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VALORIZATION OF BREWER'S SPENT GRAIN FOR SUSTAINABLE BIOETHANOL PRODUCTION VIA INTEGRATED ACID HYDROLYSIS AND ENZYMATIC FERMENTATION

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Abstract -This study explores the sustainable valorization of brewer's spent grain (BSG), a lignocellulosic agro-industrial byproduct, for bioethanol production through an integrated process of dilute acid hydrolysis and enzymatic fermentation. Comprehensive pretreatment and characterization revealed that BSG contains a high carbohydrate content (50.17%) and low moisture (9.88%), making it a promising feedstock for bioethanol generation with minimal drying requirements. Dilute sulfuric acid hydrolysis was employed to release fermentable sugars, while *Saccharomyces cerevisiae* and *Kluyveromycesmarxianus* were used as biocatalysts in the subsequent fermentation stages. Process parameters such as acid concentration, substrate dosage, temperature, and reaction time were optimized using Response Surface Methodology (RSM) and Artificial Neural Networks (ANN). Maximum glucose yield (1.67 g/L) was achieved under optimal hydrolysis conditions: 2.59 % acid concentration, 20.85 g substrate dosage, 39.94 minutes, and 76.83°C. Comparative analysis demonstrated that ANN provided superior predictive accuracy over RSM for both glucose and bioethanol yields. Optimal bioethanol outputs from *K. marxianus* and *S. cerevisiae* were 15.91 % and 18.10 %, respectively, with *S. cerevisiae* showing greater fermentation efficiency. This integrated approach underscores the potential of BSG as a low-cost, renewable substrate for bioethanol production and highlights the advantages of combining biochemical conversion with machine learning for process optimization.

Keywords: Bioethanol, fermentation, optimization, acid hydrolysis, and brewer's spent grain.

1 Introduction

The urgent global shift toward sustainable and renewable energy sources have placed biomass at the forefront of research and development for alternative fuels. Biomass is an organic material derived from plants, animals, human activities, and industrial processes, and offers a versatile and abundant source of stored solar energy (Benti et al., 2021). Among various renewable options, bioethanol stands out as a viable substitute for fossil fuels in transportation, heating, and electricity generation due to its renewability, lower environmental footprint, and compatibility with existing infrastructure (Bagherian et al., 2021; Kalak, 2023; Ben-Iwo et al., 2016; Owusu &Asumadu-Sarkodie, 2016; Ezealigo et al., 2021; Onuora et al., 2023; Okafor et al., 2022; Abbas et al., 2020; Osman et al., 2023).

A particularly underutilized feedstock for bioethanol production is brewer's spent grain (BSG), the primary byproduct of the beer brewing industry. Representing nearly 85% of all brewing byproducts, BSG is generated in vast quantities—approximately 270 kg per 1 m^3 of beer—amounting to an estimated 40 million tons globally each year (Mussatto, 2014; He et al., 2021; Pinheiro et al., 2019; Bedo et al., 2021; Lynch et al., 2016; Ahuja et

al., 2024; Parchami et al., 2021, 2022; Wagner et al., 2022). Despite its significant availability, over 70% of BSG is used as low-value animal feed, 10% is directed toward biogas production, and about 20% is disposed of in landfills, highlighting the inefficiency of current valorization pathways (Bianco et al., 2020; Steiner et al., 2015).

BSG, rich in fibers and residual starch, holds substantial promise for conversion into high-value bioethanol, providing a dual benefit of waste reduction and energy generation. However, transforming BSG into fermentable sugars requires an effective pretreatment process to break down its complex lignocellulosic structure. Pretreatment—often accounting for over 40% of total processing costs—is a critical and energy-intensive phase in biomass valorization (Sindhu et al., 2016; Hassan et al., 2018). Chemical methods such as acid hydrolysis are particularly favored for their efficiency and speed, especially under dilute conditions (2–5% acid concentration), which balance sugar yield and operational safety (Ajala et al., 2020; Kamzon et al., 2016). This method effectively exposes cellulose and hemicellulose fractions, enhancing their enzymatic accessibility (Joshi et al., 2021; Kumar & Sharma, 2017; Moodley & Trois, 2021; Santos et al., 2020; Maurya et al., 2015; Arora et al., 2019).

Following hydrolysis, microbial fermentation is used to convert reducing sugars into bioethanol. Yeasts such as Saccharomyces cerevisiae and Kluyveromycesmarxianus, along with microbes like Aspergillus niger and Zymomonasmobilis, have demonstrated efficacy in lignocellulosic ethanol production (Baki et al., 2020; Chibuzor et al., 2016; Aditomere, 2015). However, bioethanol yields from BSG are significantly influenced by multiple factors, including the type of yeast, fermentation time, substrate concentration, pH, temperature, and inoculum dosage (Smuga-Kogut et al., 2021; Fischer et al., 2017). Given the interdependence of these parameters, optimization using statistical tools has become increasingly essential.

Techniques such as Response Surface Methodology (RSM) and Artificial Neural Networks (ANN) have been employed to model and optimize fermentation conditions, enabling efficient prediction of outcomes and enhancement of process performance (Adekunle et al., 2016; Izmirlioglu& Demirci, 2016). These approaches not only reduce the cost and time of experimentation butalso support better design and control of bioreactors for industrial-scale implementation.

