Waste Technology (WasTech)
Journal homepage: http://ejournal.undip.ac.id/index.php/wastech
An International Journal

Feasibility of Bioethanol Production from Rotten Tomatoes (*Solanum Lycopersicum*) Using Saccharomyces Cerevisiae

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Abstract - *The study aimed at optimizing different fermentation conditions for bioethanol production using rotten tomatoes (Solanum lycopersicum). Rotten tomatoes were collected from sellers at Nairobi market, Kenya for six months and analyzed after each time of collection in Kenyatta University Laboratory. They were physically pre-treated and enzymatic hydrolysis was performed using commercial cellulose from Aspergillus niger. Fermentation was carried out using pure culture of Saccharomyces cerevisiae (baker's yeast). Fermentation variables were optimized at different incubation times of (24, 48, 72, 96, 120 and 144 hours) and temperatures (20°C, 25°C and 30°C). Concentration (%/v/v) of bioethanol at the end of each fermentation time was determined by the use of colorimetric method and residual sugar was determined using DNS method by Miller. Maximum bioethanol percentages of (0.17%) and (0.16%) were achieved at 24 hours and 30°C respectively. Thus, the optimum conditions for maximum bioethanol production in the study were fermentation time of 24 hours and temperature 30°C. The study results have proved the effectiveness of producing bioethanol from rotten tomatoes using baker's yeast for fermentation.*

Keywords *– bioethanol, enzymatic hydrolysis, fermentation, rotten tomatoes, Saccharomyces cerevisiae*
Submission: September 2, 2020 Correction: September 18, 2020 Accepted: October 10, 2020
Doi: http://dx.doi.org/10.14710/wastech.8.2.34-38
[How to cite this article: Osei, J.A. (2020). Feasibility of Bioethanol Production from Rotten Tomatoes (*Solanum Lycopersicum*) Using Saccharomyces Cerevisiae. Waste Technology, 8(2), 34-38 doi: http://dx.doi.org/10.14710/wastech.8.2.34-38]

1. Introduction

Tomato is in the Solanacea family which also includes species like nightshade (*Solanum nigrum L.*), brinjals (*Solanu, melongena L.*), and potatoes (*Solanum tuberosum*). It originated from South America and later spread to Europe in the 16th century and East Africa in 1900. Common names for tomato are tomate (Spain, France), tomat (Indonesia), faan ke'e (China), tomato (West Africa), tomatl (Nahuatl), jitomate (Mexico), pomodoro (Italy), nyanya (Swahili). It is the second most important vegetable after potatoes in most parts of the world ('unpublished data' Kithinji, G.V.; Dam et al., 2005). Worldwide production of tomato is estimated to be 162 million tonnes in an area of 4.8 million hectares. The lead producers are China with 50 million tonnes followed by India with 17.5 million tonnes. The total tomato production in Africa for 2012 was 17,938 million tonnes with Egypt leading with 8,625 million tonnes. Leading producers of tomato in Africa are Egypt, Nigeria, Morocco, Tunisia, Cameroon, Algeria, South Africa, Sudan, Kenya, Ghana, Tanzania, Mozambique, Benin, Libya and Niger (Arah et al.,2015).

Tomato is grown under rain fed conditions and irrigation by small scale farmers in Kenya. It is grown mainly in Kirinyaga County (Mwea area), Meru Central (Mitunguu area), Isiolo, Nyeri, Nakuru and Taita-Taveta counties with a total production of 410,033 tonnes. Tomato constitutes 7% of horticultural produce and 14% of vegetable produce in Kenya. Tomatoes are rich sources of minerals, vitamins, essential amino acids, sugars and dietary fibres, calories, phosphorus, calcium (Dam et al., 2005; 'unpublished data' Kithinji, G.V.). According to the USDA National Nutrient Database (2010), tomatoes are composed of 1.2 mg calcium, 4.7 g carbohydrate, 0.073 mg copper, 1.5 g dietary fiber, 0.2 g fat, 0.33 mg iron, 1.4 mg magnesium, 0.731 mg niacin, 0.109 mg pantothenic acid, 3 mg phosphorus, 292 mg potassium, 1.0 g protein, 0.046 g thiamine, 3.23 g total sugars and 16.9 mg vitamin C. There is high rate of tomato consumption due to its nutritional value resulting in the generation of large quantities of wastes (Ochilo et al., 2019).

