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INFLUENCE OF REACTION TEMPERATURE ON BIOETHANOL PRODUCTION BY SACCHAROMYCES CEREVISIAE USING CASSAVA AS SUBSTRATE

Musa A. A.[1]
Salim F.B.[2]
Shiaka G.P.[3]
Muhammad M.[4]
Nafisa B.[5]
Surayya M.M.[6]
Sa'adatu A.Y.[7]
Hauwa S. D.[8]
Gayus R.[9]
Gumel A.M.[10+]

[1,2,3,4,5,6,7,8,9,10]*Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, Jigawa State, Nigeria.*
[1]*Email: kalamimfor@gmail.com Tel: +2348038362360*
[2]*Email: salim.bashir@fud.edu.ng Tel: +2348069128829*
[3]*Email: shiaka.g@fud.edu.ng Tel: +2347061972844*
[4]*Email: muhammadkw89@gmail.com Tel: +2348033257654*
[5]*Email: baitanafisa@gmail.com Tel: +2349070401226*
[6]*Email: smustapha12@gmail.com Tel: +2349035434132*
[7]*Email: saadatuabba@yahoo.com Tel: +2348038760667*
[8]*Email: hauwasanid@gmail.com Tel: +2347067711194*
[9]*Email: rahabgayus0017@gmail.com Tel: +2348067092665*
[10]*Email: dr.gumel@fud.edu.ng Tel: +2348161101920*


(+ Corresponding author)

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ABSTRACT

The alarming awareness and increasing concern over effect of global warming, and environmental pollution due to the continuous combustion of fossil fuels has necessitated the call for an alternative renewable energy. The trepidation on the sustainability of the global food supply due to emphasis on corn production to meet the increased demand for bioethanol led to the need to assess the potential of alternative crops as sources of bioethanol production. On this focus, cassava proved to be an ideal crop to fulfil this need as it is more abundant and meeting requirement in serving both edible and nonedible usage than corn. However, the yield and quality of bioethanol produced from cassava can be impacted by alteration of temperature during production process. From this research, production of bioethanol from cassava was found to be optimum at a temperature of 35°C, with maximum yield of 95% and any increase beyond 40°C leads to the gelatinization of starch. The research has stroke a balance at where and when temperature needs to be altered to increase yield.

Contribution/Originality: This study contributes to the existing literature by exploring the specific influence of reaction temperature as a limiting factor in bioethanol synthesis. It is our firm believe that through this kind of study, bioethanol production process can successfully be optimized.

1. INTRODUCTION

Negative environmental impact of nonrenewable fuels such as fossil fuels has led to a detrimental effect on the biological systems (Boyle, 2004). The non-renewable nature of fossil fuels, their devastative environmental impact, and their perpetual demand have led to political and economic instability in many oil producing countries around the world (Gumel, Idris, Wada-Kura, Ibrahim, & Mustapha, 2018) thus necessitating the need for a sustainable green energy. Biofuels are liquid, gas and solid fuels preponderantly produced from biomass (Nigam & Singh, 2011). They are the most eco-friendly and sustainable energy source (Nigam & Singh, 2011). Among the sustainable green

fuel is bioethanol, which is an alcohol made through the yeast fermentation of sugars derived from starch-based feedstock, such as corn, cassava, sugar beet and sugarcane, cellulose etc. (Nitayavardhana, Rakshit, Grewell, Van Leeuwen, & Khanal, 2008). Bioethanol is regarded as one of the most dependable, clean and renewable automotive fuels. It has been used as a fuel, alone or as a blend with other fossil fuels (Nuwamanya, Chiwona-Karltun, Kawuki, & Baguma, 2012). Ethanol produced from cellulose rich crops is one of the most promising and reliable technological approach for reducing the greenhouse gases from the transportation sector (Han, Kim, Kim, Chung, & Choi, 2011). Despite its advantages, bioethanol production has suffered a setback due to high production cost. Among the strategies employed to address such setback is the utilization of affordable and renewable biomass as well as fermentation process optimization. It is now understood that it is important to use biomass energy as a means of providing modern energy as it complements solar, wind, and other intermittent energy sources in the renewable energy mix of the future (Nigam & Singh, 2011). However, the selection of feedstock to be used for the production of biofuel depends on several factors such as availability, competition between edible and inedible products, and cost (Dos Santos et al., 2015).

