https://dx.doi.org/10.4314/dujopas.v8i4b.2
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 8 No. 4b December 2022*

Effect of pH and Temperature on Bioethanol Production: Evidences from the Fermentation of Sugarcane Molasses using *Saccharomyces cerevisiae*

***Salihu UY[1], Usman UG[2], Abubakar AY[3], Mansir G[4].**

[1]Department of Microbiology and Biotechnology,
Federal University Dutse,
Jigawa, Nigeria.

[2]Department of Chemistry,
Al-Istiqama University Sumaila,
Kano, Nigeria.

[3]Department of Science Laboratory Technology,
Jigawa State Polytechnic,
Nigeria

[4]Department of Chemistry,
Bayero University Kano,
Nigeria

Email: umaryusuf712@gmail.com

Abstract

Bioethanol is increasingly seen as a competitive alternative to gasoline due to rising concerns about the greenhouse effect, the price of crude oil, and other issues. Due to improvements in the agricultural sector, millions of tons of trash and byproducts are produced annually, with the potential to be used as inexpensive energy sources and raw materials for energy production. In the sectors that produce sugar, sugarcane molasses is widely available as a feedstock for the commercial generation of low-cost bioethanol. In order to examine the impact of temperature and pH on the output of bioethanol, this study fermented sugarcane molasses to make bioethanol. In the experiment, fermentation was accomplished using the fungus Saccharomyces cerevisiae (yeast). Following fermentation, ethanol was extracted using distillation at a temperature of 78^{0}C. The impact of pH on the ethanol yield was studied using five samples at five different pH levels (3.0, 3.5, 4.0, 4.5, and 5.0) at a temperature of 35^{0}C. Five more samples were utilized to investigate the impact of temperature on the production of ethanol; the pH was maintained at 4.0 while the temperature was changed (25, 30, 35, 40, and 45^{0}C). The percentage of ethanol yield was observed to grow with increasing pH and temperature until the optimum conditions were attained, at which point it began to decline. The study also found that the best conditions for Saccharomyces cerevisiae to make ethanol were pH 4.5 and temperature 35^{0}C, yielding the highest amount of ethanol (81%).

Keywords: Bioethanol, Molasses, Fermentation, Optimization, *Saccharomyces cerevisiae*.

**Author for Correspondence*

INTRODUCTION

The impending depletion of non-renewable natural resources makes it necessary to conduct research to find new renewable natural resources as well as to increase productivity and reduce the costs of currently used procedures (De Vriesa *et al*., 2007). Biomass-based energy sources are a significant alternative that can help with this issue (Saxena *et al*., 2009). By fermenting organic substrates, such as sugarcane molasses (Cazetta *et al*., 2007), wheat stillage (Davis *et al*., 2005), fish waste (Ruanglek *et al*., 2006), and pineapple waste (Tanaka *et al*., 1999), among others, carbon sources can be transformed into ethanol, a significant and well-known biofuel (Soccol *et al*., 2005). This significantly contributes to the resolution of an environmental issue linked to residue treatment and disposal.

Since the inception of the Brazilian alcohol program, a substantial amount of bioethanol has been used as a biofuel (Goldemberg, 2008). Ethanol, often known as ethyl alcohol or grain alcohol, is a flammable, colorless, mildly poisonous chemical molecule with a unique perfume-like odor found in alcoholic beverages. In popular parlance, it is typically referred to simply as alcohol (Rao *et al*., 2004). Over the past few decades, natural energy sources like coal and oil have been used at tremendous rates. Due to their negative effects on the environment (and the resulting pressure from society) and the possibility that they could run out in the future, the modern economy's strong reliance on fossil fuels must come to an end. As a result, alternate resources such as ethanol are becoming more essential. One of the most significant renewable fuels for reducing the harm to the environment caused by the use of fossil fuels worldwide is bio-ethanol (Cardona and Sánchez, 2007). Hoefnagels *et al*. (2010) analyzed and examined the assumptions and methodological choices used to estimate the greenhouse gas emissions from the life cycle of biofuels. The hydroxyl group and the short carbon chain in ethanol are what give it most of its characteristics. The hydroxyl group in ethanol can participate in hydrogen bonding, making it more viscous and less volatile than less polar organic molecules of comparable molecular weight. With a refractive index of 1.36242 (at =589.3 nm and 18.350 C), ethanol is slightly more refractive than water (Lide, 2000).