This study, therefore, investigates the valorization of BSG through an integrated approach combining dilute acid hydrolysis and enzymatic fermentation using S. cerevisiae and K. marxianus. It further applies RSM and ANN to optimize critical process variables and assess their impact on glucose and bioethanol yields. The research aims to contribute to the development of economically viable and environmentally sustainable bioethanol production pathways using agro-industrial waste.

2 Methodology

The brewer's spent grain sample was obtained from Agbani, in Nkanu West Local Government Area, Enugu State, Nigeria. On a batch basis, 50 g of the sample was thoroughly washed with ordinary water to remove suspended particles and ground into small pieces. The initial moisture content of the sample was recorded before air drying for seven (7) days. The dried substrate was powdered with an electric grinder, packed,and stored at room temperature until used in an airtight container.

2.1 Characterization techniques

To ensure credible and reproducible results, the brewer's spent grain (BSG) was thoroughly characterized before and after pretreatment to evaluate its suitability for bioethanol production. The following characterization techniques were employed:

2.1.1 Proximate Analysis

Standard methods were used to determine moisture content, ash content, volatile matter, and fixed carbon. Moisture content was

determined using oven-drying at 105°C until constant weight was achieved. Ash content was analyzed by incineration at 550°C in a muffle furnace. Volatile matter and fixed carbon were calculated following ASTM protocols.

2.1.2 Chemical composition Analysis

Cellulose, hemicellulose and lignin contents of the sample were determined; other extractives were calculated by subtracting the total percentage of the cellulose, hemicellulose and lignin from 100%.

a) Determination of Cellulose content

The method employed by previous authors (Onyeagoro, 2012; Mbah et al., 2020) was used to determine the cellulose content of the brewer's spent grain (BSG). The sample, weighing 1.5g, was placed in a beaker. 80 ml of acetic acid, 1 ml of concentrated nitric acid, and 3 glass beads were then added to it. The content was refluxed for about 30 minutes. It was then cooled, put into a 50 ml centrifuge tube, and centrifuged at 1500 rpm for 5 minutes. 95% ethanol was added, swirled, and filtered after the liquid had been decanted. After that, the sample was washed three times using hot benzene, twice using 95% pure ethanol, and once using ether.The sample was put in a weighted crucible and then in an oven for one hour at 110 degrees Celsius. The crucible was cooled in a desiccator and weighed. The ash content was determined by placing the crucible and its content in a furnace at 500 °C for 3 hours. Thereafter, it was cooled in a desiccator, weighed, and the percentage of cellulose was calculated.

b) Determination of hemicellulose content of the sample

Hemicellulose content was determined by the gravimetric method as reported by previous authors (Onyeagoro, 2012; Mbah et al., 2020). 1g of the sample was placed into a 250 mL Erlenmeyer flask. 150 ml of 0.5 mol/dm^3 sodium hydroxide was added, and the mixture was then boiled with distilled water for 3.5 hours. It was cooled, filtered, and washed until neutral pH.The residue was dried in an oven set at 105 degrees Celsius until it reached a constant weight. The difference in sample weight before and after treatment was used to determine the sample's hemicellulose content.

c) Determination of lignin content of the sample

The gravimetric method used by previous authors (Onyeagoro, 2012; Mbah et al., 2020) was employed in determining the lignin content of the sample. Two grams of the sample were weighed and placed in a beaker. 10 mL of H_2SO_4 was added, and the sample was kept at room temperature for 2 hours with careful shaking at 30-minute intervals. 5 mL of sulphuric acid (H_2SO_4) was then added and the solution refluxed for 3 hours. Thereafter, the residue was filtered and washed several times with hot water. The sample was scraped into a weighed crucible, placed inside the oven, and dried at 105 °C for 1 hour. The weight was taken after cooling in a desiccator. The sample was ashed in a furnace at 550^oC for 3 hours, cooled in a dessicator, and weighed.

2.2 Acid Hydrolysis of the sample and Glucose content/yield determination

15g of the dry ground brewer's spent grain was weighed into a 250 ml conical flask, and 200ml of 5% dilute H_2SO_4 was used to hydrolyse the sample. The flask was covered with cotton wool, wrapped with aluminium foil, and then heated at 50 °C for 30 minutes. The sample was allowed to cool and filtered using Whatman 42 filter paper. The glucose content was determined using the titrimetric method employed by Okon et al. (2021). In the process, 4 mL of anthrone reagent was added to the supernatant and heated for 8minutesb in a boiling water bath. It was cooled rapidly and read when the green color changed to dark green at 630nm. The amount of carbohydrates (as glucose) present in the sample tube was determined from the absorbance-concentration relationship.

2.3 Investigating the Effects of Process Variables on the Glucose Yield

To evaluate the influence of critical process variables on glucose yield from brewer's spent grain (BSG), a systematic experimental design

was adopted, combining dilute acid hydrolysis with advanced statistical modeling. The process involved the following stages:

2.3.1 Experimental Design

A central composite design (CCD) under Response Surface Methodology (RSM) was employed to optimize and assess the interaction effects of four independent variables:

- Acid concentration (1–5% v/v),
- Substrate dosage (10–25 g),
- Temperature (60–90°C), and
- Reaction time (20–60 minutes).

