OPEN ACCESS

GRS Journal of Multidisciplinary Research and Studies
Abbreviate Tittle- GRS J Mul Res Stud
ISSN *(Online) - 3049-0561*
https://grspublisher.com/journal-details/GRSJMRS

Vol-2, Iss-6 (Jun- 2025)

Assessment of Horticultural Crop Sweet Potato for Ethanol production

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Received: 02.05.2025 **Accepted: 25.05.2025** **Published: 01.06.2025**

Abstract: Bangladesh is both an agricultural and energy deficient country. Sweet potatoes (Ipomoea batatus L.) are easy to grow and have a lot of starch, so they can be used to make fuel ethanol. This study looks at how to get the best amount of ethanol from sweet potatoes grown in Bangladesh. First, 100 grams of boiled sweet potato were mixed with 300 mL of clean water and then made germ-free. Two enzymes, 1750 units of α-amylase and 2000 units of glucoamylase, worked best to break down the starch. Then, 200 mL of yeast (Saccharomyces cerevisiae CCD) with a cell count of $1x10^5$ cells/mL was added to reach a total of 500 mL for fermentation. The results concluded that the optimized parameters are incubation time 6 days, pH 6.0, temperature 35◦C, sugar concentration 20 % (w/v) and inoculum concentration 10% v/v. Supplementation of external nitrogen sources (only sweet potato, urea, $(NH_4)H_2PO_4$, only peptone and all nutrients) on ethanol production were also investigated. Many things affect how much ethanol is made. The final amount, 116.33 mL per liter with 35% purity, depends on getting the best conditions during the process.

Keywords: *Sweet potato, Bioethanol, Saccharomyces cerevisiae CCD, Alcoholic fermentation.*

Introduction

Ethanol is a well-known biofuel that can be a good replacement for other energy sources. It is also used in many areas like beauty products, food and drinks, and as a popular additive in gasoline [1]. Bangladesh produces about 3,06,633 metric tons of sweet potatoes each year from 76 thousand hectares of land [2].Bangladesh is the seventh largest producer of sweet potatoes in the world. It can use sweet potatoes to make ethanol as a bioenergy source. This can help deal with problems like a growing population, high oil prices, limited natural resources, political issues in oil-producing countries, and environmental problems. Bioethanol is a good option because it breaks down naturally, is less harmful, can be used as fuel, creates new job opportunities, and reduces the need to import oil [3,4].

Sankaranarayanan and Mukarukaka [5], Sweet potatoes have about 22% starch and 5–6% sugar, making a total of 27–28% useful material for making ethanol. This shows that sweet potatoes can be a good and alternative source for biofuel. According to the FAO, in 2017, China was the top producer and exporter of sweet potatoes, growing over 72 million tons each year [6]. Sweet potato is chosen as a bioenergy crop because it is easy to grow, can adapt to different farming conditions, needs little fertilizer and water, gives high yields, and can be used as animal feed. It is also a good option because it is not a grain and its price stays more stable than other major energy crops[6-8]. Bioethanol is being used directly or blend with gasoline called "gasohol". Sweet potato contains about 35% oxygen, which helps fuel burn more completely and reduces harmful gases released from vehicles[9,10].In the USA, the most commonly used bioethanol blend is E-10, which has 10% ethanol and 90% gasoline[11]. Recently, biomass has increased its share in renewable energy and now makes up about 14% of the world's total energy[12].Jerusalem artichoke tuber have used to bioethanol production as a sustainable fuel for gasoline blends [13].Weber et al. [14],Studies show that 45% of tuber food is wasted worldwide each year. In Brazil, this means about 350,000 tons of sweet potatoes are wasted, causing 10% of greenhouse gas emissions every year. Using food waste biorefineries is suggested as a solution.

Many types of biomass have been studied for making ethanol, including sugar juice, starchy crops, and lignocellulose biomass. These are mainly grouped into first-generation sources like sugarcane, sugar beet, wheat, fruits, corn, potato, rice, sweet potato, or barley [15]; ii) The second generation of bioethanol comes from ligno-cellulosic biomass like apple pomace, waste from the agro-food industry, Taiwanese chenopod, and empty fruit bunches from oil palm. These are still being studied [16-20]; iii) The third generation of bioethanol is made from microalgae biomass and fecal waste [21-22]. The production process uses fermentation to change carbohydrates into ethanol [23]. Many pretreatment methods like chemical, physical, and biological techniques have been studied to help make bioethanol from lignocellulosic biomass by Rezania et al. [24]. Prasad et al. [25] found a good type of fungus that helps break down rice straw better after acid and alkali treatment, producing more sugar and ethanol. According to Jin et al. [26], Aspergillus fumigatus makes all the enzymes needed to break down biomass. The northern parts of Iran have great potential to produce a lot of ethanol from rice—about 648 million liters [27].Demiray et al. [28] were the first to study making bioethanol from pomegranate peel using Saccharomyces cerevisiae and Pichia stipitis, and they increased ethanol production to 44.9%.Sweet potato has a lot of starch, cellulose, hemicellulose, and sugars that can be used to make bioethanol, making it a good crop for bioenergy [29-31]. Sweet potato has 30% more starch than rice and corn, and 49% more than wheat when

grown in the same conditions [32].Sweet potato contains important pigments like β-carotene and anthocyanin, which are strong antioxidants [33].Some special types of sweet potatoes made for industry can produce 4500–6500 liters of ethanol per hectare, which is more than corn's 2800–3800 liters per hectare [34].

Bangladesh has a large population and is growing fast with more industries. Because of this, the number of cars is increasing, and they use a lot of fuel like diesel, petrol, and octane every day. In the 2018-19 year, Bangladesh spent 4.85 billion US dollars to import 7.5 million tonnes of oil. In January 2020, this cost was about 400.05 billion Bangladeshi Taka. According to the Bangladesh Economic Review 2019, the power board has lost a total of Tk 60,370 crore on buying and running power since 2007-08. Last year, the power board's loss was the highest ever at Tk 10,000 crore (https://tradingeconomics.co) [35-36]. This big spending puts a lot of pressure on Bangladesh's yearly budget. Farmers usually don't collect damaged sweet potatoes because no one buys them. But if these damaged sweet potatoes are used to make ethanol, farmers can earn extra money. Making bioethanol locally helps reduce the need for foreign fuels and can create many new jobs for people. It also gives more income to farmers who grow bioenergy crops. This study aims to check how good sweet potatoes are for making ethanol using enzymes and fermentation with *S. cerevisiae* CCD.

