https://dx.doi.org/10.4314/dujopas.v10i2b.10
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 10 No. 2b June 2024*

Effect of Tamarind (*Tamarindus indica* L.) Pulp Syrup Supplementation on the Production of Bioethanol from Sugarcane (*Saccharum officinarum* L.) Molasses at Different Temperatures and pH

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Abstract

Alternative sources of environmentally friendly energy source such as biofuels are been explored with the aim of reducing environmental pollution and cope with the growing energy demand. Bioethanol was produced from sugarcane molasses using tamarind supplements at different pH and temperatures. The volatile profile of the bioethanol produced was evaluated using Gas chromatography and mass spectroscopy (GC-MS). Bioethanol was produced from sugarcane molasses hydrolyzed using tamarind pulp syrup (TPS) and distilled water (DW) at different temperatures (26-30 ^{o}C) and pH (4.5-6.5) and utilizing Saccharomyces cerevisiae for fermentation. The results showed a significant increase in reducing sugar yield, bioethanol yield/quantity, and bioethanol volatility for the bioethanol produced using TPS hydrolysis compared to those produced using DW ($p<0.05$). The suitable temperature and pH for the production of the maximum amount of reducing sugar and bioethanol were 28 ^{o}C and 5.5 respectively. The volatility of the bioethanol produced was highest at a temperature of 28 ^{o}C and a pH of 5.5. The density of bioethanol obtained using TPS (0.797 g/cm^3) was close to the standard density of bioethanol (0.789 g/cm^3). Conclusively, TPS was found to enhance bioethanol production from sugarcane molasses through fermentation. This suggests that it could serve as an alternative hydrolyzer for the production of biofuel.

Keywords: Bioethanol, Molasses, Sugarcane, Tamarind, Temperature.

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INTRODUCTION

Environmental pollution is the exposure to harmful substances (solid, liquid and gas) leading to a reduction in the environmental quality by affecting the air and water purity as well as the soil where the plant grows (Manisalidis *et al.,* 2020). It occurs as a result of both natural disasters and man-made activities such as deforestation, erosion, flood, mining, oil/gas spillage, and burning of coal as well as industrial and automobile waste (Pona *et al.,* 2021). Environmental pollution has been a global problem that has resulted in many deaths and disability. Previous reports have shown that eighty out of one hundred disease categories are associated with environmental factors resulting in over 20% of global deaths (Ukaogo *et al.,* 2020; Pona *et al.,* 2021). Even with the numerous problems associated with pollution, the demand for oil have been projected to reach 57% by 2030. Hence, there is need for alternative source of cleaner and cheaper energy (Wong and Sanggare, 2014).

Alternative sources of environmentally friendly energy source such as biofuels are been explored with the aim of reducing environmental pollution and cope with the growing energy demand. The use of wood as a source of energy was reported to cause 50% of deforestation in developing countries (Osei, 1993). Hence, biofuels are believed to cause a significant reduction in deforestation, carbon emission, land degradation and soil erosion (Surendra *et al.,* 2014). Biofuels are used as a solvent, germicide, anti-freeze, fuel etc., (Bhatia *et al.,* 2012). For these enormous advantages of biofuels, research has been geared towards the process of producing bioethanol from many raw materials or feedstocks (Bhatia *et al.,* 2012).

First-generation biofuel (bioethanol and biodiesel) production is based on the fermentation of eatable crops such as sugarcane, corn, wheat, and sorghum (Favaro *et al.,* 2019). Lignocellulosic materials (wood, animal fat and wheat bran) are used to produce the second-generation biofuel. (Favaro *et al.,* 2019). Third-generation biofuels are derived for microalgae and cyanobacteria while the fourth-generation biofuels utilize genetic engineering to produce the desired trait in an organism for biofuel production (Cavelius *et al.,* 2023). The traits vary from high lipid synthesis, ability to utilize different sugars, and carbon fixation to improved photosynthesis (Cavelius *et al.,* 2023).

Bioethanol is a renewable fuel produced from sugar and/or starch-containing raw material by yeast fermentation where the sugar/starch is converted to ethanol (Busic *et al.,* 2018). They are produced from natural products including wheat, potato, rye, corn starch, pineapple, cassava etc. However, the rising cost of such products have made bioethanol producers to rely on the waste gotten after processing these products e.g., sugarcane molasses, pineapple peel, wheat hulk (Conesa *et al.,* 2016; Ajit *et al.,* 2017). Several previous studies have reported the production of bioethanol from sugarcane products such as molasses, bagasse, and cane juice (Cardosa *et al.,* 2010; Prasad et al., 2022). Bioethanol is considered an important biofuel to partly replace fossil-derived fuels. The reasons for the enhanced development of bioethanol are its use as a favourable and near carbon-neutral renewable fuel, thus reducing carbon dioxide (CO_2) emissions and associated climate change (Jeswani *et al.,* 2020).