These variables were selected based on preliminary trials and literature evidence indicating their significant influence on hydrolytic efficiency.

2.3.2. Pretreatment and Acid Hydrolysis

Dilute sulfuric acid hydrolysis was carried out in a thermostatic water bath reactor. The reaction mixture of ground, sieved BSG and acid solution was sealed in Erlenmeyer flasks and heated at designated temperatures and time intervals. After cooling, the hydrolysate was neutralized with calcium hydroxide and filtered.

2.3.3 Glucose Quantification

The filtrate was analyzed for glucose concentration using:

- High-Performance Liquid Chromatography (HPLC) equipped with an RI detector and Aminex HPX-87H column, and
- DNS Method (3,5-dinitrosalicylic acid assay) for cross-validation of reducing sugar content using a UV-Vis spectrophotometer.

2.3.4 Data Analysis and Modeling

The experimental data were subjected to:

- Analysis of Variance (ANOVA) to determine the statistical significance of individual and interaction effects.
- Regression modeling to develop predictive equations for glucose yield.
- 3D surface plots and contour diagrams to visualize optimal operating conditions.

2.3.5 Artificial Neural Network (ANN) Modeling

In parallel, a feed-forward back-propagation ANN model was developed using the same input variables to predict glucose yield. The model was trained, validated, and tested using normalized datasets, and its performance was compared with RSM based on metrics such as R^2, RMSE, and MAE.

2.4 Bioethanol production

Bioethanol was produced from the glucose sample by acid hydrolysis and the enzymatic fermentation process. 15 g of dry brewer's spent;/KMgrain was weighed into a 250ml conical flask, and then 200 ml of 5% dilute H_2SO_4 was used to hydrolyse the brewer's spent grain. The flask was covered with cotton wool and wrapped with aluminiumfoil, and heated at 50 °C for 30 minutes. The sample was allowed to cool and then filtered using Whatman 42 filter paper. The pH value of the sample was adjusted with sodium hydroxide before adding the yeast to the hydrolysed sample. Yeast (Saccharomyces cerevisiae) was added to the flask containing the sample and then stirred thoroughly. The substrate was fermented under various conditions. Effects of pH, yeast dosage, incubation temperature, and fermentation time on the bioethanol yield were determined. The added yeast provided enzymes (invertase and zymase) for the conversion of the sample into ethanol and carbon dioxide. The process was repeated using another yeast (Kluyveromycesmarxianus).

2.5 Optimization of Bioethanol Yield

In response surface methodology, the Central composite design tool of Design Expert Software (version 11) was used to design the experiment, which is in line with the method used by Omotioma et al (2024). On the optimization using artificial neural network, a Levenberg-Marquardt trained standard two-layer feed-forward neural network was applied.

2.6 Determination of Physico-Chemical Properties of the Bioethanol

The bioethanol was characterized to ascertain its properties in terms of viscosity, specific gravity, flash and smoke points, refractive index, cloud and pour points, and Sulphur content.

a) Specific gravity:

The specific gravity bottle method was used for the determination of the specific gravity. A clean, empty bottle was weighed on the electronic balance and the weight (W_1) recorded. It was then filled with the sample and weighed (W_2). All the determinations were at room temperature, and the volume (V) of the specific gravity bottle was recorded.

$$\text{Specific gravity} = \frac{W_2 - W_1}{V} \quad (1)$$

b) Sulphur content:

1g of the sample was mixed with 3g of a mixture of magnesium oxide and anhydrous sodium carbonate (2:1). The mixture was heated to 400 °C for 2 hours in a muffle furnace, after which it was cooled and digested in water. Barium chloride was then added to precipitate the sulphate as barium sulphate. The precipitate was filtered, and the amount of Sulphur was determined (ASTM 1992).

$$\text{Sulphur content (\%)} = \frac{\text{Ppt}\,(BaSO_4) \times 0.1373 \times 100}{\text{Weight of sample}} \quad (2)$$

c) Flash and smoke point:

A Pensky Martin Flash Point (closed) apparatus was used to measure the flash point of the sample. The sample was filled in the test cup up to the specific level, then heated and stirred at a slow and constant rate. At every 10 °C temperature rise, a flame was introduced for a moment with the help of a shutter. The temperature at which a flash appeared in the form of sound and light was recorded as the flash point.

d) Viscosity:

The appropriate spindle was selected and fixed on a digital viscometer made by SearchtechInstruments, England. The spindle was inserted in the sample till the mark on the spindle reached the surface of the sample. The enter button on the instrument was pressed, and the viscosity of the sample was displayed on the screen.

e) Refractive index:

An Abbe refractometer-bench type (Model: WYA-2S, made by SearchtechInstruments) was used to determine the refractive index of the bioethanol. The power switch was pressed on, and the illuminating lamp came up and displayed 0000. A drop of the sample was applied to the working surface of the lower refracting prism. The rotating arm and the collecting lens cone of the light-gathering illuminating units were rotated to make the light-intake surface of the upper light-intake prism to be illuminating evenly. The field of view was observed through the eyepiece, and the adjustable hand wheel was rotated to make the line dividing the dark and light areas fall in the cross line. The dispersion correction hand wheel was rotated to get a good contrast between the light and dark areas and minimum dispersion. The read button was then pressed, and the refractive index was displayed on the screen.

f) Cloud and pour point:

The cloud and pour point of the sample was determined as per IS: 1448 [P:10]:1970 using the cloud and pour point apparatus. The apparatus mainly consists of 12cm high glass tubes of 3cm diameter,which are enclosed in an air jacket filled with a freezing mixture of crushed ice and sodium chloride crystals. The glass tube containing the fuel sample was taken out from the jacket at every 10 °C interval as the temperature fell and was inspected for cloud/pour point. The point at which a haze was first seen at the bottom of the sample was taken as the cloud point. The pour point was taken to be the temperature 10 °C above the temperature at which no motion of fuel was observed for five seconds on tilting the tube to a horizontal position.

3 Results and Discussion

3.1 Proximate Analysis and Chemical Composition of the Brewer's Spent Grain

The result of the proximate analysis of the brewer's spent grain is depicted in Table 1. The ash content of brewer's spent grain was 4.63%, which signified the mineralogical level of the sample. A moisture content of no more

than 10% is considered acceptable, and this low level of moisture is ideal for the long-term preservation of biomaterials (Akubor et al., 2013). This enhances material storage stability by preventing mold growth and reducing moisture-dependent biochemical reactions (Omimawo and Allubor, 2012). The carbohydrate content was observed to be 50.17%, making the sample a suitable source of starch for bioethanol production. Additionally, saponification is not required because of the low crude fat level. However, the proximate analysis result corresponds with previously published data (Naibaho and Korzeniowska, 2021).

Table 1: Proximate analysis of brewer's spent grain

Composition	Brewer's Spent grain
Ash (%)	4.63
Crude fat (%)	14.91
Crude fibre (%)	7.26
Moisture content (%)	9.88
Protein (%)	13.15
Carbohydrate (%)	50.17

Table 2 shows the chemical composition of the brewer's spent grain. The cellulose, hemicellulose, lignin, and other extractives were recorded as 56.32%, 23.15%, 17.43%, and 3.10%, respectively. Its potential to hydrolyze into sugar enrichment is demonstrated by the large amount of cellulose content in brewer's spent grain.

Table 2: Chemical composition of Brewer's Spent Grain

Parameters	Brewer's Spent Grain
Cellulose (%)	56.32
Hemicellulose (%)	23.15
Lignin (%)	17.43
Other extractives (%)	3.10

3.2 Effects of Process Variables on the Glucose Yield

The effect of acid concentration on the yield of glucose from brewer's spent grain (Figure 1) shows that the acid concentration was varied from 1 to 3% at a step increase of 0.5% while the other parameters were kept constant. The glucose yield increased with an increase in acid concentration till it reached the maximum glucose value of 1.63g/l, at an optimum acid concentration of 2%, after which the glucose yield started decreasing with further increase of acid concentration. The reduction of glucose yield with high acid concentration results from the fact that low acid concentration is conducive for glucose production during hydrolysis. However, using a high concentration of acid for hydrolysis causes the hydrolysate to brown and char, and it frequently results in the creation of unwanted byproducts such as 5-dehydroxymethyl furfural and furfurals that prevent fermentation (Agu et al., 1997).

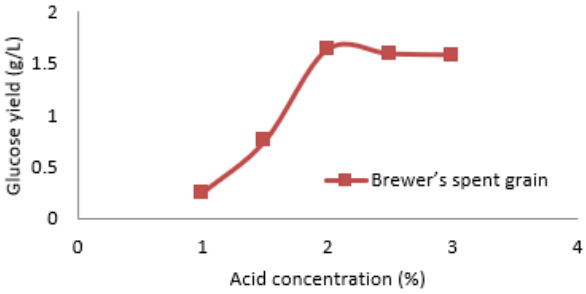

Figure 1: Effect of acid concentration on the glucose yield

In Figure 2, substrate dosage varied from 5-25g at a step increase of 5g. The yield of glucose gradually increased as the substrate dosage increases until reaching a maximum of 1.63g/l at an optimum substrate dosage of 15g at which point it began to decline with substrate dosage increase. Reduced glucose yield following the optimum substrate dosage may have resulted from the mixture's viscosity, which allowed for a gradual rise in glucose production at lower substrate dosages. However, when the optimum dosage of substrate was exceeded, the mixture's viscosity increased to the point where it may obstruct the hydrolysis reaction and reduce the yield of glucose. This fact is supported by the results showing that high dosages of biomass produce high viscosity, whereas low dosages produce low viscosity (Sanette and Tando, 2012; Onyelucheya et al., 2018; Ashish and Shalini, 2016).