Materials and Methods

Raw Material Collection

Sweet potatoes were collected from the local vegetable market in Binodpur Bazar, Rajshahi city, Bangladesh. They were boiled for 25-30 minutes, peeled, mashed, and then sterilized by autoclaving. A proper amount of this boiled sweet potato was used for the experiment.

Yeast Strain and Culture Media

The yeast strain (Saccharomyces cerevisiae CCD) was taken from the Spirit Section of Carew and Co., Darsana, Bangladesh. To grow the yeast, a modified YMPD (Yeast-Malt-Peptone-Dextrose) broth was used. Instead of dextrose, sweet potato mash was used in the broth to help the yeast grow during bioethanol production [37].

Effect of Temperature and pH for Yeast Growth

The best temperature for yeast growth was tested using YMPD media at pH 6.0, by keeping the yeast at 25˚C, 30˚C, 35˚C, and 40˚C. To find the best pH, the yeast was grown in 500 mL of modified YMPD broth with pH levels of 5.0, 6.0, 7.0, and 8.0 at 35˚C. Yeast growth was checked every 12 hours for the pH test and every 24 hours for the temperature test. Growth was measured by checking how cloudy the broth was at 610 nm.

Growth Profile and Effect of Inoculum concentration on Bioethanol Production

To study how sugar concentration affects bioethanol production by S. cerevisiae, the media was made by mixing the substrate with distilled water to get sugar levels of 5%, 10%, 15%, 20%, 25%, and 30% (weight/volume). Then, 200 mL of yeast inoculum ($1x10^5$ cells/mL) that was 24 hours old was added. To prepare the inoculum, yeast from a YMPD agar plate was grown in YMPD broth for 24 hours in an oxygen-free chamber at 35°C. The yeast growth was checked by measuring the cloudiness (OD) at 610 nm with a spectrophotometer. Only yeast cultures with an OD around 0.8 were used as inoculum. Different amounts of inoculum (5%, 10%, 15%, and 20% volume/volume) were tested by adding them to the fermentation media to see their effect.

Effect of Enzyme on Degradation of Sweet Potato Starch

To find the best amount of enzyme to break down sweet potato starch, different amounts of α-amylase enzyme (350, 700, 1050, 1400, 1750, 2100, 2450, 2800, and 3150 units) were tested. The enzyme used was from Aspergillus oryzae with an activity of 35 U/mg. The sweet potato slurry was heated at 90°C for 1 hour, stirring and mixing every 15 minutes during the process to break down the starch. After heating, it was cooled to room temperature, and the pH was set to 6.0 using 0.1 mol/L hydrochloric acid (HCl) [38]. Saccharification involves the conversion of maltodextrins into reducing sugars, (250, 500, 750, 1000, 1250, 1500, 1750, 2000 and 2250 U) of glucoamylase(Sigma, Aldrich Co, Ltd., activity was 25 U/mg)from *Aspergillus niger*, was addedat pH 4.5, temperature 55°C and incubation time 48 hours [39]. These enzymes break down the 1-4 and 1-6 alpha bonds in starch and dextrin from the ends, making single glucose molecules[40]. The broken-down starch (saccharified starch) was set to pH 6.0 and then spun in a centrifuge at 12,000 rpm for 5 minutes. The clear liquid on top (supernatant) was used for fermentation. Then, 200 mL of yeast broth culture that was 24 hours old was added, and fermentation was done at 35˚C for 6 days.

Determination of Incubation Time on Bioethanol Production

To study fermentation time, 200 mL of one-day-old yeast was added to 300 mL of substrate solution with 20% sugar. It was kept at 35˚C for five different times: 3, 4, 5, 6, and 7 days. The amount of bioethanol was measured after each time.

Effect of Sweet Potato Varieties on Bioethanol Production

Two types of sweet potatoes, red and white, were collected from the local market in Bangladesh. These varieties were tested to see how they affect bioethanol production.

Determination of Suitable Substrate Concentration on Bioethanol Production

The right amount of starch is important for making the most ethanol. So, different starch concentrations (10%, 15%, 20%, 25%, and 30% w/v) were mixed with 300 mL of distilled water. These samples were tested using the same method as described before.

Nutrient Effects on Bioethanol Production

In the study of nutrient effect, urea (0.06%), $(NH_4)H_2PO_4$ (0.2%), only peptone and all nutrients were separately added in 500 mL substrate solution (20%). 200 ml of yeast was added in each experiment.

Analytical Methods

- **Estimation of Total Sugar**

The total sugar content was measured using the anthrone method, a color-based test, as explained in the laboratory manual [41].

- **Estimation of Reducing Sugars**

The amount of reducing sugar in the wort and during fermentation was measured using the dinitrosalicylic acid (DNS) method developed by Miller (1972)[42].

- **Estimation of Non-Reducing Sugar**

The amount of non-reducing sugar was calculated using this formula: **% of Sucrose or non-reducing sugar = (% Total sugar - % Reducing sugar) × 0.95.**

Distillation Process

Distillation was done using a distillation apparatus (Mo-W4000, EURO). After fermentation, the sample was heated to 78.5°C to remove volatile compounds, which allowed alcohol to evaporate close to its boiling point. Using fractional distillation, the ethanol was concentrated up to 95.6% by volume [43]. It is difficult to increase ethanol concentration beyond 95.6% using regular distillation. However, other methods have been explored to enrich ethanol further, as reported by Kang et al. [44].

Measurement of Purity of Produced Alcohol

The purity of the bioethanol produced from sweet potato was measured using an alcohol meter (Jiujingnongduji, China). This device can measure alcohol purity from 0% to 100% by volume.

Data Analysis

Each experiment was repeated three times, and the results were shown as average values with standard deviation (mean ± SD). The data were analyzed using analysis of variance (ANOVA), and differences between treatments were compared using Duncan's Multiple Range Test (DMRT) at a significance level of P = 0.05.

Flow-chart of Bioethanol Production

The step-by-step process of producing bioethanol from sweet potato is shown in Figure 1 as a flow chart.