Molasses, a byproduct of sugar processing, is widely produced in many countries. Sucrose is lost in sugarcane molasses, reducing factory profit; thus, converting molasses to ethanol is a viable option for maximizing molasses utilization. Ethanol is widely used as a fuel additive in automobiles (Perry's, 1987). In 2010, the United States became the world's largest ethanol producer, producing 49.2 billion liters of ethanol fuel (Lichts, 2011).

For ethanol fermentation, yeasts are the most commonly used microorganisms. The main byproducts of Saccharomyces cerevisiae's anaerobic fermentation are carbon dioxide, glycerol, and cell biomass in addition to ethanol. Although carbon dioxide is an unavoidable byproduct of fermentation, the off-gas can be sold as a high-quality raw material. Osmotic stress can cause the production of glycerol as a compatible solute (Brandberg *et al*., 2007). Saccharomyces cerevisiae, a fermentative yeast, is widely used in the production of ethanol using renewable biomass as the primary carbon source, such as sugar cane, sugar beet, and molasses (Laluce, 1991). This is because the said strain exhibits typical values for fermentation parameters, such as the ability to ferment in both low sugar (5% of sugar) and high sugar (30% of sugar) (Nishida *et al*., 2004). Sugar-cane blackstrap molasses is a particularly suitable raw material for this purpose, as it is both inexpensive and abundant in the sugar business. A batch, fed-batch, or continuous manner of ethanol fermentation can be used (Vitolo, 1996). Fermentation is the primary method of producing ethanol used in alcoholic beverages and the vast majority of ethanol used in fuel. In the absence of oxygen, certain yeast species, most notably Saccharomyces cerevisiae, metabolize sugar to create ethanol and carbon dioxide

(Morias *et al*., 2007). The chemical transformation of sugar into ethanol and carbon dioxide is depicted in the following equation: $C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$ (Morias *et al*., 2007). Although there have been numerous reviews of the literature for the production of bio-ethanol from various sources over the past few decades (Beatriz Palmarola *et al*., 2005; Dale, 1987; Ferrari *et al*., 1992; Martin *et al*., 2006; Nigam, 1999; Olsson & Hahn-Hagerdal, 1996), only a small number of authors (De Vasconcelos *et al*., 1998; Doelle and Greenfield, 1985; Huertaz-Daz *et al*., 1991) have studied the kinetics of ethanol production from sugar cane using yeast cells (*Saccharomyces cerevisiae*). Hence, in the current study, an effort has been made to maximize the factors (temperature and pH) that influence the generation of bio-ethanol from sugar molasses.

MATERIALS AND METHODS

Sample Collection

The sample (sugarcane molasses) was obtained at Malam Alu Agro Allied Company Limited, which is situated at Maiduguri Road in the Birnin Kudu local government area of Jigawa State, Nigeria and was then placed in a clean bottle and kept at room temperature (20 to 25^{0}C) before being used. The strain of baker's yeast (Saccharomyces cerevisiae) used was obtained from Sahad Store Dutse.

Mash Preparation and Fermentation

Using a weighing scale, 75 grams of sugarcane molasses were measured out and put into a clean one-liter beaker (1000 ml). A 500 ml measuring cylinder was used to measure 250ml of distilled water, which was then placed into the beaker containing the molasses sample. Using a clean glass rod and an electric magnetic stirrer, the mixture was thoroughly stirred for two minutes. The pH of the medium was adjusted to the required level. The contents of the beaker were then transferred to a 500ml Erlenmeyer flask. 3.0 gm. of baker's yeast (Saccharomyces cerevisiae) was weighed and introduced to the flask. The cap of the Erlenmeyer flask was secured with clean cotton, followed by aluminum foil. The flask mixture was fermented for 72 hours in an incubator at the specified temperature. The same approach was used to optimize factors like pH and temperature (Gasmalla *et al*., 2012).