Difficulties in separating rotten tomatoes from waste mass, their high water content and easily degradability call for sustainable ways to manage them. One of the practical and convenient ways of tomato waste management is through bioethanol production which will cause reduction in waste volume in addition to fuel generation (Nwosu-

Obieogu et al., 2016). Bioethanol is a universal organic solvent produced mainly in Brazil and USA from sugarcane and corn respectively. There has been increased rate of bioethanol production from 24.8 million tonnes in 2001 to 74 billion tonnes in 2009 and 85 billion tonnes in 2011 (Khraisheh and Li, 2010; 'unpublished data' Oleskowiczn-Popiel, P, Thomsen, A.B and Schmidt, J.E.; Saini et al., 2015).

Bioethanol production from feed stock rich in sucrose and starch is first generation bioethanol, feedstock rich in lignocellulose and algal biomass are second generation and third generation bioethanol respectively (Azhar et al., 2017). Lignocellulosic biomass is the biodegradable portion of organic products, waste and residues obtained from agriculture, forestry and industries (Muktham et al., 2016). Raw materials involved in first generation bioethanol production are inadequate for meeting higher fuel demands leading to deforestation to obtain enough farmland (Saini et al., 2015). Ethanol production from wastes will enhance the effective use of agricultural land and help solve issues of food insecurity especially in developing countries (Braide et al., 2016). The study reports the production of bioethanol from rotten tomatoes under different incubation conditions using baker's yeast for fermentation.

2. Methodology

2.1 Raw Materials

Rotten tomatoes were collected from tomato sellers in Nairobi market. Cellulose and *Saccharomyces cerevisiae* (baker's yeast) were purchased from (Sigma Aldrich, Kobian Scientific Limited, Nairobi, Kenya) and Nairobi market respectively. All chemicals used were analytical grade and procured from Chemistry and Biochemistry departments in Kenyatta University.

2.2 Separate Hydrolysis and Fermentation Process

SHF was carried out anaerobically using pure culture of *Saccharomyces cerevisiae* for fermentation and commercial cellulose from *Aspergillus niger* for enzymatic hydrolysis. 50 grams of blended rotten tomatoes was dissolved in 250 ml distilled water and pH was adjusted to 4.5. The solution was subjected to autoclaving at 120^{o}C for 15 minutes. Cellulose solution was inoculated into the medium under room temperature conditions and incubated using an incubating shaker at 150 rpm and 30^{o}C for 48 hours. Media were centrifuged at 5,000 rpm for 30 minutes after incubation to obtain tomato hydrolysates. The percentage total sugar was determined using DNS method by Miller.

Tomato hydrolysates were sterilized at 120^{o}C for 15 minutes before yeast inoculation. Approximately 10^{7} cells/ml *Saccharomyces cerevisiae* suspension was used as an inoculum for fermentation. Optimization of fermentation variables was done at different temperatures of (30^{o}C, 25^{o}C, 20^{o}C) and incubation times of (24, 48, 72, 96, 120 and 144 hours). Centrifugation was carried out after each time of fermentation at 4000 rpm for 15 minutes.

2.2.1 Preparation of Yeast and Enzyme Inoculum

Enzyme inoculum was prepared by dissolving 0.05 M citrate buffer solution with pH 4.8 in 2 g commercial cellulase from *Aspergillus niger* for 30 minutes.

Inoculum of *Saccharomyces cerevisiae* was cultured in sterilized YPD broth (containing 20 g/l dextrose, 4 g/l yeast extract and 3 g/l peptone) with adjusted pH of 6.5. Media was kept in an incubating shaker at 30^{o}C and agitation rate of 150 rpm for 48 hours.