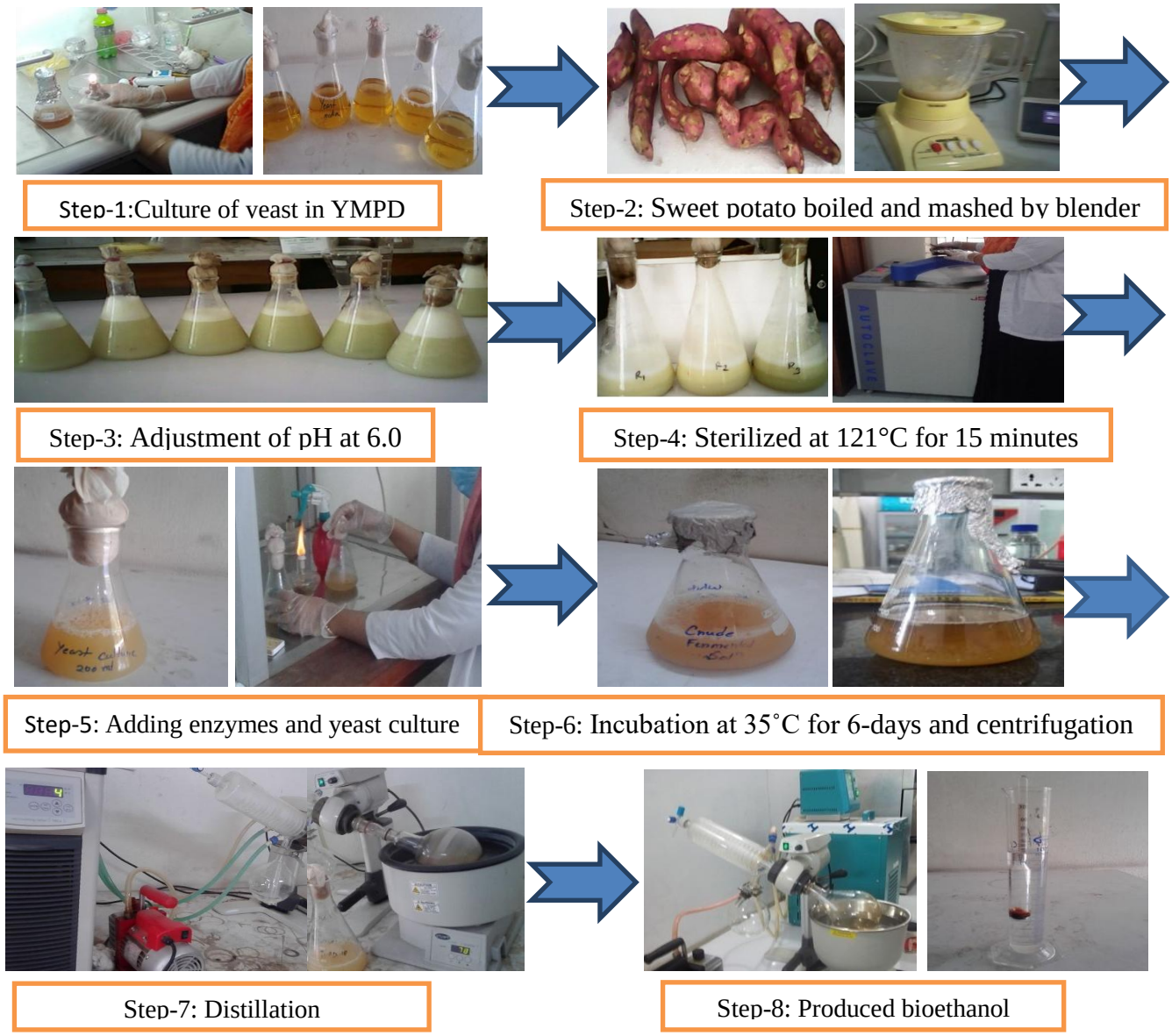


Fig. 1.Flow-chart of bioethanol production from sweet potato

Results and Discussion

Effect of Temperature on Bioethanol Production

Temperature is very important for making ethanol. To find the best temperature, solutions with 20% sugar were kept at 25˚C, 30˚C, 35˚C, and 40˚C. The sugar concentration was 20% (w/v), and fermentation happened in 500 mL flasks. Two things were checked at the same time: yeast growth and ethanol amount. Samples were taken every 12 and 24 hours, and fermentation continued for 72 hours (Table 1).

Table 1. Effect of Temperature on Bioethanol production

Time (h)	Alcohol (mL/L)			
	Temp 25˚C	**Temp 30˚C**	**Temp 35˚C**	**Temp 40˚C**
0	0	0	0	0
12	12 ± 0.67d	13.67 ± 0.44d	15 ± 0.67d	10.67 ± 1.11c
24	34 ± 0.67c	51.67 ± 1.11c	76.67 ± 1.11c	20 ± 0.67b
48	51.67 ± 1.11b	66 ± 0.67b	93 ± 0.67b	24.33 ± 1.11a
72	63.66 ± 1.11a	82 ± 2.22a	102.5 ± 6a	9.5 ± 0.5d
96	54 ± 1.33b	80 ± 0.67ab	94.33± 2.44b	9 ± 0.67d

The values shown are the averages ± standard deviation from three repeats. In each column, numbers with the same letter(s) are not significantly different from each other at the 5% level according to DMRT.