Cassava (*Manihot esculenta*), sometimes also called manioc, grown in northern Nigeria has met these requirements, it also grows on marginal soils, has high yield, optimum management cost, and its farming is not labor intensive (Nuwamanya et al., 2012). Cassava is the third largest source of carbohydrates for human consumption in the world, with an estimated annual world production of 208 million tons (Leen et al., 2007). The largest cassava market by far is in Nigeria, responsible for 18% of world cassava production (Leen et al., 2007). Because of its high carbohydrate content, it is ideal biomass for the production of bioethanol but requires effective pretreatment because of its ligno-cellulosic nature and complexity of plants cell wall (Han et al., 2011). Pretreatment makes hydrolysis more rapid and efficient (Han et al., 2011). Hence the chosen crop for this research. Because of the continuous need for green energy, bioethanol needs to be produced rapidly and efficiently. While the production methodology is somewhat generalized, optimization of parameters such as temperature could lead to increase or decrease in the yield, quality and time frame of the production of bioethanol from cassava. This research stroke a balance at the optimum temperature for possible highest yield of ethanol production from this substrate.

2. METHODOLOGY

Freshly harvested cassava roots were gotten from Laraba village in Dutse local government of Jigawa State, Nigeria. The *Saccharomyces cerevisiae* used in this study was obtained from a purchased 500g commercial baker's yeast pack. The chemical hydrolysis was carried out using 0.4M H_2SO_4 prepared in the Biotechnology Laboratory, Federal University Dutse, Nigeria.

2.1. Pretreatment and Starch Extraction

One (1) kg of the harvested cassava roots were peeled off and then washed in clean water to remove residual peels. The washed roots were then grated thoroughly to obtain a milk pulp. Extraction of starch from the milk pulp was carried out using the method of Ajibola, Edema, and Oyewole (2012). The pulp was sieved through a screen mesh to suspend the starch into a clean water and the residual coarse root materials and fiber were discarded. Sedimentation of the starch milk was then carried out for 4-6 hours before decanting the supernatant. The bright-white thick starch that caked at the bottom of the container was pressed to remove the remaining water, sun dried for 72 hours, and then taken to the Biotechnology laboratory of Federal University Dutse.

2.2. Hydrolysis

Acidic hydrolysis using 1400ml of dilute 0.4M H_2SO_4 and 500g of cassava starch was carried out. The prepared acid was poured into a 2000ml conical flask. On addition of the starch, the hydrolysate was heated at 80°C on a

magnetic stirrer with continuous stirring until formation of a smooth gel. The hydrolysate was then passed through a muslin cloth and the solution's pH was adjusted to 4.0 using NaOH solution.

2.3. Activation of Saccharomyces Cerevisiae

Thirty (30) gram of baker's yeast was placed in a beaker containing 90ml of distilled water (1:3 w/v).The solution was warmed at 35°C in a water bath for 15 minutes with continuous stirring to activate the yeast before adding it into the 2000ml conical flask containing the cassava starch hydrolysate. It was then stirred on a magnetic stirrer for 2minutes without heating.

2.4. Temperature Based Fermentation of Hydrolysate

For temperature evaluation, the bioethanol production was observed according to Ajibola et al. (2012) with slight modification. Briefly, the solution was divided into six (6) sets of 250ml, each capped in 500ml conical flask. They were fermented individually using Labotech® incubator shaker that was set at 250rpm for three days under varied temperature treatments as indicated in Table 1.

Table-1. Fermentation treatments of samples.

