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Lactic acid production from Brewer's Spent Grain by *Lactobacillus plantarum* ATCC 8014

Yohannes Assefa and S Anuradha Jabasingh*

Process Engineering Division, School of Chemical and Bio Engineering, Addis Ababa Institute of Technology, Addis Ababa University, Addis Ababa, Ethiopia.

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The objective of this research was to produce lactic acid from Brewery Spent Grain using *Lactobacillus plantarum* ATCC 8014. The production was carried out in four main stages, including the pretreatment, hydrolysis, fermentation and recovery of lactic acid. Box Behnken Design (BBD) (Design expert® 7 software) was used to investigate the effect of temperature (115–130°C), reaction time (25–35 min) and acid concentration (1.5–2.0 M) during the hydrolysis. Fermentation of the hydrolyzate was performed at 35°C, pH 5.0–5.5 and 200 rpm for 72 h. Optimization results proved the suitability of BSG to be used as a feedstock for the lactic acid production.

Keywords: Barley spent grain, Box behnken design, Fermentation, Hydrolysis, Optimization

Introduction

Brewer`s spent grain (BSG) is the solid residue remaining after the mashing and lautering, of the barley grains. It consists mainly of grain husk and other residues, which are not converted to fermentable sugars by the mashing process.[1] Brewer's spent grain (BSG) is the most abundant brewing by-product, corresponding to about 85% of total by-products generated. Traditionally, the material has been landfilled or sold as animal feed.[2] Chemically, BSG is rich in polysaccharides, protein and lignin. Currently, there is a rapid growth of the brewery industries in Ethiopia. There are about eleven breweries in full function and production. There are also some breweries being expanded and expected to be completed for the next few years. In Ethiopia, around 263,736 kg barley spent grain is generated daily.[3] Lactic acid, which is commonly used in food, chemical and pharmaceutical industries, has a wide range of applications ranging from medical devices, such as suture threads and scaffolds, to commodity products like bottles and films for food packaging.[4] The optimization of fermentation processes requires profound knowledge of the factors determining microbial metabolism, and the influence of process parameters.[5] Temperature, incubation period and pH are the key environmental parameters that affect the fermentation process. The present study is an attempt to produce lactic acid using BSG as substrate, using the *Lactobacillus plantarum* ATCC 8014. This also utilizes the BSG or the waste generated in the breweries into a value-added product, as a part of expanding sustenance in the country.[6–8]

*Author for Correspondence
E-mail: anu3480@gmail.com

Materials and Methods

Materials

Sodium hydroxide, sulfuric acid, beef extract, yeast extract, tween 80, manganese sulphate, acetone, *MRS Broth were purchased from Merck, Germany*. Brewer's spent grain (BSG) was gifted by the BGI brewery Addis Ababa, Ethiopia. *Lactobacillus plantarum* ATCC 8014 was procured from the Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia.

Feedstock preparation and characterization

BSG was successively washed with distilled water to remove impurities, and dried at 75°C. It was treated with 0.5 M NaOH for 4 h at room temperature. The excess of the alkaline solution was removed by washing with distilled water. The material was further dried in an oven at 75°C for 24 h. The dried biomass was ground and sieved to 250–500 μm and stored in an air-tight container, until use. Experiments were conducted to determine the moisture content, fixed carbon content, ash content and volatile matter content of the samples.[2] Compositional analysis was carried out to find the yield of extractives, hemicelluloses, lignin, and cellulose.[6,9]

Acid hydrolysis

Response surface methodology, with three-level-three-factororial Box-Behnken Design was employed during the acid hydrolysis. BSG was hydrolyzed using H_2SO_4 in the autoclave at a temperature of 115°C, 122.5°C and 130°C, with acid concentration 1.5 M, 1.75 M and 2.0 M and time of 25 min, 30 min and 35 min. After the hydrolysis, the solid part was separated from the liquid by vacuum filtration unit and the filtered hydrolyzate was neutralized with 4M NaOH until the pH of 5.0–5.5. After hydrolysis, the liquid fraction of the hydrolyzate samples was analyzed for their reducing sugar content using the phenol-sulfuric acid method. The absorption values of the samples were recorded at 490 nm on a spectrophotometer.[10]