Optimization of pH

Five (5) Erlenmeyer flasks of samples were prepared, fermented, and utilized to investigate the effect of pH on the amount of bioethanol produced. The pH of the flasks was adjusted to range between 3.0, 3.5, 4.0, 4.5, and 5.0 while the temperature was maintained at 35^{0}C (Wong and Sanggari, 2014).

Optimization of Temperature

To investigate how temperature affects how much bioethanol is produced, five (5) Erlenmeyer flasks of samples were prepared and fermented. The pH was kept constant at 4.0 while the temperature was varied between 25^{0}C, 30^{0}C, 35^{0}C, 40^{0}C, and 45^{0}C (Wong and Sanggari, 2014).

Distillation of Ethanol

The samples were filtered to get rid of any remaining yeast after 72 hours of fermentation. To obtain the ethanol, the filtered fermented sample was then heated to 78^{0}C in a rotary evaporator (Gasmalla *et al*., 2012).

Identification of Bioethanol

A small amount of potassium dichromate ($K_2Cr_2O_7$) and a few drops of concentrated H_2SO_4 were added to about 2 mL of the distilled samples. The sample's color changes from orange to green, indicating the presence of bioethanol (Periyasamy *et al*., 2009).

Flammability Test

The bioethanol produced was tested to see if it was flammable or not. It was discovered to be flammable after igniting a spirit lamp with about 10ml of the distilled sample (Edjekouane *et al*., 2020).

Calculating the Percentage of Ethanol Yield

The following formula was used to calculate the percentage of ethanol yield:

$$\textbf{\textit{Percentage yield}}\ (\%) = \frac{\textbf{\textit{Volume of ethanol recovered}}}{\textbf{\textit{Total volume of the sample}}}\ x\ 100$$

The **volume of ethanol recovered** represents the amount (ml) of ethanol retrieved after distillation, while the total **volume of the sample** indicates the amount of distilled water and molasses used for fermentation, which is 310 ml.

RESULTS AND DISCUSSION

Results of pH Optimization

Figure 1 below illustrate how pH affects ethanol production yield.