Bioethanol is usually produced through fermentation using different strain of yeast. However, considering some factors like temperature, stirring, pH, and the addition of certain substances have been shown to affect the quality and quantity of the bioethanol produced (Sanchez et al., 2021). Reports have shown that the addition of tamarind pulp as supplements for bioethanol production improve bioethanol yield by 40% (Patil *et al.,* 1998). The current study was aimed at producing bioethanol from sugarcane molasses using tamarind supplements at different pH and temperatures as well as evaluating the volatile profile of the bioethanol using GC-MS.

MATERIALS AND METHODS

Source of Materials

Sugarcane molasses were obtained from Savannah Sugar Company Limited, Numan Local Government Area, Adamawa State, Nigeria (Latitude 9º 35.398'N; Longitude 11º54.707'E) and were then placed in a clean bottle and kept at room temperature (20-25 ^{o}C) before being used. Fresh and ripe *Tamarindus indica* L. fruit were purchased from Monday market, Maiduguri, Nigeria while Baker's yeast *Saccharomyces cerevisiae* was obtained from the Department of Microbiology, University of Maiduguri.

Tamarind Pulp Preparation

The fruits were rinsed in clean water to remove the extraneous components and air dried. The extract was prepared by boiling 10 g of the fruit pulp in 100 mL of distilled water for 30 mins followed by filtration. The filtrate (triplicate) was used as a supplement to the fermentation medium.

Determination of Reducing Sugar

Reducing sugar was estimated as described by Rabah *et al.* (2011). The filtrate was mixed with dinitro salicylic acid (DNSA) reagent in a ratio of 1:1. A blank sample (1 mL distilled water and 1 mL DNSA reagent) was prepared. The solutions were boiled in a water bath for 10 min and allowed to cool while observing the development of a reddish-brown colour. 40% sodium potassium tartrate (1 mL) was added to stabilize the colour and absorbance was read at 540 nm using an ultraviolet-visible spectrophotometer. The concentration of reduced sugar was determined by a glucose standard curve.

pH Adjustment and Sterilization of the Hydrolyzed Samples

The pH was adjusted to 5.0 by either adding sodium hydroxide (NaOH) or hydrochloric (HCl) acid and monitoring with a digital pH meter. This was done to prevent denaturing of the yeast by a hyper acidic or basic state. All the hydrolyzed samples were then sterilized by autoclaving at 121 ^{o}C for 15 minutes before fermentation.

Reactivation of Baker's Yeast (*Saccharomyces cerevisiae)*

One (1) gram of baker's yeast was put in a flask containing a solution of warm distilled water, glucose broth media and yeast extract. The solution was then subjected to incubation for 24 hours. The reactivated yeast was then inoculated into Potato Dextrose Agar (PDA) and subjected to incubation at 30 ^{o}C for 5 days. After 5 days, the growth observed was used to form a pure culture (Kwarkwai et al., 2024).

Mash Preparation and Fermentation

Seven (7) millilitres of sugarcane molasses were suspended into thirty (30) sets of 250 mL conical flasks. Fifteen (15) were suspended with 50 mL of tamarind pulp syrup and fifteen (15) with 50 mL of distilled water. Then, 10 mL of the reactivated baker's yeast was aseptically inoculated into the hydrolyzed samples. Each of the fifteen (15) conical flasks set was divided into five (5) sets, and the temperature readings (26, 27, 28, 29 and 30 °C) for each set were recorded in triplicate. In the meantime, the medium pH of the inoculated conical flasks was adjusted (4.5-6.5) in triplicates across the temperature ranges to evaluate the effect of pH. The flasks were then covered with cotton wool and wrapped with aluminium foil paper, and the fermentation was carried out at room temperature for five (5) days. The fermentation medium was aseptically removed from thermostatically controlled water baths at every 1 hour respectively.

Distillation of Bioethanol Produced
In this case, the Soxhlet extractor was used in distillation. All trials were conducted at 78 ^{o}C. The fermentation broth was suspended into round-bottom conical flasks that were attached to a distillation column that was encased in tape water. To collect the distillates, another conical flask was attached to the other end of the distillation column. The round bottom flask containing the fermented broth was heated using a heating mantle set to 78 ^{o}C (Li *et al.,* 2017).