Sample	1	2	3	4	5	6
Temperature (°C)	25	30	35	40	40	40
Supplement					Sucrose (5g).	Alpha-amylase (5ml)

After fermentation, each sample was first filtered through Whatman's filter paper before taking the clear liquids for distillation at 80-85°C and reduced pressure using a rotary evaporator at 150rpm.

2.5. Analytical Tests

2.5.1. Ethanol Yield

Ethanol concentration was monitored using ATC AZ116 refractometer via the refractive index method. A calibration curve was obtained initially by diluting pure 98% ethanol in water to obtain different standard concentrations of ethanol and their corresponding refractive indexes obtained from the refractometer readings.

2.5.2. Physico-Chemical Analyses

2.5.2.1. Flammability

Individual bioethanol samples were tested by placing 5mls into a spirit lamp containing cotton wool at the top. Commercial matches were lit to get some lightening flame. Samples were then tested for flammability by lightening the flame on the cotton wool.

2.5.2.2. Colorimetric Ethanol Detection:

The colorimetric reaction used for the ethanol detection was based on the redox reaction of acidified Potassium dichromate generating acetic aldehyde and Cr (III) (green) or Cr (II) (blue), depending on the ethanol concentration, as adopted from Dos Santos et al. (2015).

Briefly, bioethanol samples were individually added in drops to test tubes containing potassium dichromate (VI) solution acidified with dilute sulfuric acid. The tubes were then warmed in a hot water bath. And after heating, the orange colored solutions turned green.

2.5.2.3. pH Test:

pH meter was first inserted in a buffer solution to standardize the apparatus then placed into the sample (ethanol) and the readings were obtained as mentioned in literature (Ademiluyi & Mepba, 2013).

2.5.2.4. Clarity Test

A portion (1 ml) of all samples of produced bioethanol was placed in a cuvette which was placed in a spectrophotometer and transmittance of light was measured at 650nm. Absolute ethanol was used as standard as described by Nuwamanya et al. (2012).

2.5.2.5. Sulfate and Chloride Ions Test

Presence of sulfate and chloride in the produced samples were assessed by means of precipitation analysis. A 0.1M barium chloride solution was added drop wise (up to 0.5 ml) to 0.5 ml of individual bioethanol samples to examine possible formation of barium sulfate which does not dissolve in water solutions. The presence of chloride ions was examined using similar approach by adding up to 0.5ml silver nitrate drop wise into 0.5ml of individual ethanol samples (possible precipitate of solid silver chloride was studied).

2.5.2.6. Electrical Conductivity

PC200 digital electrical conductivity meter (Thomas Scientific, United States) was used. A known quantity of the produced ethanol was poured into a beaker. The electrode was immersed to the censor market point. The meter was turned on and the electrical conductivity was recorded.

2.6. Determination of Purity via Density, Specific Gravity and Percentage Ethanol

Densities of all produced ethanol samples were determined using hydrometer and mass per volume measurement as indicated in Equation 1.

$$Density\ \left(\frac{g}{ml}\right) = \frac{mass\ (g)}{Volume\ (ml)} \quad (1)$$

On the other hand, the specific gravity was calculated according to Equation 2:

$$Specific\ gravity\ (spg) = \frac{density\ of\ ethanol}{density\ of\ water} \quad (2)$$

Ten samples of varying proportions of ethanol were then prepared separately (ranging from 10%, to 90% and absolute ethanol), the density of each was determined and recorded. A standard curve of density against percentage ethanol was then plotted to get the individual percentage ethanol of the six produced samples using the knowledge of the prepared samples. The density of Ethanol used as standard is 0.80 g/ml.

3. RESULTS AND DISCUSSION

3.1. Bioethanol Presence and Yield

The efficiency of the fermentation depends on the temperature and the ability of the yeast to utilize particular feedstocks based on their characteristics and compositional differences. All samples were first analyzed for the presence of ethanol using a refractometer, the brix index of each sample was determined, and the yield in volume was determined accordingly and presented in Table 2.