2.3 Analytical Method

2.3.1 Determination of Yeast cells numbers

Number of viable yeast cells was determined by haemocytometer together with microscope and 0.4 % trypan blue solution. 0.4% trypan blue solution was prepared using 4 g/l trypan blue, 8 g/l NaCl, 11.2 g/l KCl, 1.44 g/l Na_2HPO_4 and 0.24 g/l of KH_2PO_4. 50 ul trypan blue solution was mixed with 50 ul yeast culture media out of which 5 ul was viewed on a haemocytometer under a microscope and non-stained cells were counted. The number of viable yeast cells in yeast inoculum was determined by the formula:

Cells/ml=average number of cells per corner square ×dilution factor ×10^{4}

2.3.2 Determination of Residual sugar concentration

The dinitrosalicylic acid (DNS) method of Miller was used to estimate the percentage total sugar and residual sugar after fermentation. 0.5 ml of DNS reagent was added to 0.5 ml of sample solution to be analysed and mixture was incubated in a boiling water bath for 10 minutes. 5 ml of distilled water was added afterwards and allowed to cool at room temperature. Optical density of samples was recorded at 575 nm using UV-vis spectrophotometer. The percentage of residual sugars present after every 24 hours fermentation time was extrapolated from standard glucose curve.

2.3.3 Determination of Bioethanol concentration

Analysis of bioethanol was carried out using potassium dichromate, sulphuric acid and UV-vis spectrophotometer at 575 nm. Prepared standards were used to estimate the concentration of bioethanol. Standard curve was drawn from known standard concentrations and their corresponding absorbance values. Bioethanol concentration of rotten tomato hydrolysate was determined from the standard curve and expressed as percentage volume per volume (%v/v).

2.4 Statistical analysis

Experiment was carried out in triplicates and data was analyzed by one way analysis of variance using Genstat statistical package (Discovery version 4).

3. Result and Discussion

3.1 Effects of different fermentation times on ethanol production

The percentage total sugar present in the rotten tomato hydrolysates was determined to be 0.4%. Table 1

shows the concentration of bioethanol and the percentage residual sugar present after each fermentation time.

The low residual sugars at the end of each fermentation time show the suitability of *Saccharomyces cerevisiae* (baker's yeast) in the production of bioethanol from rotten tomatoes. Ethanol concentration decreased with increase in fermentation times due to the accumulation of toxic metabolic by-products leading to the decline of yeast cell biomass (Zain et al., 2012). *Saccharomyces cerevisiae* also assimilated the ethanol produced as a source of energy due to reduction in hydrolysate sugar level as incubation time increased. According to (Mayzuhroh et al. 2016), *Saccharomyces cerevisiae* easily forms organic acids with longer fermentation times which interact with alcohol accumulated in samples to form ester compounds and hence decrease ethanol content.

Ali and Kemat (2017) reported decrease in bioethanol with increasing fermentation time in *Moringa oleifera* seeds husk. Shahzad et al. (2019) and Woldesenbet et al. (2016) also reported decreases in ethanol concentration from cotton stalk and raw coffee wet processing wastes respectively.

Table 1. Residual sugar and bioethanol percentages at different fermentation times

Fermentation time (hours)	Residual sugar (%)	Ethanol Concentration (%/v/v)
24	0.05	0.1711
48	0.03	0.1629
72	0.00	0.1452
96	0.00	0.1234
120	0.00	0.1008
144	0.00	0.0982

3.2 Ethanol production at different incubation temperatures

Temperature 30^{o}C was optimum for fermentation using *Saccharomyces cerevisiae*. Tahir et al. (2010) also found 30^{o}C to be the optimum temperature for ethanol fermentation by Saccharomyces cerevisiae. Mutreja et al. (2011); Hosny et al. (2016); Hossain et al. (2015) and Benjamin et al. (2014) all reported maximum ethanol production at temperature 30^{o}C using agricultural wastes.

Temperature 20^{o}C was low for effective bioethanol fermentation by *Saccharomyces cerevisiae*. According to Gibson et al. (2007), low temperature conditions cause reduction in membrane fluidity of yeast cells leading to stresses in yeast growth.