Identification of Bioethanol Produced
Two grams of potassium dichromate ($K_2Cr_2O_7$) and two drops of concentrated H_2SO_4 was added to about 2 mL of the distilled samples. A colour change from orange to green indicated the presence of bioethanol (Edjekouane *et al.,* 2020).

Determination of Concentration of the Bioethanol Produced
The percentage of the bioethanol produced was carried out by quantitative analysis using acid potassium dichromate reagent as described by Oniya *et al.* (2016). Each of 0, 2, 4, 6, 8 and 10 mL of 1% bioethanol were diluted in 10 mL of distilled water to produce 0, 0.2, 0.4, 0.6, 0.8 and 1.0 concentration. 1 mL of each of the different bioethanol concentrations was mixed with 1 mL of the acid potassium dichromate and allowed to stand for an hour for colour development absorbance was measured at 588 nm using an ultraviolet-visible spectrophotometer and the reading was used to develop a standard bioethanol curve.

Density Measurement
The density of the bioethanol produced was carried out using a density bottle as described by Li *et al.* (2017). The bottle was placed on a level surface which was filled with bioethanol. An empty density bottle was weighed on weighing balance and the readings were taken. The empty density bottle was then filled with bioethanol produced by the addition of tamarind pulp supplement and weighed. Another empty-density bottle was filled with bioethanol produced by the addition of distilled water and it was also weighed. The specific density of the bioethanol produced was calculated as follows: Density of bioethanol produced by the addition of distilled water = X_2-X_1 while Density of bioethanol produced by the addition of tamarind pulp supplement = X_3-X_1. Where X1= Weight (g) of empty density bottle, X2= Weight (g) of empty density bottle filled with bioethanol produced by adding distilled water, and X3= Weight (g) of empty density bottle filled with bioethanol produced by adding tamarind pulp.

Determination of Volume (Quantity) of the Bioethanol Produced
The quantity of bioethanol produced was determined by collecting the distillate (bioethanol) over a slow heat at 78 ^{o}C (for 40 minutes), measuring it with a measuring cylinder, and expressing it as the quantity of bioethanol produced in litre by multiplying the volume of distillate collected at 78 ^{o}C by the density of bioethanol produced (Humphrey and Caritas, 2007).

Determination of Compounds Present in the Bioethanol Produced
Gas chromatography and mass spectroscopy (GS-MS) analysis were conducted per the procedure developed in the Department of Analytical Chemistry Laboratory, Yobe State University Damaturu, Yobe State. Agilent Technologies 6890N Network GC system and Agilent Technologies 5973 Network mass selective detector coupled with 7683B series injector. The carrier gas used was helium at a flow rate of 1.2 mL/min. The injection volume was one (1) Nanolitre (nL). The inlet temperature was maintained at 230 ^{o}C. The oven temperature was programmed initially at 50 ^{o}C for 5 min. It was then programmed again to increase to 300 ^{o}C at a rate of 10 ^{o}C with 25 min and this temperature was held for 15 min. The

total run time was 15 minutes. The MS transfer line was then maintained at a temperature of 250 °C. The source temperature was also maintained at 230 °C and the MS was Gauged at 150 °C. The ionization mode used was electron ionization mode at 70 Ev. Total ion count (TIC) was used to evaluate for compound identification and quantification. The spectrum of the separated compound was then compared with the database of the spectrum of known compounds saved in the NISTO2 Reference Spectra Library. Data analysis and peak area measurement were carried out using Agilent software.