The low yield of the 40°C sample could be attributed to low microbial activity of the yeast at the elevated temperature beyond which, there would be no significant production. This is in line with the work of Rhee, 1984 where at 40°C, ethanol production was apparently inhibited. The sucrose supplement in the 40S sample improved the yield slightly due to the readily available glucose for the yeast to ferment.

Table-2. Individual brix index and yield volumes of produced bioethanol samples.

Serial Number	Sample Identity	Brix Index	Ethanol Presence	Yield (Ml/100ml Broth)
1	Absolute ethanol	21	Positive	N/A
2	25°C	15	Positive	67
3	30°C	18	Positive	78
4	35°C	20	Positive	95
5	40°C	11	Positive	80
6	40S (Supplemented with sucrose)	14	Positive	82
7	40A (supplemented with alpha amylase)	16	Positive	89

3.2. Physico Chemical Analyses

3.2.1. Flammability

All the samples were found to be flammable, with flash point ranging from 16°C to 23°C, and this obervation was found to be in accord with Ademiluyi and Mepba (2013) who reported a flash point that spans between 15°C and 24°C in obioethanol derived from whole cassava flours.

3.2.2. Influence of Reaction Temperature on Electrical Conductivity and pH

The electrical cobductivity of fuel ethanol has been a basic parameter in assessing its quality. On this note, a conductivity of less than 500 µs/m is considered to be appropreate (Ademiluyi & Mepba, 2013; Moriarty, 2013). In this research, regardless of the fermentation temperature, the ethanol conductivity were found to be within the acceptable values (Table 3). Additionally, the pH values, were found to be in agreement with the pH of standard 95% ethanol as well as those reported in literatures (Azad, Yesmin, Sarker, Sattar, & Karim, 2014; Kumar & Singh, 2016; Liyakathali, Muley, Aita, & Boldor, 2016).

Table-3. Electrical conductivity and pH of bioethanol produced at different temperature.

Fermentation Temp (°C)	Electrical Conductivity (µs/m)	pH
25	340	6.21
30	300	6.72
35	280	6.75
40	200	6.49
40S	210	6.81
40A	215	6.67

3.2.3.Clarity Test

Reference to standard ethanol, the optical density of the produced ethanol based on the fermentation temperature is presented in Figure 1.

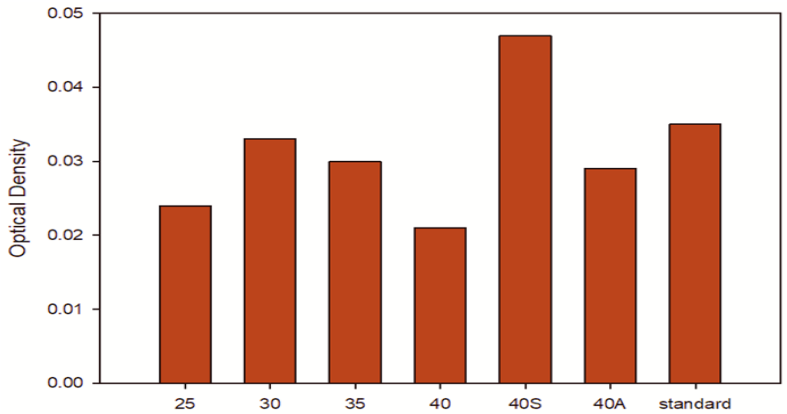

Figure-1. Ethanol samples based on fermentation temperature as a function of optical density. Note: 40S and 40A are sample produced at 40°C with supplementation of sucrose and alpha amylase, respectively.

From the presented Figure 1, the optical densities of the produced samples fall within the range of the standard and in good agreement with previous reports (Hassan, Mona, & Tagelsir, 2018).