Culture media

Lactobacillus plantarum ATCC 8014 was employed for the conversion of glucose to lactic acid. Fermentation broth-liquid medium (1 L) contained in g: 10, Peptone; 10, Beef extract; 5, Yeast Extract; 20, Dextrose; 1, Tween 80; 2, Ammonium citrate; 5, Sodium acetate; 0.1, Magnesium sulphate; 0.05, Manganese sulphate; 2, Dipotasium phosphate. *Lactobacillus plantarum* ATCC 8014 was grown in 25 mL test tubes containing 10 mL MRS broth. After 48 h of static incubation at 37°C, 1 mL of this culture was transferred to a new test tube with 10 mL MRS broth, and incubated at the same conditions. The cells were harvested by centrifugation (1,100 rpm, 15 min), resuspended in the sterilized water and inocu lated into the 150 mL of the production medium.[11]

Fermentation

All the fermentation experiments were carried out at 35°C and pH (5.0–5.5), optimum for the culture. The culture media and the hydrolyzate sample were mixed in a proportion of 1:10 in Erlenmeyer flask. Then the mixture was placed in a shaker incubator at 200 rpm for 72 h. The fermented liquor was centrifuged at 5000 rpm for 5 min and filtered through 0.2 µm paper filter. Lactic acid in fermented broth was identified according to the method of Borshchevskaya *et al*., 2016 by spectrophotometric determination.[12]

Results and Discussion

Proximate analysis and chemical composition analysis of BSG

Moisture content, volatile matter, ash content and fixed carbon content of BSG were 10.8, 73.0, 3.9 and 12.3% respectively. The raw BSG sample was rich in volatiles (73.0%) and fixed carbon but low in ash content (3.9%). The BSG supplied had a moisture content of about 10.8%, which is a well-tolerated value for production of lactic acid.[11] Biomass with higher fixed carbon content has a higher lactic acid yield. Chemical composition analysis of BSG showed extractives, cellulose, lignin and hemicellulose to be 13.1, 28.7, 16.9 and 41.3 % respectively. The low lignin content indicates the credibility of BSG towards hydrolysis.[2]

Total reducing sugar and Lactic acid yield in percentage

The variation in the lactic acid yield at different reaction times, temperature and acid concentration is shown in Table 1. To analyze the experimental

Table 1 — Total reducing sugar (%) and Lactic acid yield (%) from BSG using *Lactobacillus plantarum* ATCC 8014

Run	A:Reaction time (min)	B:Reaction temperature (ºC)	C:Acid concentration (M)	Total reducing sugar (%)	Lactic Acid yield (%)
1	35.00	130.00	1.75	52.42	23.90
2	30.00	130.00	2.00	51.26	27.56
3	25.00	122.50	2.00	43.21	24.00
4	25.00	122.50	1.50	33.75	27.19
5	30.00	122.50	1.75	42.63	23.90
6	30.00	130.00	1.50	39.85	27.78
7	30.00	115.00	1.50	37.65	25.63
8	25.00	130.00	1.75	58.65	26.51
9	35.00	122.50	2.00	35.22	23.89
10	30.00	122.50	1.75	45.11	23.56
11	25.00	115.00	1.75	55.98	27.61
12	30.00	122.50	1.75	45.23	24.29
13	30.00	122.50	1.75	43.46	24.00
14	30.00	122.50	1.75	43.26	25.08
15	35.00	115.00	1.75	48.50	20.19
16	35.00	122.50	1.50	24.60	17.03
17	30.00	115.00	2.00	48.85	26.63