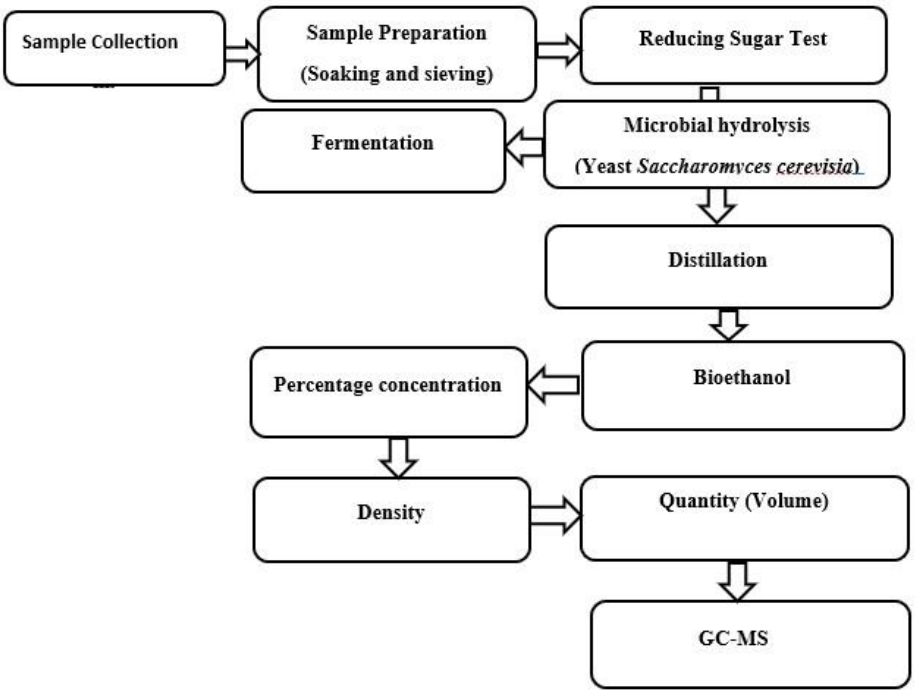

Figure 1: A Schematic Design of Bioethanol Production from yeast *Saccharomyces cerevisiae*

Statistical Analysis

The data obtained were statistically analyzed using a student t-test with Minitab statistical software to test the concentration of the reducing sugar and the purity of the bioethanol produced. The values obtained were then represented as Mean ± SD. $P < 0.05$ was considered statistically significant.

RESULTS

Reducing Sugar Produced from Sugarcane Molasses Hydrolyzed using Tamarind Pulp Syrup (TPS) and distilled water (DW) at Different Temperature and pH

The concentration of reducing sugar produced from sugarcane molasses as a substrate was maximized after 5 days of incubation by adjusting all ferment broth conditions such as pH (4.5-6.5) and temperature (26-30 °C). The highest yield of reducing sugar after hydrolysis was achieved on day 3 of incubation (28 °C, pH 5.5). The reducing sugar produced using TPS was 0.448±0.07 g/L while that produced using DW was 0.378±0.05 g/L (Table 1). The amount of reducing sugar produced from sugarcane molasses hydrolyzed with TPS on days 1-3 (26-28 °C, pH 4.5-5.5) was significantly higher ($p<0.05$) compared to the reducing sugar produced using DW. However, the reducing sugar produced on days 4 & 5 (29-30 °C, pH 6.0-6.5) using DW was significantly higher compared to those produced using TPS at $p<0.05$ (Table 1).

Table 1: Reducing Sugar yield from Sugarcane Molasses Hydrolyzed using TPS and DW at Different Temperature and pH

Day	pH	Temp. (°C)	Reducing Sugar produced using TPS (g/L)	Reducing Sugar produced using DW (g/L)
1	4.5	26	0.335 ± 0.07[a]	0.263± 0.05[b]
2	5.0	27	0.394 ± 0.06[a]	0.343 ± 0.06[b]
3	5.5	28	0.448 ± 0.07[a]	0.378 ± 0.05[b]
4	6.0	29	0.247 ± 0.07[b]	0.311 ± 0.06[a]
5	6.5	30	0.161 ± 0.06[b]	0.258 0.06[a]

Values presented as Mean ± SD. Values in the same row with different superscripts indicate significant differences at $p < 0.05$. TPS= tamarind pulp syrup, DW= distilled water.

Bioethanol Yield from Sugarcane Molasses Hydrolyzed using TPS and DW at Different Temperature and pH

The highest yield of bioethanol from sugarcane molasses hydrolyzed using TPS and DW were 0.395±0.07 mL/L and 0.365±0.06 mL/L respectively. These yields were obtained after fractional distillation at 28 °C and pH 5.5. Fermentation was influenced by temperature and pH. The ideal temperature and pH for the *Saccharomyces cerevisiae* fermentation reaction ranges from 27 °C – 28 °C and pH 5.0-5.5 respectively. The bioethanol yield using TPS was significantly higher compared to those produced using DW at all temperatures and pH (Table 2).