Table 1. Residual sugar and ethanol percentages present at different temperatures

Temperature	Residual sugar (%)	Ethanol Concentration (%/v/v)
20	0.05	0.1004
25	0.08	0.1325
30	0.08	0.1627

4. Conclusion

Rotten tomatoes were feasible for bioethanol production using *Saccharomyces cerevisiae* and hence should be utilized in energy production instead of allowing them to pollute our environment. Baker's yeast was very efficient in ethanol fermentation and should be widely employed due to its economical nature.

Acknowledgement

The research was fully funded by Intra Africa Mobility Program under European Union.

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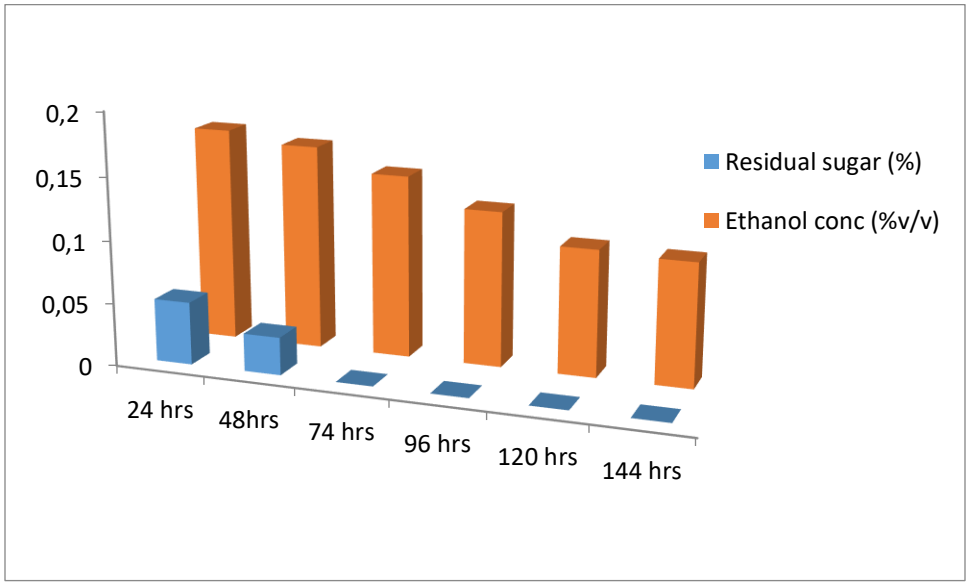

Figure 1. Effects of different incubation times on the amount of residual sugars and ethanol

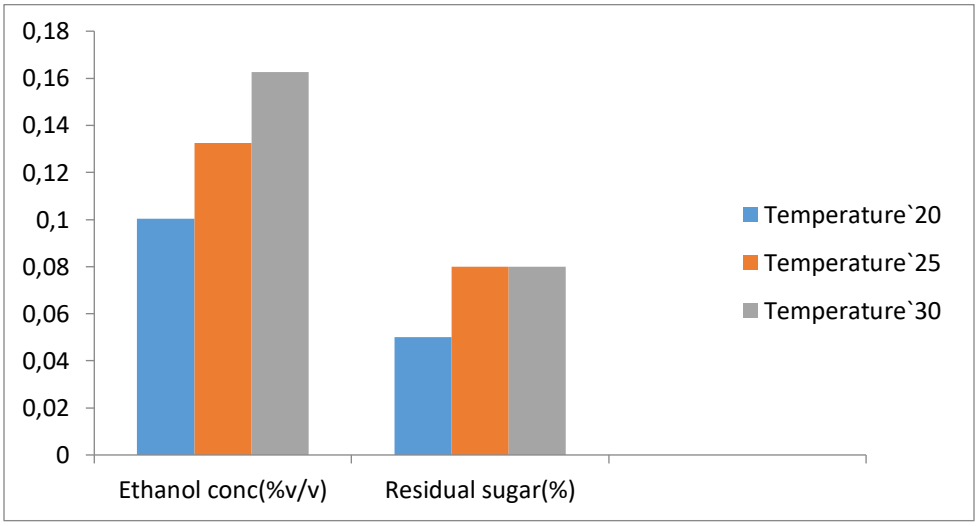

Figure 2. Effects of different temperatures on percentage ethanol and residual sugars