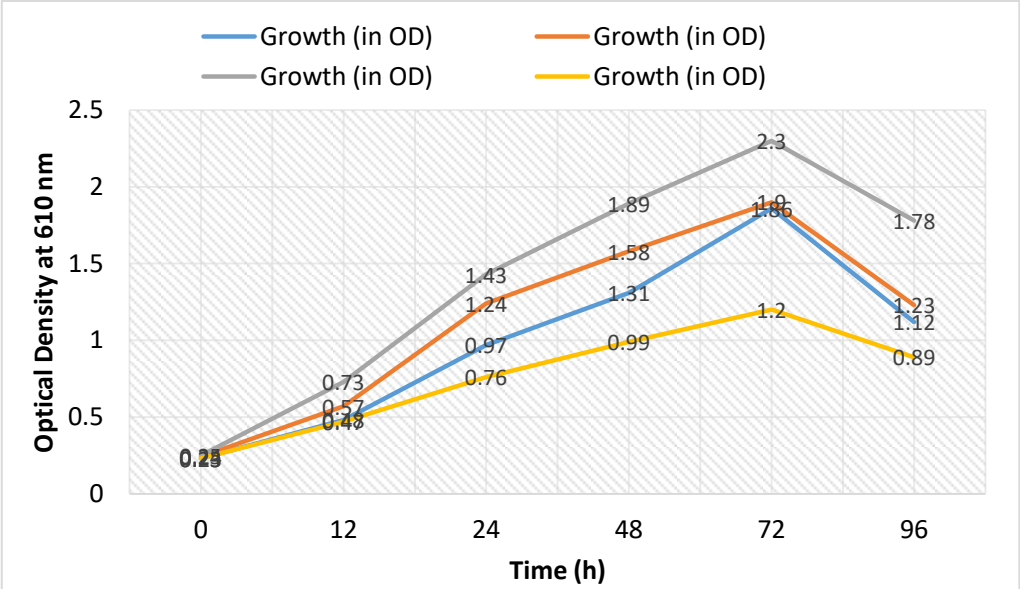


Fig.2.Effect of Temperature on Bioethanol production

A lower ethanol yield (63.66 mL/L) was recorded at 25 °C, whereas the highest yield (102.5 mL/L) was achieved at 35 °C after 72 hours. The maximum cell growth (OD 2.3) also occurred at 35 °C, outperforming the growth observed at 40 °C, as illustrated in Fig. 2. However, when the temperature exceeded 35 °C, both cell growth (OD 1.2) and ethanol production (9 mL/L) significantly declined. Therefore, 35 °C was determined to be the optimal temperature for ethanol production. These findings are consistent with the results reported by Lin et al. [46], observed that The highest specific cell growth rate and ethanol productivity were observed within the temperature range of 30–40 °C. However, a marked decline in both cell growth and ethanol yield occurred at 50 °C. According to Sujit et al. [47], Bioethanol concentration, productivity, and fermentation efficiency increased with temperature from 25°C to 30°C, declined gradually between 30°C and 35°C, and dropped sharply at temperatures above 35°C. A similar study was conducted by Sharma et al. [48] on bioethanol production from kinnow waste and banana peels via fermentation, which reported a decline in ethanol yield at temperatures above 30°C. This reduction in yield at elevated temperatures was attributed to the thermal inactivation of yeast involved in the ethanol production pathways.[49].

Effect of Optimum pH on Bioethanol Production

Among the tested pH levels (5.0, 6.0, 7.0, and 8.0), maximum yeast growth was observed at pH 6.0 (Table 2). Hence, pH 6.0 is regarded as the optimal condition for yeast proliferation and substrate fermentation. Khan et al. [50] reported an optimal pH of 6.0 for bioethanol production from substrate, which aligns with the optimum pH identified in the present study.

Table 2.Effect of pH onBioethanol production

Time (h)	Alcohol (mL/L)			
	pH 5.0	pH 6.0	pH 7.0	pH 8.0
0	0	0	0	0
12	12 ± 0.67d	16.33 ± 1.11d	17 ± 0.67d	11.33 ± 1.11c
24	35 ± 1.33c	72.67 ± 1.78c	55.67 ± 2.44c	20 ± 0.67b
48	51.67 ± 1.11bc	80.66 ± 4.88b	67 ± 1.33b	24.33 ± 1.11a
72	61.67 ± 1.11a	115± 4.66a	88.33 ± 2.22ab	11.65 ± 1.11c
96	52 ± 0.67b	79 ± 0.66bc	92.33± 2.44a	8 ± 0.65d

Data are presented as means ± standard deviation from three replicates. Within each column, values sharing the same letter(s) are not significantly different at the 5% significance level according to Duncan's Multiple Range Test (DMRT). According to Chohan et al. [51], Under optimal conditions—40°C temperature, pH 5.78, and 12.25% (w/v) substrate concentration—starch-based waste such as potato peel produced the highest bioethanol concentration (22.54 g/L) and yield (0.32 g/g).

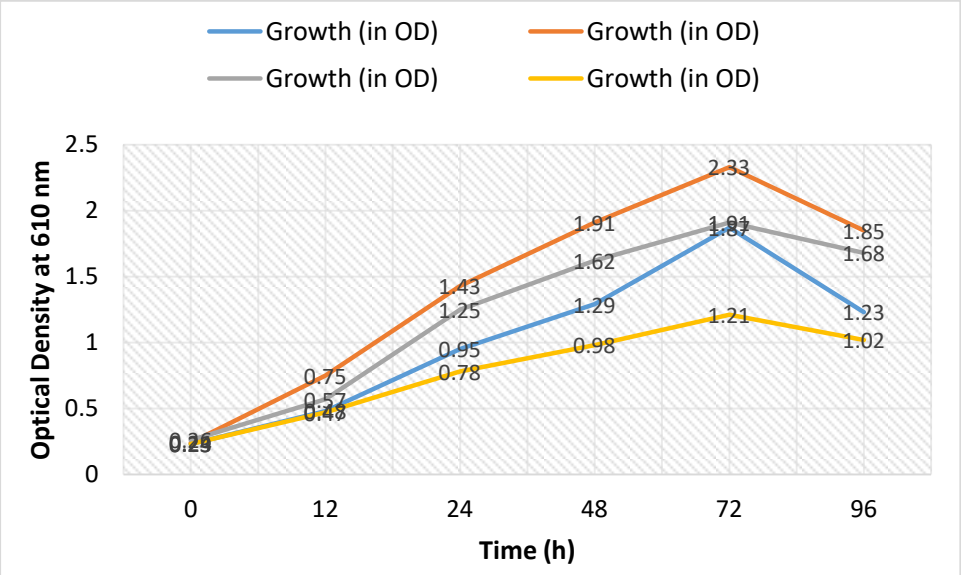


Fig.3. Effect of pH on Bioethanol production

Atitallah et al. [52] utilized date palm sap for ethanol production using *Wickerhamomyces anomalus* and observed that while fermentation occurred at pH 5.0, it resulted in low ethanol yield due to inhibited yeast multiplication at lower pH levels. Optimal results were achieved at pH 6.0, where the highest ethanol production (115 mL/L) was recorded (Table 2), along with the maximum yeast growth (OD 2.33), which was greater than that observed at pH 7.0 and pH 8.0 (Fig. 3). Moreover, both batch and fed-batch fermentations achieved high yields (73 g/L) without requiring pH adjustment. Vishwakarma et al. [53], found that Optimal ethanol production from fruit waste via *Saccharomyces cerevisiae* fermentation was achieved at pH 5.5, a temperature of 32°C, a specific gravity of 0.865, and a concentration of approximately 6.21% (w/v). According to Lin et al. [46], most fermentation media used for bioethanol production maintain a pH range between 4.5 and 5.5, accompanied by varying sugar concentrations. However, based on fermentation efficiency observed in the present study, pH 6.0 was selected for subsequent experiments.