Table 2: Bioethanol Yield from Sugarcane Molasses Hydrolyzed using TPS and DW at Different Temperature and pH

Treatment	pH	Temp. (°C)	Bioethanol yield using TPS (mL/L)	Bioethanol yield using DW (mL/L)
A	4.5	26	0.327 ± 0.05[a]	0.241 ± 0.06[b]
B	5.0	27	0.290 ± 0.05[a]	0.283 ± 0.05[b]
C	5.5	28	0.395 ± 0.07[a]	0.365 ± 0.06[b]
D	6.0	29	0.218 ± 0.04[b]	0.257 ± 0.06[a]
E	6.5	30	0.265 ± 0.03[a]	0.229 0.06[b]

Values presented as Mean ± SD. Values in the same row with different superscripts indicate significant differences at $p < 0.05$. TPS= tamarind pulp syrup, DW= distilled water.

Densities of Bioethanol Produced from Sugarcane Molasses Hydrolyzed using Tamarind Pulp Syrup and using Distilled Water

The density of bioethanol produced using TPS and DW increased with increasing temperature and pH. Densities of 0.797±0.07 g/cm^3 and 0.814±0.06 g/cm^3 were recorded for bioethanol produced using TPS and DW respectively. The density of bioethanol produced using DW was significantly higher relative to those produced using TPS in all the temperature and pH ranges $P<0.05$ (Table 3).

Table 3: Bioethanol Densities Produced from Sugarcane Molasses Hydrolyzed using TPS and DW

Treatments	pH	Temp. (°C)	Bioethanol Density using TPS (g/cm^3)	Bioethanol Density using DW (g/cm^3)
A	4.5	26	0.813 ± 0.0601[a]	0.845 ± 0.0557[b]
B	5.0	27	0.824 ± 0.0572[b]	0.862 ± 0.0649[a]
C	5.5	28	0.797 ± 0.0693[b]	0.814 ± 0.0637[a]
D	6.0	29	0.845 ± 0.0721[b]	0.873 ± 0.0685[a]
E	6.5	30	0.863 ± 0.0674[b]	0.886 ± 0.0589[a]

Values presented as Mean ± SD. Values in the same row with different superscripts indicate significant differences at $p < 0.05$. TPS= tamarind pulp syrup, DW= distilled water.

Bioethanol Quantity Produced from Sugarcane Molasses Hydrolyzed using TPS and DW

The results revealed that bioethanol created from sugarcane molasses hydrolyzed with TPS had a significantly higher ($p<0.05$) quantity compared to those hydrolyzed using DW. The highest amount of bioethanol produced was at 28°C and pH 5.5 for both TPS (27.61±1.32 mL/L) and DW (25.58±1.27 mL/L), see Table 4.

Table 4: Bioethanol Quantity Produced from Sugarcane Molasses using TPS and DW

Treatments	pH	Temp (°C)	Bioethanol Quantity using TPS (mL/L)	Bioethanol Quantity using DW (mL/L)
A	4.5	26	23.67 ± 1.00^{a}	22.31 ± 1.43^{b}
B	5.0	27	24.32 ± 1.53^{a}	23.01 ± 1.51^{b}
C	5.5	28	27.61 ± 1.32^{a}	25.58 ± 1.27^{b}
D	6.0	29	22.21 ± 1.07^{a}	21.64 ± 1.07^{b}
E	6.5	30	21.35 ± 1.25^{a}	$20.47 \quad 1.15^{b}$

Values presented as Mean ± SD. Values in the same row with different superscripts indicate significant differences at $p < 0.05$. TPS= tamarind pulp syrup, DW= distilled water.

Gas Chromatography and Mass Spectroscopy Analysis of the volatile profile of Bioethanol Produced using TPS and DW

The results revealed a significantly higher ($p<0.05$) percentage of bioethanol output produced from sugarcane molasses hydrolyzed using TPS compared to that hydrolyzed using DW The highest percent of bioethanol output produced from sugarcane molasses hydrolyzed using both TPS and DW was observed at 28 °C and pH 5.5. they were found to be 27.41% v/v and 10.70% v/v for TPS and DW respectively (Table 5).

Table 5: Volatile Profile of the Bioethanol Produced from Sugarcane Molasses Hydrolyzed using TPS and DW

Treatment	pH	Temp (°C)	Peak area (n=3)	Bioethanol Produced using TPS (%)	Peak area (n=3)	Bioethanol Produced using DW (%)
A	4.5	26	134163942.8	26.22^{a}	71553472.94	10.30^{b}
B	5.0	27	212646526.2	16.54^{a}	83575732.82	8.82^{b}
C	5.5	28	222333357.9	27.41^{a}	86751190.7	10.70^{b}
D	6.0	29	232734387.7	24.74^{a}	882633655.2	10.23^{b}
E	6.5	30	252764374.3	23.43^{a}	894527367.4	9.47^{b}

Values presented as Mean ± SD. Values in the same row with different superscripts indicate significant differences at $p < 0.05$. TPS= tamarind pulp syrup, DW= distilled water.