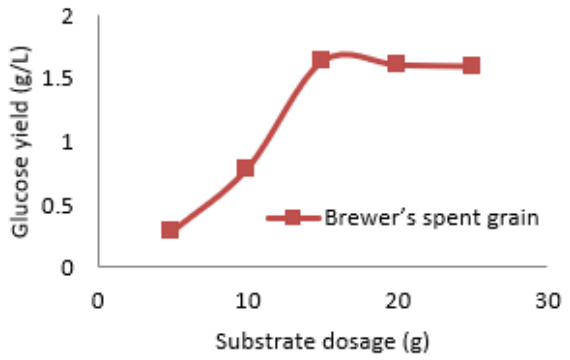

Figure 2: Effect of substrate dosage on the glucose yield

Figure 3 illustrates how the hydrolysis time of brewer's spent grain was adjusted from 20 to 60 minutes with a 10-minute increment while maintaining all other parameters constant. As the hydrolysis time increased, the glucose yield increases as well, reaching its maximum of 1.63g/l at the optimum 40-minute time. The glucose yield decreased as the time increased after surpassing the optimum time. This may be explained by the breakdown of glucose into a degradation product over a long hydrolysis period, which is in conformity with research by Onyelucheya et al. (2018).

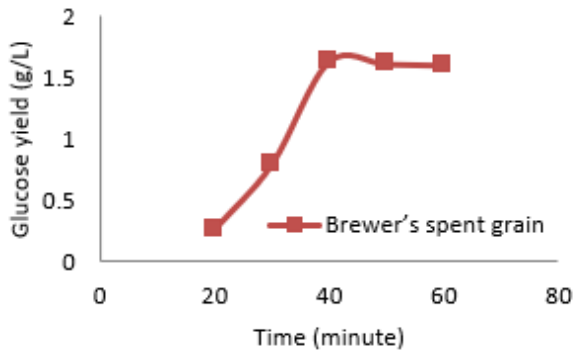

Figure 3: Effect of time on the glucose yield

With all other factors held constant, the temperature was adjusted in steps of 10 degrees Celsius from 55 to 95 degrees Celsius.The glucose yield reached its highest value of 1.63g/l at the ideal temperature of 75^{o}C, as shown in Figure 4, and then decreased with additional temperature increases after increasing progressively with reaction temperature during the early phases of hydrolysis. This may be explained by the fact that at higher temperatures, xylose is converted to glucose (Zhong et al., 2015). Hence, increasing the temperature favorably increase the yield of glucose within a particular range, but increasing the temperature too much could harm the conversion process and ultimately reduce the yield of glucose. This finding conforms with the research conducted by Lenihan et al. (2010) and Hernandez et al. (2012).

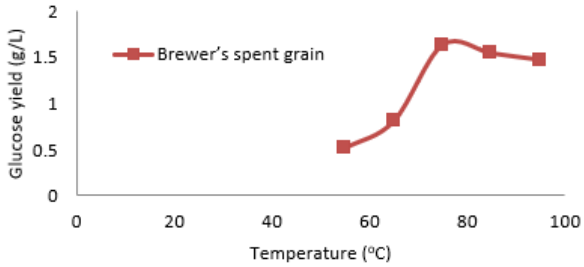

Figure 4: Effect of temperature on the glucose yield

3.3 ANN and the Corresponding RSM Results of Glucose Yield

Experimental glucose yield from the brewer's spent grain was shown in Table 3 alongside the RSM and ANN predicted yields. However, the ANN prediction was relatively closer to the experimental/actual glucose yield.

Table 3: Actual, RSM and ANN data predictions of glucose yield from brewer's spent grain

Std	Run	F 1 A: Acid conc. %	F 2 B: Substrate dosage g	F 3 C: Time min.	F 4 D: Temp. ^{o}C	Actual glucose yield g/L	RSM predicted glucose yield g/L	ANN predicted glucose yield g/L
19	1	15	1	40	75	0.87	1.04	0.855
27	2	15	2	40	75	1.63	1.58	1.615
13	3	5	1	60	95	0.28	0.2849	0.265

9	4	5	1	20	95	0.3	0.2102	0.285
20	5	15	3	40	75	1.52	1.45	1.505
10	6	25	1	20	95	0.68	0.7071	0.665
30	7	15	2	40	75	1.63	1.58	1.615
12	8	25	3	20	95	1.39	1.37	1.375
15	9	5	3	60	95	0.63	0.6208	0.615
26	10	15	2	40	75	1.63	1.58	1.615
24	11	15	2	40	95	1.59	1.56	1.575
4	12	25	3	20	55	0.89	0.9005	0.875
22	13	15	2	60	75	1.61	1.62	1.595
11	14	5	3	20	95	0.42	0.496	0.405
6	15	25	1	60	55	0.61	0.5494	0.595
8	16	25	3	60	55	1.02	1.07	1.005
29	17	15	2	40	75	1.63	1.58	1.615
3	18	5	3	20	55	0.31	0.2585	0.295
21	19	15	2	20	75	1.41	1.5	1.395
28	20	15	2	40	75	1.63	1.58	1.615
25	21	15	2	40	75	1.63	1.58	1.615
5	22	5	1	60	55	0.22	0.1974	0.205
2	23	25	1	20	55	0.46	0.4296	0.445
14	24	25	1	60	95	0.86	0.8719	0.845
23	25	15	2	40	55	1.15	1.28	1.135
17	26	5	2	40	75	0.73	0.8462	0.715
18	27	25	2	40	75	1.48	1.46	1.465
1	28	5	1	20	55	0.18	0.1677	0.165
7	29	5	3	60	55	0.35	0.3383	0.335
16	30	25	3	60	95	1.56	1.59	1.545