Growth Profile and Effect of Inoculum concentration on Bioethanol Production

The kinetic growth analysis of *Saccharomyces cerevisiae* with gradually increasing sugar concentrations in YMPD medium revealed a rise in optical density (OD 2.16) up to a 20% sugar concentration, as illustrated in Fig. 4. Beyond this concentration, yeast growth was inhibited. Growth measurements were recorded every 12 hours at 610 nm to monitor kinetics, and a decline in production was observed over time. Therefore, the highest yeast growth was achieved at a 20% (v/v) sugar concentration, as shown in Fig. 4. Additionally, bioethanol production increased with rising yeast concentrations; however, exceeding 3 g/L of yeast led to a reduction in fermentation efficiency. This trend is consistent with findings reported by Sharma et al. and Reddy and Reddy [48,54]. Similar result was obtained by Sandesh et al. [55]. He observed

that fermenting sugarcane with *Saccharomyces cerevisiae* under optimized conditions—pH 6.0, 20% sugar concentration, and a temperature of 30°C—resulted in enhanced ethanol production and improved fermentation efficiency.

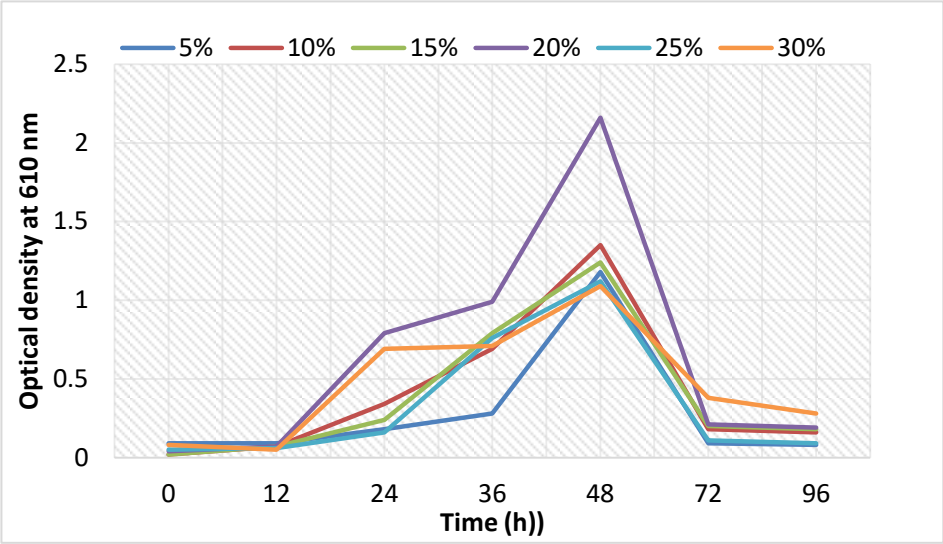


Fig.4. Effect of Sugar Concentration on Growth Studies

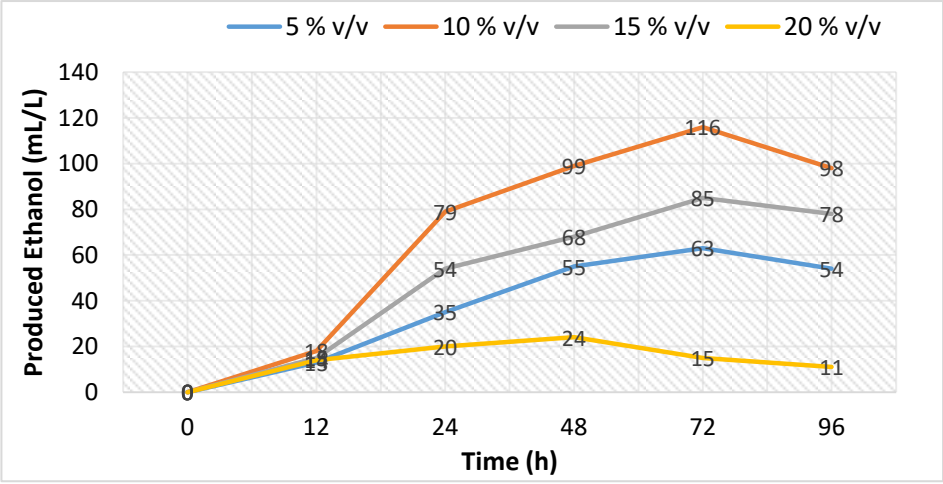


Fig. 5. Effect of inoculum concentration on bioethanol production

Ethanol production at varying inoculum concentrations (5%, 10%, 15%, and 20% v/v) is presented in Fig. 5. The highest ethanol yield was obtained at 10% v/v inoculum, reaching 116 mL/L, followed by 15% v/v with 85 mL/L. In contrast, the lowest ethanol production was observed at 20% v/v and 5% v/v, yielding only 15 mL/L and 63 mL/L, respectively, after 72 hours of fermentation. After 96 hours, ethanol production began to decline gradually across all inoculum concentrations. According to Azhar et al. [56], In bioethanol production, commonly used inoculum sizes are 5% and 10% (v/v). Similar findings were reported by Kumar et al. [57], who achieved 7.95% (v/v) bioethanol yield from raw sweet potato using *Saccharomyces cerevisiae* MTCC-170 with a 10% inoculum size at pH 6.0 after 48 hours of fermentation. Zhang et al. [58], reported The highest ethanol concentration (128.5 g/L) and ethanol productivity (4.76 g/L/h) were likely achieved due to favorable conditions that supported optimal yeast activity for bioethanol production. Sharma et al. [48], reported that the increased of inoculum concentration was to be linearly increased with ethanol production. However, according to Zabed et al. [59], Ethanol production increased with rising cell densities from 1×10^4 to 1×10^7 cells/mL; however, no significant improvement was observed between 10^7 and 10^8 cells/mL. This is because increasing cell concentration within a certain range accelerates fermentation by promoting rapid sugar consumption and ethanol conversion. While inoculum concentration does not significantly influence the final ethanol yield, it primarily affects the sugar consumption rate and overall ethanol productivity [60].