3.2.4. Sulfate and Chloride Ions Test

The absence of sulfate ions in all produced samples indicates high purity of the ethanol. On the other hand, the positive formation of solid silver chloride precipitate in the 30°C sample makes it unfit for use in motor engines (Nuwamanya et al., 2012). The cloudy nature of the sample could be attributed to insufficient distillation. Absence of sulfate and chloride ions in the other studied samples suggest high ethanol quality and purity (Teixeira, Chaves, Guimarães, Pontes, & Teixeira, 2009) thus making cassava an efficient substrate.

3.2.5. The Bioethanol Density as a Function of Fermentation Temperature

The individual densities obtained for all samples fell within 0.815 g/ml, this indicates the suitability of cassava as a substrate for producing high quality bioethanol Table 4.

The density of ethanol used as standard at 25°C is 0.80 g/ml.

Table-4. Individual densities of bioethanol produced at various temperatures.

Serial Number	Bioethanol Sample	Density (G/Ml)
1.	25°C	0.86
2.	30°C	0.83
3.	35°C	0.82
4.	40°C	0.84
5.	40S	0.84
6.	40A	0.86

3.3. Influence of Fermentation Time and Temperature on Bioethanol Production

The influence of fermentation time and temperature on ethanol yield was studied (Figure 2). It was observed that increasing fermentation time beyond 72 hours resulted in decrease production of ethanol. Similar observation on reduced ethanol production with prolong reaction time was reported earlier (Liyakathali et al., 2016). Furthermore, fermentation at 35°C proved to be the optimum fermentation temperature for this process. Similarly, the percentage ethanol of the 35°C sample from the curve has the best yield as a result of optimum *Saccharomyces cerevisiae* activity at that temperature. This outcome is similar to the work of Nuwamanya et al. (2012) on bioethanol production from non-food parts of cassava. In the same vain, addition of alpha amylase improve starch hydrolysis to release the sugar, thus the observed improved rate. This observation was also found to be in tandem with Azad et al. (2014) and Kumar and Singh (2016).

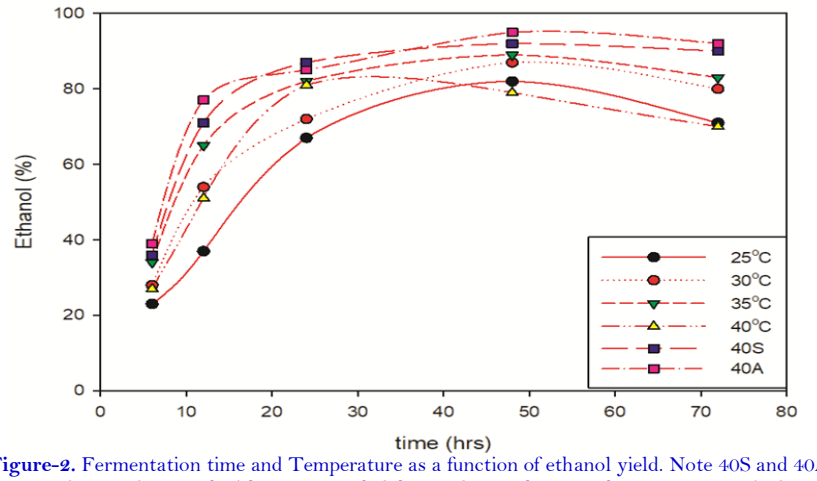

Figure-2. Fermentation time and Temperature as a function of ethanol yield. Note 40S and 40A are samples supplemented with sucrose and alpha amylase and operated at 40°C, respectively.

4. CONCLUSION

From this research, it can be said that temperature is a significant parameter for bioethanol production from cassava using Saccharomyces cerevisiae. Its variation does influence the yield, purity, concentration, clarity, and overall quality of bioethanol. Cassava starch fermented at 35°C produced the best bioethanol overall in yield and quality, implying why further production from this substrate should be executed at this optimum temperature while sugar supplemented fermentation at 40°C produces unappreciable bioethanol yield.

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