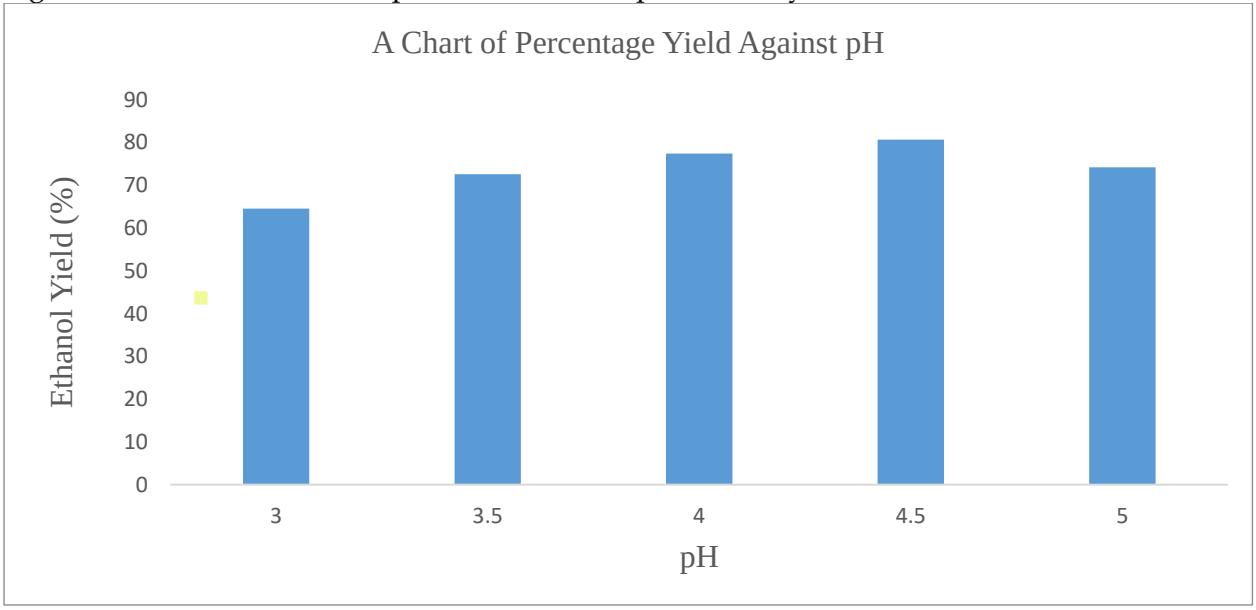

Figure 1 A graph depicting how pH affects the amount of ethanol produced.

Results of Temperature Optimization

A graph indicating how temperature influences ethanol yield is shown in figure 2 below.

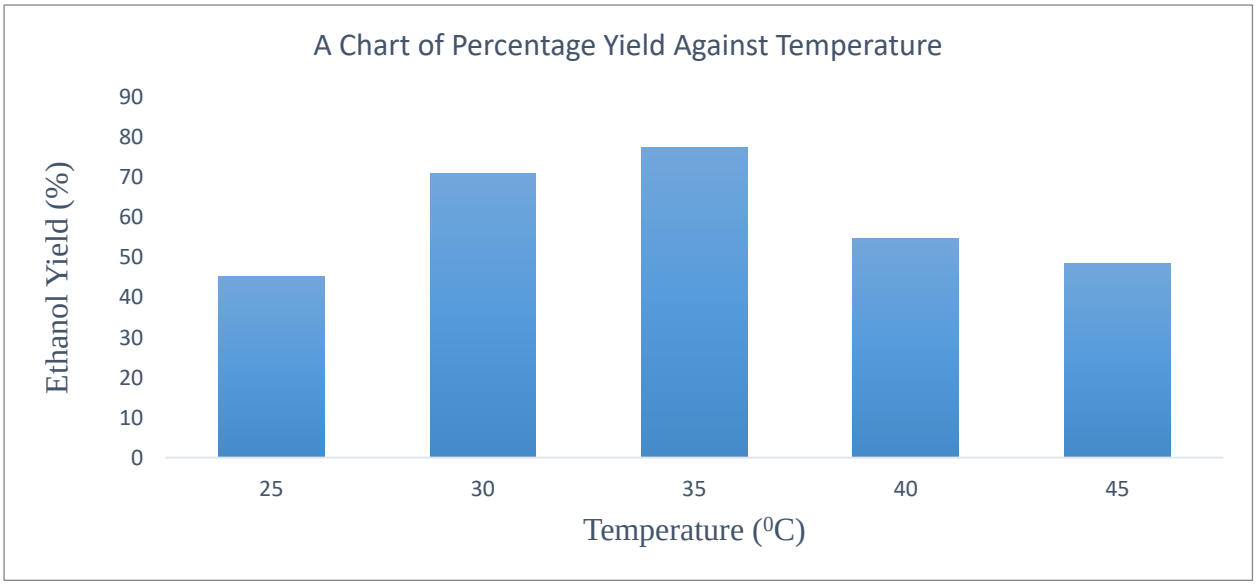

Figure 2 A graph depicting how temperature affects the amount of ethanol produced.

Discussion

Optimization of pH

The maximal ethanol output at pH 4.5 represents enzyme function in an environment, but the lower ethanol yields at pH 3.0, 3.5, 4.0, and 5.0 represent less yeast activity. In general, yeast is an acidophilic organism that thrives in acidic environments. The optimal pH range for yeast growth can range from 3.0 to 6.0, depending on temperature, oxygen availability, and the strain used. Plasma membrane-bound proteins, such as enzymes and transport proteins, require optimal pH conditions to function (Narendranath and Power, 2005). It is critical for the yeast to maintain a consistent intercellular pH during growth. Many enzymes are active within yeast cells during growth and metabolism. Each enzyme performs optimally at its optimum pH, which is acidic due to the acidophilic nature of yeast. When the pH of the extracellular enzymes deviates from the appropriate level, the yeast cell must use energy to either pump in or pump out hydrogen ions to preserve the optimal intercellular pH (Narendranath and Thomas, 2001).