3.4 Effects of Process Parameters on the Yield of Bioethanol

Effects of process variables on the yield of bioethanol using Saccharomyces cerevisiae (SC) and Kluyveromycesmarxianus (KM) are presented in Figures 5 - 8. In each case, bioethanol yield increased as pH, yeast dosage, temperature, and time increased till maximum yield was attained. The results are for the one factor at a time, and from Figure 5, with a 1.0 step increase, the pH was varied from 2.0 to 6.0.Cell growth and fermentation are optimally facilitated by a pH of 4. Saccharomyces cerevisiae (SC) gave the highest bioethanol yield than that from Kluyveromycesmarxianus (KM). Similarly, in Figure 6, at a dosage of 4.5%wt/v, bioethanol yield obtained using Saccharomyces cerevisiae is greater than that from Kluyveromycesmarxianus. Additionally, Figure 7 illustrates how temperature affects the yield of bioethanol. At 40°C, the highest bioethanol yield was achieved; at 45°C and above, the yield progressively dropped, as previously reported by Fakruddin et al. (2013).An increase in temperature often has a favorable impact on fermentation rates due to an increased rate of bacterial growth and the formation. Temperature increases can have adverse effects when the temperature increases more than the optimum level required for bacterial activity, thus leading to the death of cells, reduction in product formation speed, and denature of enzymes. Furthermore, the effect of time enhances the mass transfer rate of glucose to bioethanol, but an extension of time can negatively affect the degree of saccharification, probably due to enzyme denaturation (Figure 8). Nevertheless, the rate of product formation becomes

inversely proportional to the increase in time when fermentation goes above a limit of 72 hours. From the experimental results obtained, the highest bioethanol yield using Saccharomyces cerevisiae, followed by Kluyveromycesmarxianus, at 72 ^{o}C of fermentation was observed.

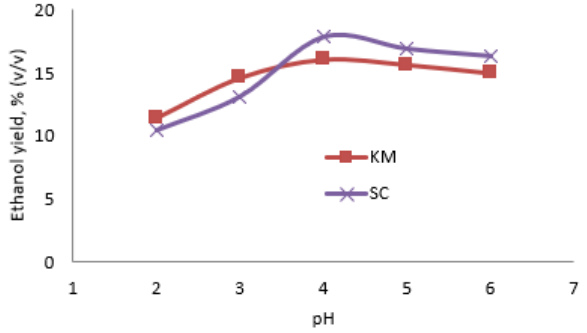

Figure 5: Effect of pH on the bioethanol yield from brewer's spent grain aided by Saccharomyces cerevisiae (SC) and Kluyveromycesmarxianus (KM) respectively**.**

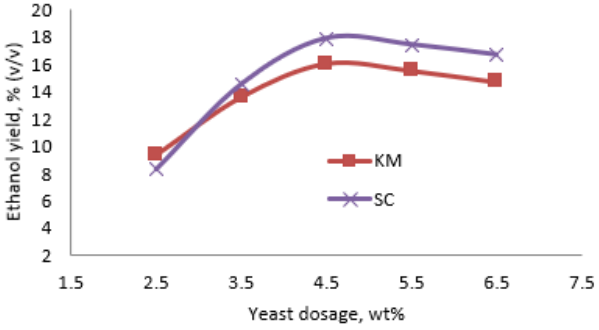

Figure 6: Effect of yeast dosage on the bioethanol yield from brewer's spent grain

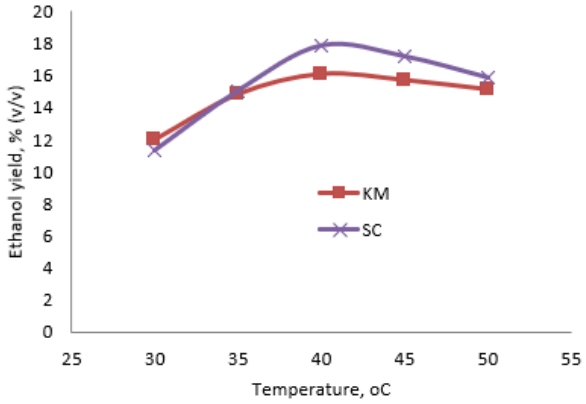

Figure 7: Effect of incubation temperature on bioethanol yield from brewer's spent grain

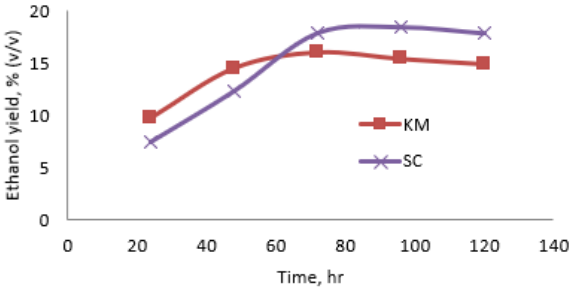

Figure 8: Effect of fermentation time on bioethanol yield from brewer's spent grain

3.5 ANN and RSM data of bioethanol yield

ANN and RSM results of bioethanol yields from brewer's spent grain aided by saccharomyces cerevisiae and kluyveromycesmarxianus are presented in Tables 4 and 5 respectively. In each case of the experimental or actual bioethanol yields, RSM predicted bioethanol yields and ANN predicted bioethanol yields were reported, with ANN appearing to be closer to the actual/experimental yield of bioethanol. This aligns with the findings of the Onukwuli et al. (2021).