Effect of Enzymatic Hydrolysis on Bioethanol Production

This experiment plays a crucial role in evaluating the effect of enzymes on bioethanol production. The success of ethanol production largely depends on two key processes: the efficient conversion of starch into fermentable sugars and the subsequent fermentation of these sugars by a suitable microorganism. To achieve starch-to-sugar conversion, the substrate undergoes enzymatic hydrolysis [61-63]. Although yeast is vital for the fermentation process, the incorporation of enzymes greatly improves the breakdown of starch into fermentable sugars, which in turn speeds up fermentation and boosts bioethanol production

[58]. In the absence of enzymes, *Saccharomyces cerevisiae* CCD fermented the substrate very slowly and produced an undesirable red or purple coloration. However, the addition of small amounts of α-amylase and glucoamylase significantly accelerated fermentation, accompanied by the characteristic aroma of alcohol. This study determined that optimum bioethanol production levels of 115 mL/L and 116 mL/L were achieved with 1750 U of α-amylase and 2000 U of glucoamylase, respectively (**Fig. 6**and **Fig. 7**).

Lareo et al. [64], found that A 90-minute treatment with α-amylase was sufficient to achieve complete starch hydrolysis (100%). Thus, it can be concluded that using 1750 U of α-amylase and 2000 U of glucoamylase effectively breaks down the starch in a 20% (w/v) sweet potato substrate into simpler disaccharides and monosaccharides, enabling optimal bioethanol production.

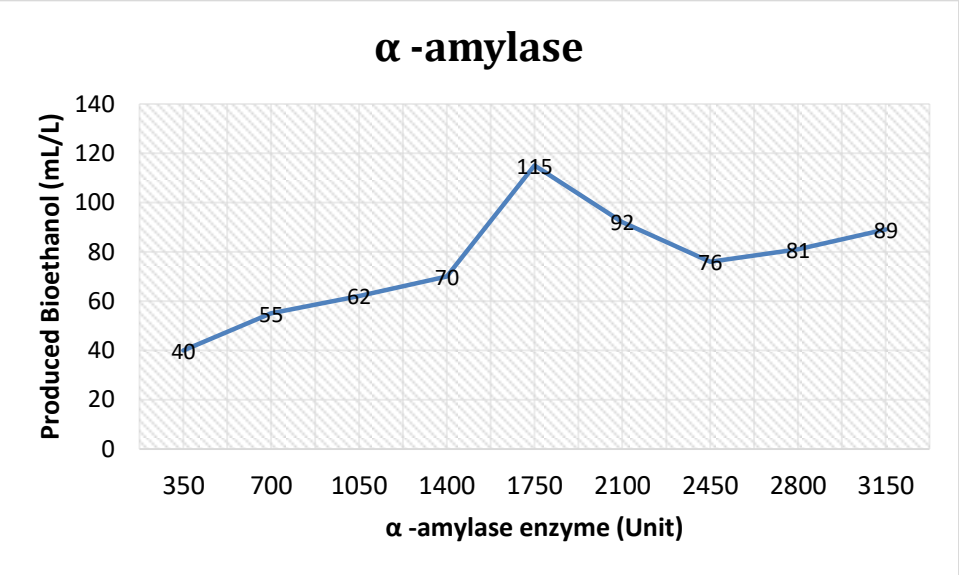


Fig. 6. Effect of α-amylase on Bioethanol Production

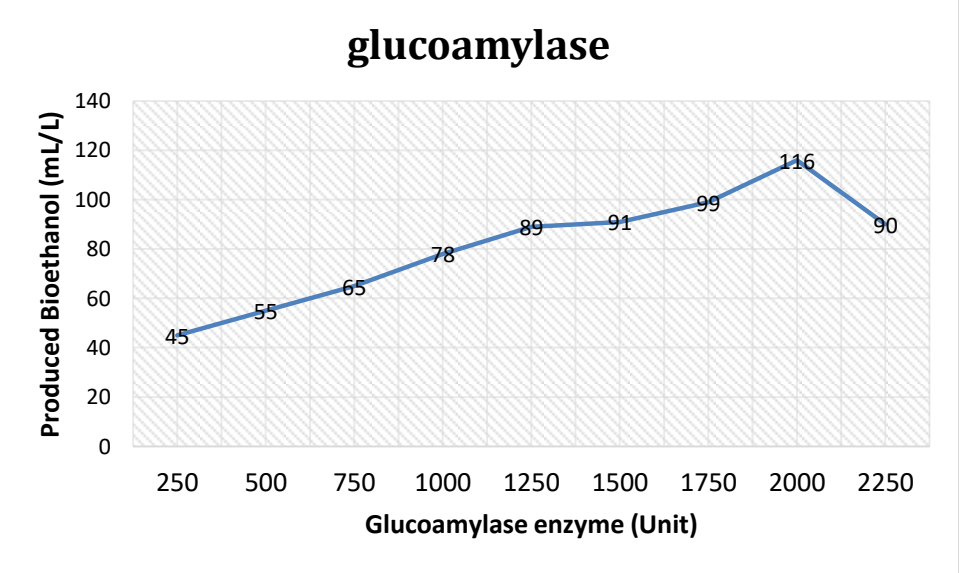


Fig. 7. Effect of Glucoamylaseon Bioethanol Production

Excessively high enzyme concentrations can lead to feedback inhibition, reducing the conversion of substrate into ethanol, as observed when 3150 U of α-amylase and 2250 U of glucoamylase were used during fermentation. Ochaikul and Suwannaposri [65] hydrolyzed sweet potato starch using 0.05% (w/v) α-amylase at 90°C for 2 hours and 0.015% (w/v) glucoamylase at 60°C for 4 hours, employing *Saccharomyces cerevisiae* YRK 017 for fermentation. They reported a maximum ethanol concentration of 14.55 g/L. Meanwhile, Pereira et al. [66] observed that α-amylases produced from sweet potato peel exhibited optimal activity at 60°C and pH 4.5. According to Jagatee et al. [67], the optimum conditions for dextrinization and saccharification using α-amylase and glucoamylase are an incubation time of 45 minutes and 24 hours, pH values of 5.5 and 4.5, temperatures of 90°C and 65°C, and enzyme concentrations of 20 µL and 224 µL, respectively.