If the extracellular pH deviates too far from the optimal pH range, the cell may find it difficult to maintain a steady intracellular pH and the enzyme may fail to function normally. Furthermore, if the enzymes get deactivated, the yeast cell will be unable to proliferate and produce ethanol efficiently (Narendranath and Power, 2005).

However, at pH 3.0, this study found the lowest ethanol output. This could be owing to the yeast strain's inability to handle pH 3.0. The pH range at which yeast strains activate and generate ethanol varies. There are other possibilities; the yeast used in the experiment could be old. In comparison to new yeast, old yeast will not carry out the fermentation process efficiently. According to Misono and Yamaguchi 1990, at pH 5.0, the rate of ethanol production increases. This assertion does not apply to this study because pH 5.0 does not produce the best results.

Optimization of temperature

The best yield was found at 35^0C, out of the five temperature ranges used. A proper temperature is required for the yeast to react throughout the fermentation process (Rivera and

Cardona, 2006). Yeast is killed by excessive heat, whereas yeast activity is slowed by low temperatures. As a result, maintaining a precise temperature range is essential.

However, at 45^0C, the ethanol production declined. This suggests that the optimal temperature for ethanol production is 35^0C. This finding is consistent with previous research on the effect of temperature on ethanol production (Pramanik, 2003; Redzepovic *et al*., 2002; Roehr, 2001). This study's findings also contradict the findings of Fakruddin *et al*. (2012), who discovered that the optimum temperature for ethanol production is 30 degrees Celsius. We can assume from this that the higher the temperature, the lower the ethanol amount. The rate of an enzyme-catalyzed reaction increases with temperature until a particular point is reached, at which point the enzymes begin to denature. Higher temperatures hinder cell development and drastically reduce fermentation. In this investigation, ethanol production decreased significantly at 40^0C, indicating that higher temperatures impede cell growth.
Enzymes are sensitive to temperature changes. When the temperature rises above 40^0C, the rate of respiration slows and declines. At a high temperature, the yeast enzyme releases energy and gets denatured, thereby producing less ethanol. The optimum temperature for yeast enzyme activity is around 35^0C; below this temperature, the rate of reaction is slow, and above 45^0C, the yeast enzyme denatures.

Less ethanol production at low temperatures may be owing to enzymes' reduced tolerance for producing ethanol at low temperatures (Gao and Fleet, 1988; Torija *et al*., 2003). Furthermore, at low temperatures, the enzyme deactivates and the reaction slows or ceases completely (Togarepi *et al*., 2012). The molecules travel slower at lower temperatures than at higher temperatures. These explanations explain why the enzyme might not have enough energy to cause a chemical reaction. Therefore, it is evident that 350 °C was the optimum temperature for ethanol production.

CONCLUSION

The results of this study demonstrated that sugarcane molasses is a good source of bioethanol, as shown by the high yield obtained, and that temperature and pH have an effect on the amount of bioethanol produced during the fermentation process. Following this research, it was found that, the amount of ethanol produced increases from pH 3.0 to 4.5 and declines when the pH hits 5.0. This indicates that pH 4.5 is the ideal pH for yeast (Saccharomyces cerevisiae) to make bioethanol, as seen by the highest percentage yield (81%).
The study also found that at 35^0C, ethanol yield was at its peak (77%). This study concluded that the optimum temperature and pH for producing bioethanol from sugarcane molasses were 35^0C and 4.5, respectively.

REFERENCES

Beatriz Palmarola-Adrados, Mats galbe, and Guido Zacchi. (2005). Pretreatment of barley husk for bio-ethanol production. J. of Chemical Technology and Biotechnology, 80, 85-91.

Brandberg, T., Gustafsson, A., and Franzén, C.J. (2007). The impact of severe nitrogen limitation and microaerobic conditions on extended continuous cultivations of *Saccharomyces cerevisiae* with cell recirculation. *Enzym Microb Technol*. **40**: 585-593.