Table 4: ANN and RSM data of bioethanol yield from brewer's spent grain aided by saccharomyces cerevisiae

Std	Run	F 1 A: pH	F 2 B: Yeast dosage, %wt/v	F 3 C: Temp., ^{o}C	F 4 D: Time, hr	Actual bioethanol yield % (v/v)	RSM predicted bioethanol yield % (v/v)	ANN predicted bioethanol yield % (v/v)
29	1	4	4.5	40	72	17.94	17.8	17.94

10	2	6	2.5	30	120	7.56	7.73	7.56
2	3	6	2.5	30	24	5.39	4.83	5.39
15	4	2	6.5	50	120	7.96	8.06	7.96
18	5	6	4.5	40	72	17.75	18.35	17.75
9	6	2	2.5	30	120	4.68	4.28	4.68
24	7	4	4.5	40	120	17.23	17.47	17.23
22	8	4	4.5	50	72	17.07	16.22	17.07
25	9	4	4.5	40	72	17.94	17.8	17.94
26	10	4	4.5	40	72	17.94	17.8	17.94
20	11	4	6.5	40	72	16.96	16.45	16.96
4	12	6	6.5	30	24	9.19	9.62	9.19
21	13	4	4.5	30	72	12.82	13.96	12.82
11	14	2	6.5	30	120	6.07	6.52	6.07
19	15	4	2.5	40	72	11.46	12.26	11.46
27	16	4	4.5	40	72	17.94	17.8	17.94
30	17	4	4.5	40	72	17.94	17.8	17.94
3	18	2	6.5	30	24	4.79	4.51	4.79
6	19	6	2.5	50	24	7.87	7.8	7.87
7	20	2	6.5	50	24	6.28	6.49	6.28
14	21	6	2.5	50	120	10.42	10.25	10.42
16	22	6	6.5	50	120	17.37	17.87	17.37
5	23	2	2.5	50	24	4.02	4.41	4.02
23	24	4	4.5	40	24	15.19	15.24	15.19
17	25	2	4.5	40	72	13.21	12.9	13.21
8	26	6	6.5	50	24	13.98	13.92	13.98
12	27	6	6.5	30	120	14.86	14.02	14.86
1	28	2	2.5	30	24	3.87	3.76	3.87
13	29	2	2.5	50	120	4.53	4.48	4.53
28	30	4	4.5	40	72	17.94	17.8	17.94

Table 5: ANN and RSM data of bioethanol yield from brewer's spent grain aided by kluyveromycesmarxianus

Std	Run	F 1 A: pH	F 2 B: Yeast dosage, %wt/v	F 3 C: Temp., ^{o}C	F 4 D: Time, hr	Actual bioethanol yield % (v/v)	RSM predicted bioethanol yield % (v/v)	ANN predicted bioethanol yield % (v/v)
29	1	4	4.5	40	72	16.05	15.91	16.05
10	2	6	2.5	30	120	5.64	5.83	5.6402
2	3	6	2.5	30	24	3.47	2.9	3.4702
15	4	2	6.5	50	120	6.06	6.16	6.0602
18	5	6	4.5	40	72	15.84	16.44	15.84
9	6	2	2.5	30	120	2.79	2.38	2.7902
24	7	4	4.5	40	120	15.38	15.6	15.38
22	8	4	4.5	50	72	15.17	14.32	15.17
25	9	4	4.5	40	72	16.05	15.91	16.05
26	10	4	4.5	40	72	16.05	15.91	16.05
20	11	4	6.5	40	72	15.06	14.55	15.06

4	12	6	6.5	30	24	7.26	7.69	7.2602
21	13	4	4.5	30	72	10.92	12.06	10.92
11	14	2	6.5	30	120	4.17	4.64	4.1702
19	15	4	2.5	40	72	9.56	10.36	9.5602
27	16	4	4.5	40	72	16.05	15.91	16.05
30	17	4	4.5	40	72	16.05	15.91	16.05
3	18	2	6.5	30	24	2.89	2.6	2.8902
6	19	6	2.5	50	24	5.97	5.9	5.9702
7	20	2	6.5	50	24	4.38	4.59	4.3802
14	21	6	2.5	50	120	8.54	8.36	8.5402
16	22	6	6.5	50	120	15.47	15.97	15.47
5	23	2	2.5	50	24	2.12	2.51	2.1202
23	24	4	4.5	40	24	13.29	13.35	13.29
17	25	2	4.5	40	72	11.32	11.01	11.32
8	26	6	6.5	50	24	12.06	12.01	12.06
12	27	6	6.5	30	120	12.97	12.12	12.97
1	28	2	2.5	30	24	1.95	1.84	1.9502
13	29	2	2.5	50	120	2.63	2.59	2.6302
28	30	4	4.5	40	72	16.05	15.91	16.05

3.7 Characterization of Bioethanol from Brewer's Spent Grain

The physicochemical characterization of bioethanol produced from BSG using *Saccharomyces cerevisiae* and *Kluyveromycesmarxianus* is presented in Table 8, with comparisons to ASTM standards where applicable. These results provide insights into the suitability of the produced bioethanol for fuel and industrial applications.