DISCUSSION

The outcome of this study revealed that sugarcane molasses hydrolyzed with TPS produced a higher concentration of bioethanol when compared to sugarcane molasses hydrolyzed with DW. This suggests that the inclusion of tamarind pulp syrup has the potential to increase bioethanol production. This is in agreement with a previous study by Patil *et al*. (2017) who reported an increase in bioethanol production when supplemented with tamarind wastes such as husk, pulp, seeds, fruit, and effluent generated after tartaric acid extraction using yeast cultures.

Our results showed that pH 5.5 enhanced the conversion of sugar present in the medium to ethanol by yeast (*Saccharomyces cerevisiae*). Previous studies reported that a slightly acidic pH between 5.0 and 5.5 enhanced yeast growth but prevented bacterial multiplication (Eskes *et al*., 2018; Lund *et al*., 2020; Salas-Navarrete *et al*., 2023). We then hypothesized that pH 5.5 was suitable

for promoting yeast growth and resulting in more ethanol production through fermentation stimulated by the yeast. As the number of days increased, the sugar concentration fell. This might be because the cellulose from the sugar molasses was being utilized to produce bioethanol and as the days went by the quantity of the sugarcane molasses was depleted. The current study showed that using TPS as a hydrolysis agent improved the yield of bioethanol suggesting that waste production high in sugar can be utilized as a hydrolyzing agent for bioethanol production. Therefore, apart from sugarcane molasses, other sugars-rich waste can be used for bioethanol production either as the main source or as a hydrolyzing agent. According to Irfan *et al.* (2014) and Okella *et al.* (2017), three distinct substrates sugarcane bagasse, rice straw and wheat straw were used for bioethanol production using *Saccharomyces cerevisiae* in a 500 mL Erlenmyer flask at 30 ^{o}C for four (4) days of the fermentation period. Among all these tested substrates, sugarcane bagasse (77 g/L) produced more bioethanol as compared to rice straw (62 g/ L) and wheat straw (44 g/ L) using medium composed of (%) 0.25 $(NH_4)2SO_4$, 0.1 KH_2PO_4, 0.05 $MgSO_4$ and 0.25 yeast extract by *Saccharomyces cerevisiae.* This difference in bioethanol production was due to the availability of fermentable sugars from cellulose present in biomasses. Studies (Sarkar *et al.,* 2019; Khandaker *et al.,* 2020) on fruit wastes, mainly fruit peels, have yielded bioethanol in recent years, demonstrating their abundance and including components that can be converted into fermentable sugars.

The current study showed that the density of bioethanol obtained using TPS (0.797 g/cm^3) was close to the standard density of bioethanol is 0.789 g/cm^3. It also showed an increase in bioethanol density with temperature increase. Jiang *et al.* (2019) pointed out that the temperature has been considered an important factor in influencing the density during bioethanol production. We discovered that increasing bioethanol production without getting the standard density will not produce an effective biofuel. Hence, while looking for ways to enhance bioethanol production the density should be considered to produce energy-friendly and efficient biofuel.

The significantly higher quantity of bioethanol produced from sugarcane molasses hydrolyzed using TPS 27.61 mL/L is an indication that TPS can be a good hydrolysis agent in bioethanol production. This might be due to the high sugar content in tamarind as reported in earlier studies (Van den Bilcke *et al.,* 2014; Nayik and Gull, 2020; Hemalatha and Parameshwari, 2021). This is in line with the work of Irfan *et al*. (2014) who observed an increase in bioethanol produced from the three different agro-industrial biomass residues (sugarcane bagasse, rice husk, and corn cob) using tamarind as a hydrolyzer and yeast (*Saccharomyces cerevisiae*) for fermentation. The high percentage of bioethanol output produced from sugarcane molasses hydrolyzed using TPS suggests that TPS can be used to produce a more efficient biofuel for both domestic and industrial use.