results, Design expert® 7.0.0 software was used. The maximum lactic acid yield of 27.78% was obtained at run 6, at 130°C, 1.5 M acid concentration, and at 30 min. While the minimum yield of 17.03% was obtained during the run 16, at a temperature of 122.5°C, 2 M acid concentration, and 35 min hydrolysis time (Table 1). The maximum yield of glucose at 1.75 M of acid concentration, at 130°C and 25 min was 58.65%. The decrease and increase of the yield depends on the level of factors. In order to analyze the regression equation of the model, 2D contour plots were obtained by plotting the response against any two variables while keeping the other variable at center level. The maximum effect was observed on the yield of lactic acid at 27.5 min, when the temperature reached 122.5°C. Beyond this temperature, the yield gradually decreased, due to the non-conversion of glucose to lactic acid.[12] At high hydrolysis temperature, the amount of lactic acid yield was low compared to the middle maximum point, due to the conversion of reducing sugar to other by products. The contour plot developed as a function of temperature and time, while the acid concentration was kept constant at 1.75 M is shown in Fig. 1a whereas Fig. 1b shows the interaction effect of acid concentration and time on lactic acid yield at a temperature of 122.5°C. The maximum yield of lactic acid was observed at a moderate time until the acid concentration reached 1.75 M and the reaction time was 29 min. But, beyond 1.75 M acid concentration at fixed time, the yield of lactic acid decreased. As well as, upon increasing the acid concentration, from 1.5 M to 1.75M with a decrease of hydrolysis time from 35 min to 25 min, the yield of lactic acid increased. The highest yield was obtained at 30 min of hydrolysis time and 1.75M sulfuric acid concentration. The yield of lactic acid increased slightly with hydrolysis temperature from 115 to 122.5°C and acid concentration from 1.5 M to 1.75 M (Fig. 1c). However, upon increasing the hydrolysis temperature beyond 122.5°C, and acid concentration, beyond 1.75 M there was a gradual decline in the yield, due to the degradation of reducing sugars (Fig. 2).[13]