Ash content, which indicates the inorganic residue left after combustion, was found to be low in both samples-0.10% and 0.11% for *S. cerevisiae* and *K. marxianus*-derived ethanol, respectively. These values are favorable, as lower ash content is associated with cleaner combustion and minimal engine deposits (Abdul Kareem et al., 2021).

The cloud temperature at which crystals begin to form was slightly lower than the ASTM limit of 23 °C, with values of 18.91 °C (*S. cerevisiae*) and 18.93 °C (*K. marxianus*), indicating better cold flow properties. Similarly, pour point values were within acceptable limits (4.95 °C and 4.89 °C), showing potential for performance in moderately cold climates, as also reported in similar studies on bioethanol from agro-residues (Ajala et al., 2020; Ezealigo et al., 2021).

Flash point, a critical safety parameter, was measured at 16.51 °C and 15.98 °C, respectively. These values align with ASTM limits (10-15 °C), particularly for denatured ethanol fuels, supporting their safe storage and handling under ambient conditions (Osman et al., 2023).

In terms of refractive index, both bioethanol samples showed slightly lower values (1.349 and 1.346) than the ASTM standard range (1.360-1.364). This deviation may be attributed to trace impurities or the water content, and has been similarly observed in ethanol derived from lignocellulosic feedstocks (Mussatto, 2014; Moodley and Trois, 2021).

The specific gravity values (0.866 and 0.864) were slightly above the ASTM standard (0.750-0.850). While this may influence blending characteristics, it still reflects a fuel-grade ethanol with good density and combustion potential (Kamzon et al., 2016).

Sulphur content, which affects environmental emissions, was particularly low-0.010% and 0.011%, well within the ASTM limit of 0.05%. This confirms the low-sulfur nature of

BSG-derived ethanol and supports its use in clean energy transitions (Bianco et al., 2020).

Lastly, the viscosity values (1.71 and 1.72 mPa.s) slightly exceeded the ASTM range (1.200 mPa.s), but remain within acceptable bounds for bioethanol fuels, suggesting good flow and atomization characteristics in internal combustion engines (Pinheiro et al., 2019).

Table 8: Characteristics of the bioethanol produced using *Saccharomyces cerevisiae* and *Kluyveromycesmarxianus*

Parameters	S. cerevisiae Ethanol	K. marxianus Ethanol	ASTM Standard
Ash content (%)	0.10	0.11	—
Cloud point (°C)	18.91	18.93	23
Flash point (°C)	16.51	15.98	10-15
Pour point (°C)	4.95	4.89	—
Refractive index	1.349	1.346	1.360-1.364
Specific gravity	0.866	0.864	0.750-0.850
Sulphur content (%)	0.010	0.011	0.05
Viscosity (mPa.s)	1.71	1.72	1.200

4 Conclusion

This study has demonstrated the promising potential of brewer's spent grain (BSG), a readily available agro-industrial by-product, as a sustainable feedstock for bioethanol production through integrated acid hydrolysis and enzymatic fermentation. The high carbohydrate and cellulose content of BSG, particularly its starchy fractions, provided a strong substrate basis for fermentable sugar and subsequent ethanol production. The application of dilute sulfuric acid hydrolysis revealed that glucose yield significantly increased with rising substrate dosage, reaction time, acid concentration, and temperature until optimal conditions were attained. Notably, the highest glucose yield of 1.67 g/L was achieved at an acid concentration of 2.59%, substrate dosage of 20.85 g, hydrolysis time of 39.94 minutes, and a temperature of 76.83 °C.

Artificial Neural Networks (ANN) proved superior to Response Surface Methodology (RSM) in modeling and predicting glucose yield, capturing nonlinear relationships with greater precision and minimizing prediction errors. Similarly, in the fermentation phase, bioethanol yields varied with yeast species and process parameters. *Saccharomyces cerevisiae* demonstrated a higher bioethanol yield (18.10%) compared to *Kluyveromycesmarxianus* (15.91%), affirming its efficacy and robustness in fermenting glucose derived from BSG.

This research reinforces the value of integrating process optimization tools such as ANN in biomass valorization studies and highlights the efficiency of *S. cerevisiae*-mediated fermentation in sustainable bioethanol production. Given the scalability and abundance of BSG, this approach offers a viable pathway for bioenergy generation, waste valorization, and circular economy advancement, especially in regions seeking affordable and eco-friendly energy alternatives. Future work should explore techno-economic assessments and lifecycle analysis to evaluate the full-scale viability of this conversion system within industrial frameworks.

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