8.33mL/50g BSG (average), which were close to the RSM result of 8.38mL/50g of BSG. The experimental values were found to be close to the predicted values and hence the model was validated.

3.4. FT-IR Characterization of Bioethanol

The alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film, the region 3500 cm^{-1} -3200cm^{-1} shows a very intense and broadband indicated the O-H stretch of alcohols, while the region 1260 cm^{-1} -1050cm^{-1} confirms the C-O stretch. The bands at around 2880 cm^{-1} and 2930 cm^{-1} were assigned as the symmetric stretching modes of the $-CH_2$ and $-CH_3$ groups, respectively.[7]

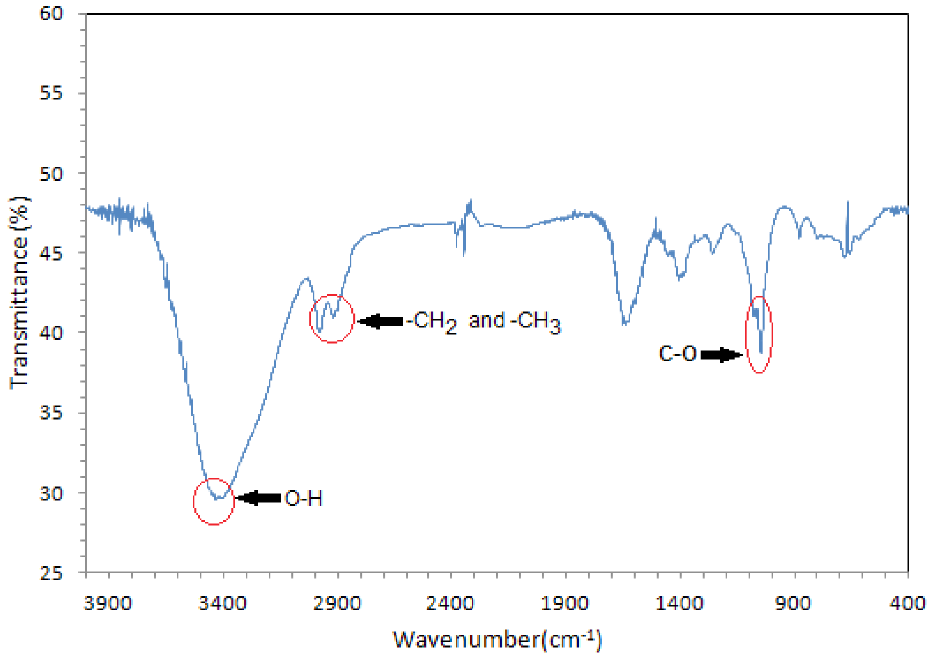


Figure 4. FT-IR spectrum of ethanol from Barley spent grain

The presence of these regions ascertains that the product obtained from Barley spent grain (BSG) is ethanol. Fig. 4 shows the FT-IR spectrum of the ethanol obtained from BSG. The biomass conversion process adopted in this study for the fuel production clearly evidences the expanding sustenance in Ethiopia based on renewable energy sources, Barley spent grains, in this case.[19,20]

Conclusions

This study was performed to reveal the potential of barley spent grain as a prospective input for the ethanol production. Barley spent grain (BSG) is promising lignocellulosic feedstocks for the production of bioethanol. The two-stage diluted acid hydrolysis is the limelight of this study. Response surface methodology and ANOVA analysis shows that the hydrolysis temperature, time and acid concentration have a significant effect on the yield of ethanol. Process optimization at 144.29°C, 26.26 min and 1.68% acid concentration, yielded 47.6 % (w/w) reducing sugar and 8.33 mL ethanol per 50 g of dry BSG, respectively. The present study recommends the further research capabilities to utilize this largely available by-product, Barley Spent Grain.

Acknowledgment

None

Declaration

The author declares that this contribution is original and is not published elsewhere in part or in whole, elsewhere.

Conflict of Interest

The author declares no conflict of interest.

Authorship (contribution or attribution)

All authors have read and equally contributed to this article.

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