Nutrient Effects on Bioethanol Production

In this study, the effects of different nutrients on bioethanol production were evaluated by adding urea (0.06%), ammonium dihydrogen phosphate [$(NH_4)H_2PO_4$] (0.2%), peptone (5 g/L), and a combination of all nutrients [urea (0.06%) + $(NH_4)H_2PO_4$ (0.2%) + peptone (5 g/L) + yeast extract (3 g/L)] separately to 500 mL of

substrate (20% w/v). Each experiment was inoculated with 200 mL of yeast. Sreekumar et al. [68] demonstrated that key chemical factors influencing ethanol production include nitrogen and phosphorus sources, yeast extract, and inoculum size, as microorganisms require nitrogen for growth and metabolism. Results from the present study showed that the addition of all nutrients significantly increased bioethanol production to 116.33 mL/L, whereas the substrate alone produced a much lower yield of 59.66 mL/L. This highlights the critical role of nutrients, particularly nitrogen sources such as urea, $(NH_4)H_2PO_4$, and peptone, in enhancing bioethanol production (**Table 3**).

Table 3. Nutrient Effects on Bioethanol Production

Parameter (Different Nutrients)	**O.D. of fermented crude ethanol at 610 nm**	**Volume after fermentation (mL/L)**	**Volume after distillation (mL/L)**	**Purity of Bioethanol % (v/v)**
Only Sweet potato	0.11 ± 0.08b	435.00 ± 4.00d	59.66 ± 4.52c	17.66 ± 1.53c
Urea	0.34 ± 0.05a	491.00 ± 1.00a	94.66 ± 1.53a	30.00 ± 1.00b
Peptone	0.23 ± 0.07ab	444.67 ± 4.51c	65.00 ± 2.65c	29.00 ± 1.00b
All Nutrients	0.12 ± 0.07b	491.67 ± 1.53a	116.33 ± 2.00a	35.00 ± 1.00a
$(NH_4)H_2PO_4$	0.16 ± 0.08b	464.67± 4.93b	82.33 ± 3.06b	28.67 ± 0.57b

Values represent the means ± standard deviation of three replicates. Within each column, values sharing the same letter(s) are not significantly different at the 5% level according to Duncan's Multiple Range Test (DMRT).

The fermentation process can be enhanced through appropriate supplementation of nutrients—such as various nitrogen sources, vitamins, and metal ions—in the medium, which may lead to an increased final ethanol concentration [68,69Many nutrient supplements commonly used in laboratory research—such as amino acids, vitamins, sterols, and unsaturated fatty acids—are often too costly for industrial applications. Therefore, ethanol-tolerant yeast strains are necessary for efficient fermentation [70]. As yeast grows and multiplies, it requires substantial nitrogen for continued growth and ethanol production. While adding urea can promote yeast growth, excessive amounts may become toxic. Demiray et al. [28] increased ethanol yield to 44.9% (v/v) from pomegranate peel by supplementing *S. cerevisiae* fermentation with nitrogen sources (yeast extract, peptone, and $(NH_4)_2SO_4$) and metal salts (K^+, Mg^{2+}, Ca^{2+}, Zn^{2+}). Similarly, Izmirlioglu and Demirci [71] reported that enzymatic hydrolysis of industrial potato mash (40.4 g/L) released sugars that supported ethanol production (11.63 g/L) by *S. cerevisiae*, with supplements such as yeast extract, malt extract, and $MgSO_4 \cdot 7H_2O$ further enhancing yield. Benerji et al. [72] observed increased ethanol production (13.29% w/v) from mahula flower with 0.06% urea supplementation using *S. cerevisiae*. Additionally, Kumar et al. [56] tested three nitrogen sources for ethanol production and found peptone at 1.5 g/L to be the most effective, yielding 7.93% (v/v) ethanol.

Suitable Concentration of Sweet potato on Bioethanol Production

Substrate concentration plays a crucial role in maximizing ethanol production. Among five tested concentrations—10%, 15%, 20%, 25%, and 30% (w/v)—all treatments except 25% and 30% (w/v) formed a clear white layer on the surface of the fermented solution, indicating complete conversion of starch into ethanol during the incubation period. In contrast, the 30% starch treatment resulted in an opalescent and turbid solution with an optical density (OD) of 0.69. As shown in Table 4, the optimal concentration of sweet potato starch for bioethanol production was 20%, yielding the highest ethanol volume of 115.33 mL/L. Ethanol production decreased at higher concentrations, with 83.33 mL/L at 25% (w/v), and further dropped to 51.33 mL/L at 30% (w/v), suggesting that very high substrate concentrations negatively affect fermentation efficiency.

Table 4. Bioethanol from Different Concentrations of Sweet Potato Solution

Sweet Potato Concentrations % (w/v)	**O.D. of fermented crude sol. at 610 nm**	**Volume after fermentation (mL/L)**	**Volume after distillation (mL/L)**	**Purity of Bioethanol % (v/v)**
10%	0.29 ± 0.02d	435.00 ± 4.00c	56.66 ± 3.79c	9.00 ± 1.00d
15%	0.27 ± 0.04d	486.67 ± 5.86a	89.66 ± 4.16b	16.33 ± 3.21c
20%	0.37 ± 0.03c	491.67 ± 1.53a	115.33 ± 1.53a	34.66 ± 1.53a

25%	0.52 ± 0.03b	461.67 ± 9.45b	83.33 ± 4.51b	30.00 ± 1.00b
30%	0.69 ± 0.03a	211.67 ± 12.58d	51.33 ± 2.08c	15.00 ± 1.00c

Values represent the means ± standard deviation of three replicates. Within each column, values sharing the same letter(s) are not significantly different at the 5% level according to Duncan's Multiple Range Test (DMRT).

Low concentrations of sweet potato resulted in low bioethanol yields, but production increased with substrate concentration up to 20% (w/v). However, bioethanol production declined at 25% and 30% (w/v) sweet potato concentrations due to reduced free glucose availability caused by incomplete starch saccharification. The high viscosity of these concentrated solutions creates handling challenges and may hinder the full hydrolysis of starch into fermentable sugars [73,74]. Swain et al. [75] reported maximum ethanol production of 172 g/kg substrate under optimized conditions of 80% moisture, 0.2% ammonium sulfate, pH 5.0, 10% inoculum size, and fermentation at 30ºC for 72 hours.

Determination of Incubation Time on Bioethanol Production

For optimal substrate conversion by yeast, an adequate fermentation time is essential. Potato samples were fermented under anaerobic conditions and monitored over 3, 4, 5, 6, and 7 days to determine the maximum bioethanol yield. As shown in Table 5, bioethanol production increased with longer incubation periods. The highest ethanol concentration of 111 mL/L was observed on day 6, followed by 82.66 mL/L on day 5 and 65 mL/L on day 4.