CONCLUSION

The current study demonstrated the effect of supplementation with tamarind pulp syrup for enhancing bioethanol production from sugarcane molasses fermentation at different levels of pH and temperature. The Bioethanol produced with supplementation of TPS had a higher concentration of reducing sugar, bioethanol output and a bioethanol density close to the standard. TPS was found to enhance bioethanol production from sugarcane molasses fermentation. This suggests that it could serve as an alternative hydrolyzer for the production of biofuel which is a renewable energy source.

REFERENCES

Ajit, A., Sulaiman, A.Z. and Chisti, Y. (2017). Production of bioethanol by *Zymomonas mobilis* in high-gravity extractive fermentations. *Food and Bioproducts Processing,* 102: 123-135.

Bhatia, L., Johri, S. and Ahmad, R. (2012). An Economic and Environmental Analysis of Bioethanol Production from Sustainable Agricultural Waste: a review. *AMB Express,* 2:65.

Bušić, N., Marđetko, S.K.G., Morzak, H., Belskaya, M.I., Šantek, D. *et al.* (2018). Bioethanol Production from Renewable Raw Materials and its Separation and Purification: A Review. *Food Technology and Biotechnology,* 56:289-311.

Cardona, C.A., Quintero, J.A. and Paz, I.C. (2010). Production of Bioethanol from Sugarcane Bagasse: Status and Perspectives. *Bioresources Technology,* 101: 4754-4766.

Cavelius, P., Engelhart-Straub, S., Mehlmer, N., Lercher, J., Awad, D. et al. (2023). The potential of biofuels from first to fourth generation. *PLoS Biology,* 21:e3002063.

Conesa, C., Seguí, L., Laguarda-Miró, N. and Fito, P. (2016). Microwaves as a pretreatment for enhancing enzymatic hydrolysis of pineapple industrial waste for bioethanol production. *Food and Bioproducts Processing,* 100: 203-213.

Edjekouane, M., Lansari, F., Khelifi, O., Boukheteche, I. and Laksaci, H. (2020). Production of Bioethanol from a Local Natural Resource. *Algerian Journal of Renewable Energy and Sustainable Development,* 2:56-59.

Eskes, A., Deprez, M.A., Wilms, T. and Winderickx, J. (2018). pH homeostasis in yeast; the phosphate perspective. *Current Genetics,* 64:155-161.

Favaro, L., Jansen, T. and van Zyl, W.H. (2019). Exploring industrial and natural Saccharomyces cerevisiae strains for the bio-based economy from biomass: the case of bioethanol. *Critical Reviews in Biotechnology,* 39:800-816.

Hemalatha, S. and Parameshwari, S. (2021). The scope of tamarind (Tamarindus indica L.) kernel powder in diverse spheres: A review. Materials Today: *Proceedings,* 45:8144-8148.

Humphrey. C.N. and Caritas U.O. (2007). Optimization of ethanol production from Garcinia kola (bitter kola) pulp Agro-waste. *African Journal of Biotechnology,* 6.17: 2033-2037.

Irfan, M., Nadeem, M. and Syed, Q. (2014). Ethanol Production from Agricultural Wastes using *Saccharomyces cerevisiae*. *Brazilian Microbiology Journal,* 45:457- 465.

Jeswani, H.K., Chilvers, A. and Azapagic, A. (2020). Environmental sustainability of biofuels: a review. *Proceedings of the Royal Society A,* 476:20200351.

Jiang, S., Qi, J., Hu, Y., Ren, C., Jiang, Y. *et al.* (2019). Predicting the Density and Viscosity of Biodiesels and Biodiesel Blends by the Regular-Solution Theory. *Industrial and Engineering Chemical Research,* 58:17038-17048.

Khandaker, M.M., Abdullahi, A.U., Abdulrahama, M.D., Badaluddin, N.A. and Mohd, K.S. (2020). Eds: Inambao, Bioethanol Production from Fruit and Vegetable Waste by Using *Saccharomyces cerevisiae*, In: Bioethanol Technologies, Intechopen.

Kharkwai A.C., Joshi, H., Shandilya, C., Dabral, S., Kumar, N. *et al.* (2024). Isolation and characterization of a Newly Discovered Plant growth-promoting Endophytic Fungal Strain from the genus Talaromyces. *Scientific Report,* 14; 66022.

Li, G., Li, J., Li. S., Zu, X., Zhang L. *et al.* (2017). Batch distillation is used to extract bioethanol from fermented sweet sorghum bagasse. *Journal of Bioscience and Bioengineering,* 34:127-132.

Li, G., Li. J., Li. S., Zu, X., Zhang, L., Q, L. and Xu, W. (2017). Batch distillation is used to extract bioethanol from fermented sweet sorghum bagasse. *Journal of Bioscience and Bioengineering,* 34: 127-132.