Cardona, C.A., and Sánchez, O.J. (2007). Fuel ethanol production: process design trends and integration opportunities. *Bioresour Technol*. **98**: 2415-2457.

Cazetta, M.L., Celligoi, M.C., Buzato, J.B., and Scarmino, I.S. (2007). Fermentation of molasses by *Zymomonas mobilis*: effects of temperature and sugar concentration on ethanol production. *Bioresour Technol*. **98**(15):2824-8.

Dale BE, (1987). Lignocellulose conversion and the future of fermentation biotechnology. TIBTECH, 5, 287-291.

Davis, L. Jeon, Y.J., Svenson, C., Rogers, P., Pearce, J., and Peiris, P., (2005). Evaluation of wheat stillage for ethanol production by recombinant *Zymomonas mobilis*. *Biomass Bioenerg.* **29**(1):49-59.

De Vasconcelos, J.N., Lopes, C.E., and de França, F.P. (1998). Yeast immobilization on cane stalks for fermentation. International Sugar J, 100(1190), 73-75.

De Vriesa, B.J.M., Van Vuurenb, D.P., and Hoogwijk, M.M. (2007). Renewable energy sources: their global potential for the first-half of the 21st century at a global level: an integrated approach. *Energ Policy*. **35**(4):2590-610.

Doelle, H.W., and Greenfield, P.F. (1985). The production of ethanol from sucrose using zymomonas mobilis. Appl. Microbial. Biotecnol, 22, 405-410.

Edjekouane M, Lansari F, Khelifi O, Boukheteche I, Laksaci H. Production of Bioethanol from a Local Natural Resource. Algerian Journal of Renewable Energy and Sustainable Development, 2020, 2(1), 56-59. https://doi.org/10.46657/ajresd.2020.2.1.8

Fakruddin, M.D., Abdul-Quayum, M.D., Ahmed, M.M., and Choudhury, N. 2012. Analysis of Key Factors Affecting Ethanol Production by *Saccharomyces cerevisiae* IFST-072011. *Biotechnology, **11**: 248-252.*

Ferrari, M.D., Neirotti, E., Albornoz, C., and Saucedo, E. (1992). Ethanol production from eucalyptus wood hemicellulose hydrolysis by Pichia stipitis. Biotechnol. Bioeng, 40, 753-759.

Gao, C., and Fleet, G. H. (1988). *Journal of Applied Bacteriology*. **65**:405– 410.

Gasmalla, M. A. A., Yang, R., Nikoo, M., and Man, S. (2017). Production of Ethanol from Sudanese Sugar Cane Molasses and Evaluation of Its Quality. *Journal of Food Processing &Amp; Technology*, *03*(07). https://doi.org/10.4172/2157-7110.1000163.

Goldemberg, J. (2008). In proceeding of the conference on ecological dimensions of Biofuels.

Hoefnagels, R., Smeets, E., and Faaij, A. (2010). Greenhouse gas footprints of different biofuel production systems. *Renew Sustain Energ Rev*. **14**:1661-1694.

Huertaz-Díaz, H., Cacho, C.L., and Bernard, L. (1991). Fermentation of sugarcane juice and blackstrap molasses by zymomonas mobilis. J. Agric. Univ.P.R, 75(1), 43-50.

Laluce, C. (1991) Current aspects of fuel ethanol production in Brazil. *Critic Rev Biotechnol* **11**: 149-161.

Lichts, F.O. Industry Statistics: 2010 World fuel Ethanol Production, *Renewable Fuels Association*. Retrieved **2011**-04-30.

Lide, D.R. (2000). *CRC handbook of chemistry and physics. 8th ed* CRC press.

Martin, C., Lopez, Y., Plasencia, Y., and Hernandez. (2006). Characterization of agricultural and agro-industrial residues as raw materials for ethanol production. Chem.Biochem.Eng, 20 (4), 443-447.

Misono, H. M., and Yamaguchi, Y. (1990). *Journal of Fermentation Technology.* **8**:210-218.