Table5. Determination of Fermentation Time on Bioethanol Production

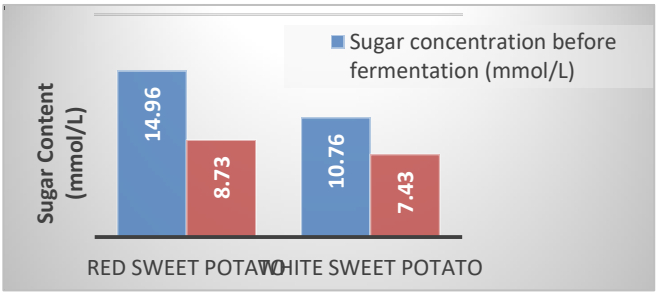
Incubation Time (Days)	**O.D. of fermented crude ethanol at 610 nm**	**Volume after fermentation (mL/L)**	**Volume after distillation (mL/L)**	**Purity of Bioethanol % (v/v)**
3 days	0.29± 0.016b	435.00 ± 2.31d	59.66 ± 2.61c	17.66 ± 0.88c
4 days	0.34 ± 0.05b	491.00 ± 0.57a	65.00 ± 1.53c	29.00 ± 0.58b
5 days	0.23 ± 0.07b	444.67 ± 2.61c	82.66 ± 0.88a	30.00 ± 0.58b
6 days	0.09 ± 0.01c	491.67 ± 0.88a	111.00 ± 1.16a	35.00 ± 0.57a
7 days	0.76 ± 0.09a	464.67± 2.85b	55.33 ± 1.76b	28.67 ± 0.33b

Values represent the means ± standard deviation of three replicates. Within each column, values sharing the same letter(s) are not significantly different at the 5% level according to Duncan's Multiple Range Test (DMRT).

This experiment showed that a 6-day incubation period is optimal for bioethanol production. Beyond this period, bioethanol yield gradually decreases with longer fermentation times. After 7 days, the ethanol concentration dropped to its lowest value of about 55.33 mL/L, accompanied by an unpleasant odor and reduced ethanol quality. The decline in production with extended incubation is likely due to the substrate containing not only glucose but also starch, protein, and fat. The energy from protein and fat enables yeast to convert ethanol into other by-products, resulting in lower ethanol levels after 7 days. Breisha [76] reported that increasing yeast concentration from 3.0% to 6.0% reduced fermentation time from 72 to 48 hours. Similarly, Khandaker et al. [77] found that bioethanol production peaked on day 5 at 16.2% (v/v), with lower yields of 14.5% and 12.8% (v/v) observed on days 3 and 1, respectively.

Effect of Sweet Potato Varieties on Bioethanol Production

In Bangladesh, the two common local sweet potato varieties are the purple-red skin with yellow flesh (Red) and the yellow skin with white flesh (White). Both varieties were tested to evaluate their effect on bioethanol production (Fig. 8).



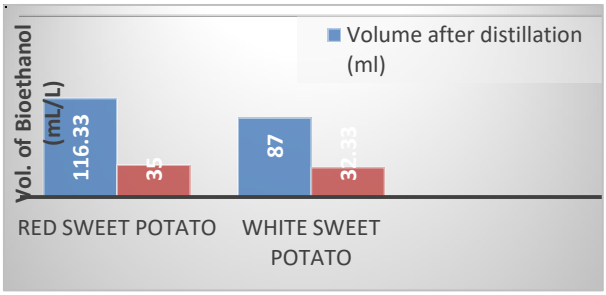


Fig.8.Effect ofSweet Potato Varieties on Bioethanol Production

The nutrient composition differs between the red and white varieties of sweet potato, which can significantly impact bioethanol production. After optimizing all processes, the red sweet potato produced more bioethanol (116.33 mL/L with 35% v/v purity) compared to the white variety (87 mL/L with 32.33% v/v purity), as shown in Fig. 8. This yield is notably higher than that reported by Lee et al. [9], who studied bioethanol production from sweet potato using co-immobilization of fungi and yeast, achieving a maximum ethanol yield of 4.08% (v/v) at an Aspergillus to Monascus ratio of 1:2. Similarly, the highest ethanol yield from a red potato variety in Nepal was 5.2% [78]. These differences may be due to the higher fermentable sugar content in sweet potatoes. Martins et al. [12] observed that conversion efficiency significantly increases during sweet potato ripening, reaching a peak 25 days after harvest. Silva et al. [15] studied an integrated process for bioethanol and biodiesel production from sweet potato, reporting an average bioethanol yield of 161.4 L per ton. Biochemical analysis of red sweet potato before and after fermentation with α-amylase and Saccharomyces cerevisiae showed a decrease in total sugar (from 15.80 ± 1.30 to 10.37 ± 1.00 g/100g), reducing sugar (from 9.63 ± 1.14 to 7.17 ± 0.64 g/100g), and non-reducing sugar (from 6.17 ± 0.49 to 3.20 ± 1.14 g/100g), confirming the conversion of sugars into ethanol, consistent with Girisha et al. [79]. Schweinberger et al. [80] proposed a simple equation to estimate total reducing sugars in sweet potatoes based on moisture content and found ethanol potential increases non-linearly with increasing sweet potato mash concentration (22% ethanol from 10 kg/L of sweet potato with 66% moisture). According to Oliveira et al. [81], starch concentration in sweet potatoes directly affects alcohol production, and higher starch content leads to greater profitability. However, further research is necessary to standardize protocols and improve the cost-effectiveness of bioethanol production from sweet potatoes compared to other substrates like sugarcane or beet molasses.

Conclusions

The results show that sweet potato is a good raw material for making bioethanol. It is cost-effective and better for the environment. Unlike cellulosic biomass, it does not need pretreatment or hydrolysis before use. Recent improvements in Saccharomyces cerevisiae CCD make it better at tolerating alcohol. It can efficiently convert starch with a concentration of 20% (w/v). This makes it very useful for bioethanol production. The study used specific conditions to get the best ethanol yield. These include pH 6.0, temperature 35°C, and 20% sugar concentration. With 6 days of incubation, enzymes, nutrients, and 10% inoculum size, the ethanol produced was 116.33 mL/L with 35% purity. Present bottlenecks can be removed and the anhydrous alcohol could be produced by high grade distillation setup.

Acknowledgment

The corresponding author acknowledged the National Science and Technology (NST) fellowship for this research from the Ministry of Science & Technology, Government of the People's Republic of Bangladesh.

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