Lund, P.A., De Blasé, D., Liran, O., Scheler, O., Mira, N.P. *et al.* (2020). Understanding how Microorganisms Respond to Acid pH is Central to their Control and Successful Exploitation. *Frontiers in Microbiology,* 11:5566140.

Manisalidis, I., Stavropoulou, E., Stavropoulos, A. and Bezirtzoglou, E. (2020). Environmental and Health Impacts of Air Pollution: A Review. *Frontiers in Public Health,* 8:14.

Nayik, G.A. and Gull, A. (2020). Antioxidants in Fruits: Properties and Health Benefits. Springer Nature Singapore Pte Ltd.

Okello, J., Okullo, J.B., Eilu, G., Nyeko, P. and Obua, J. (2017). Mineral Composition of Tamarindus indica LINN (Tamarind) Pulp and Seeds from Agro Ecological Zones of Uganda. *Journal of Food Science and Nutrition,* 5:959-966.

Oniya, O.O., Adebayo, A.O. and Ogunkunle, O. (2016). Optimization of Biodiesel Production from Milk Bush (Thevetia peruviana) Oil Using Shell as Catalyst. *Advances in Multidisciplinary and Scientific Research Journal,* 3:9-22.

Osei, W.Y. (1993). Wood fuel and deforestation - answers for a sustainable environment. *Journal of Environmental Management,* 37:51-62.

Patil, B.G., Gokhale, D.V., Bastawde K.B., Puntambekar, U.S. and Patil, S.G. (2017). An Improved Process for the Production of Alcohol. *Journal of Enzyme Microbiology Technology,* 12:141-148.

Patil, B.G., Gokhale, D.V., Bastawde, K.B., Puntambekar, U.S. and Patil, S.G. (1998). The use of tamarind waste to improve ethanol production from cane molasses. *Journal of Industrial Microbiology & Biotechnology,* 21:307-310.

Pona, H.T., Xiaoli, D., Ayantobo, O.O. and Tetteh, N.D. (2021). Environmental health situation in Nigeria: Current status and future needs. *Heliyon,* 7:e06330.

Prasad, S., Gupta, V.C., Bhatt, R. and Dhanya, M.S. (2021). Ethanol production from Sugarcane: An Overview. In: Input use Efficiency for Food and Environmental Security. Springer Nature Singapore Pte, Pp. 6665-686.

Rabah, A.B., Oyeleke, S.B., Manga, S.B. and Hassan, L.G. (2011). Utilization of Millet and Guinea Corn Husk for Bioethanol Production. *African Journal of Microbiology Research,* 5:5721-5724.

Salas-Navarrete, P.C., Rosas-Santiago, P., Suarez-Rodriguez, R., Martínez, A. and Caspeta, L. (2023). Adaptive responses of yeast strains tolerant to acidic pH, acetate, and supraoptimal temperature. *Applied Microbiology and Biotechnology,* 107:4051-4068.

Sanchez, N., Cobo, M., Rodriguez-Fontalvo, D., Uribe-Laverde, M.Á. and Ruiz-Pardo, R.Y. (2021). Bioethanol Production from Sugarcane Press-Mud: Assessment of the Fermentation Conditions to Reduce Fusel Alcohol. *Fermentation,* 7: 194.

Sarkar, S. Prajapati, K. Poddar, R. and Biswas, A.S. (2019). International Biodeterior and Biodegradation. 145:104795.

Surendra, K.C., Takara, D., Hashimoto, A.G. and Khanal, S.K. (2014). Biogas as a sustainable energy source for developing countries: Opportunities and challenges. *Renewable and Sustainable Energy Reviews,* 31:846-859.

Ukaogo, P.O., Ewuzie, U. and Onwuka, C.V. (2020). Environmental pollution: causes, effects, and the remedies. *Microorganisms for Sustainable Environment and Health,* 2020:419-429.

Van den Bilcke, N., Alaerts, K., Ghaffaripour, S., Simbo, D.J. and Samson, R. (2014). Physico-chemical properties of tamarind (Tamarindus indica L.) fruits from Mali: selection of elite trees for domestication. *Genetic Resources and Crop Evolution,* 61:537-553.

Wong, Y.C. and Sanggare, V. (2014). Production of Bioethanol from Sugarcane Bagasse using Fermentation process. *Oriental Journal of Chemistry,* 30: 507-513.