Morias, P.B., Rosa, C.A., Linardi, V.R., Carazza, F., Nonato E.A. (2007). Production of fuel alcohol by *Saccharomyces starins* from tropical habitats. *Biotechnol Lett* **18**: 1351-1356.

Narendranath, N.V., and Power, R. (2005). *Applied and Environmental Microbiology*. **71**: 2239-2243.

Narendranath, N.V., and Thomas, K.C. (2001). Ingledew, W. M.;*Journal of the American Society of Brewing Chemists*. **59**:187-194.

Nigam, J.N. (1999). Continuous ethanol production from pineapple cannery waste. J. of Biotechnol, 72, 197-202.

Nishida, O., Kuwazaki, S., Suzuki, C., Shima, J. (2004). Superior molasses assimilation, stress tolerance, and trehalose accumulation of baker's yeast isolated from dried sweet potatoes (hoshi-imo). *Biosci Biotechnol Biochem*. **68**:1442-1448.

Olsson, L., and Hahn-Hägerdal, B. (1996). Fermentation of lignocellulosic hydrolysis's for ethanol production. Enzyme Microb. Technol, 18, 312-331.

Periyasamy, S., Venkatachalam, S., Ramasamy, S., and Srinivasan, V. (2009). Production of Bio-ethanol from Sugar Molasses Using Saccharomyces Cerevisiae. *Modern Applied Science*, *3*(8). https://doi.org/10.5539/mas.v3n8p32.

Perry's, R.H. (1987). *Chemical Engineers' Handbook, (6thEdn), International student edition, McGraw-Hill Chemical Engineering Services.*

Pramanik, K. (2003). *Institution Chemistry Engineers*. **34**:487-492.

Rao, R.S., Prakasham, R.S., Prasad, K.K., Rajesham, S., and Sarma, P.N. (2004). Xylitol production by *Candida* sp. Parameter optimization using Taguchi approach. *Process Biochem.* **39**: 951-956.

Redzepovic, S., Orlic, S., Sikora, S., Majdak, A., and Pretorius, I. S. (2002). *Whiley Inter-science Journals*. **350**.

Rivera, M., and Cardona, C. A. (2006). *Ingenier1´a y Competitividad*. **6**:17–25.

Roehr, M. (2001). *The Biotechnology of Ethanol: Classical and Future Applications. Chichester: Wiley-VCH.* **232**.

Ruanglek, V., Maneewatthana, D., and Tripetchkul, S. (2006). Evaluation of thai agro-industrial wastes for bioethanol production by *Zymomonas mobilis*. *Process Biochem*. **41**(6):1432-7.

Saxena, R.C., Adhikari, D.K., and Goyal, H.B. (2009). Biomass-based energy fuel through biochemical routes: a review. *Renew Sust Energ Rev*. **13**(1):167-78.

Soccol, C.R., Vandenberghe, L.P.S., Costa, B., Woiciechowski, A.L., Carvalho, J.C., and Medeiros, A.B.P. (2005). Brazilian biofuel program: an overview. *J Sci Ind Res India*. **64**(11):897-904.

Tanaka, K., Hilary, Z.D., and Ishizaki, A. (1999). Investigation of the utility of pineapple juice and pineapple waste material as low-cost substrate for ethanol fermentation by *Zymomonas mobilis*. *J Biosci Bioeng*. **87**(5):642-6.'

Togarepi, E., Mapiye, C., Muchanyereyi, N., and Dzomba, P. (2012). *International Journal of Biochemistry Research & Review*. **2**(2):60-69.

Torija, M.J., Roze's, N., Poblet, M., Guillamo´n, J.M., and Mas, A. (2003). *Antonie van Leeuwenhoek*. **79:**345– 352.

Vitolo, M. (1996). Production of ethanol and invertase by *S. cerevisiae* grown in blackstrap molasses.

Wong, Y. C., and Sanggari, V. (2014). Bioethanol Production from Sugarcane Bagasse using Fermentation Process. *Oriental Journal of Chemistry*, *30*(2), 507–513. https://doi.org/10.13005/ojc/300214.