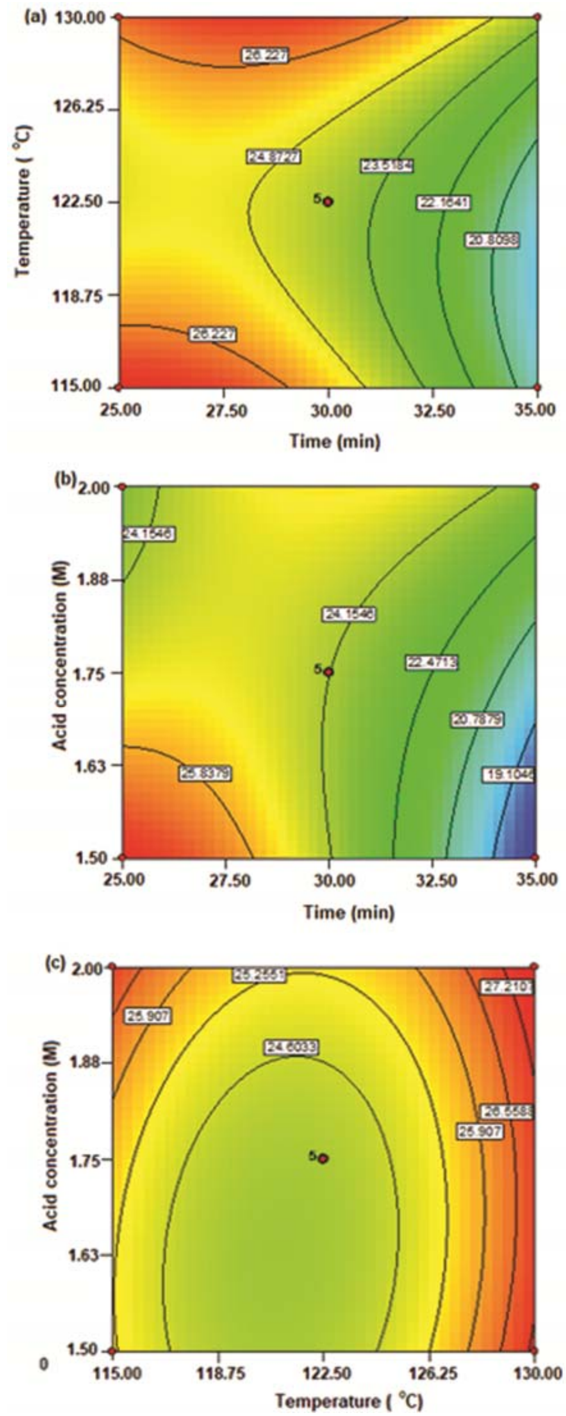

Fig.1 — Contour plots showing the effect of time, temperature and acid concentration on the lactic acid yield

Model validation and adequacy check

According to the BBD using Design-Expert® 7, the experiment was carried out at the optimized conditions. The optimal value of the test factors were 1.89 M acid concentration, 129.75°C and 32.39 min. At these optimized conditions, the lactic acid yield of 26.71% was obtained and it was in good agreement with the predicted one (run 2 with a lactic acid yield of 27.56%). Therefore the model is considered to be accurate and reliable for predicting the yield of lactic acid. The model equation that correlates the response (lactic acid yield) to the hydrolysis process variables in terms of the coded factors is given in Eq.1.

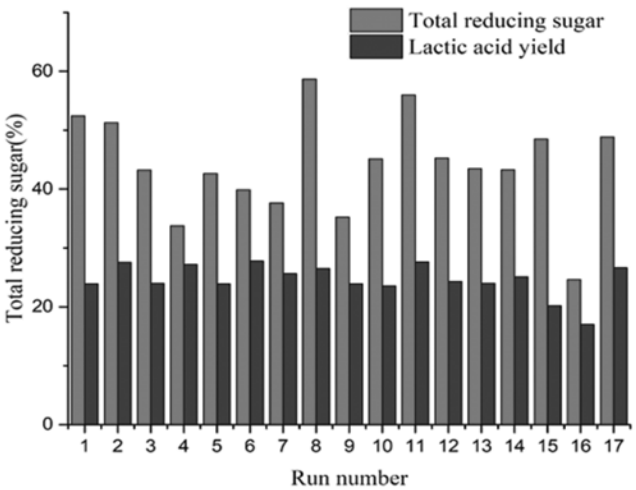

Fig. 2 — Total reducing sugar and the lactic acid yield from BSG

$$Lactic\ acid\ yield(\%) = 24.17 - 2.54A + 0.71B + 0.56C + 1.0AB + 2.51AC - 0.31BC - 1.74A^2 + 2.13B^2 + 0.60C^2 \quad \ldots (1)$$

Where, A is the reaction time (min), B is the reaction temperature (°C) and C is the sulfuric acid concentration (M). The model was tested for adequacy by analysis of variance.[14] The regression model was found to be highly significant with the correlation coefficients of determination of R-Squared, adjusted R Squared and predicted R-Squared having a value of 0.9807, 0.9558 and 0.8440 respectively. The Model F-value of 39.43 implies the model is significant. There is only a 0.01% chance that a "Model F Value" this large could occur due to personal error or disturbance.[3,7] Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, A^2, and B^2 are significant model terms. The "Lack of Fit F-value" of 1.09 implies, it is not significant relative to the pure error. BSG employed in this study could thus be a potential feedstock lactic acid production using *Lactobacillus plantarum* ATCC 8014.

Conclusions

The conversion of brewery spent grain to lactic acid was carried out after the pretreatment using dilute acid hydrolysis. The optimum conditions during hydrolysis were 1.89 M acid concentration, 129.75°C and 32.39 min. At these operating conditions, the maximum yield of reducing sugar and lactic acid was found to be 51.26% and 26.71%, respectively. From this result, it can be concluded that Barley Spent Grain (BSG) has the potential to be used as a low-cost feedstock for the production of lactic acid using *Lactobacillus plantarum* ATCC 8014.

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