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# Effects of Enzymes on Bioethanol Generation in A Bioreactor System

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**Abstract - The production of bioethanol is an important process due to its wide applications in renewable energy. It also plays a significant role in waste management, as most agricultural by-products, including kitchen waste, can be converted into useful bioethanol. This process involves the use of enzymes for ethanol production, and enzyme activity can influence the yield and quality of the ethanol produced. Therefore, this study investigated the effect of different enzymes on bioethanol generation. The enzymes used were alpha-amylase, cellulase, and *S. cerevisiae* (baker's yeast). The slurry produced was transferred into a 1000 mL beaker, and the pH was measured and adjusted to 5.0. Alpha-amylase and cellulase were added simultaneously to facilitate hydrolysis, after which *S. cerevisiae* was introduced for fermentation. Ethanol production was carried out in a prefabricated bioreactor. The enzyme concentrations ranged from 0.1 g/L to 0.7 g/L, while pH, temperature, and substrate concentration were kept constant to evaluate the effect of enzyme concentration on bioethanol yield. The results showed that a continuous increase in enzyme concentration under optimal conditions led to a corresponding increase in bioethanol yield. The highest yield (80.4%) was obtained at the maximum enzyme concentration of 0.7 g/L. Additionally, the highest bioethanol concentration (15.4 g/L) was achieved at 35°C and a pH of 5.0.**

**Keywords: Bioethanol, Bioreactor, Kitchen waste, Enzyme,**

## 1 INTRODUCTION

First-generation (1G) ethanol production uses feedstock like corn and sugarcane juice, but second-generation (2G) ethanol production uses lignocellulosic biomass (Rastogi and Shrivastava 2017; Junqueira *et al.*, 2017 & Edenseting *et al.,* 2022). Also, 2G ethanol faces substantial technological and financial obstacles, whereas 1G ethanol is currently widely commercialized (Siqueira et al. 2020). In this regard, enzymes are essential to the manufacture of ethanol because they function as biocatalysts, increasing the rate at which biomass is converted into fermentable sugars and then into ethanol (Sales et al., 2025).

The agro-domestic, chemical, and pharmaceutical industries frequently use enzymes which are naturally produced by microorganisms like bacteria, fungi, and yeasts to catalyse particular steps in a variety of biotechnology processes (Raveendran *et al.,* 2018, Farhan et al., 2025, Brundiek, H.; Höhne 2016 & Meghwanshi *et al.,* 2020).

Enzyme catalytic properties are tremendously important to the production of paper and cardboard from plant lignocellulose (Arsalan et al., 2025), production of fermented beverages like wine, cheese, enzyme-modified milk, etc. (Fernandes 2010 & Edenseting *et al.,* 2020), refine or structure oils and derivatives, make detergents, and more (Bourlieu, et al., 2020). For technological, environmental, and financial reasons, the industrial demand for process-adapted enzymes has significantly increased (Prasoulas et al., 2020). Enzymes also function as biocatalysts, promoting sustainable biomass transformation processes in environments with minimal environmental impact (low temperatures and the absence of harsh chemicals or solvents),

In order to develop better biocatalysts, there is already a constant effort to learn more about the structure-function relationships of enzymes (Rocha et al., 2022). However, recently, there has been a growing interest in using enzymes as analytical tools to better understand the structure and composition of bioproducts (Prasoulas et al., 2020). Similar to how antibodies recognize their epitopes, using an enzyme as a probe (EP) takes use of each enzyme's specificity for a particular recognition site on its substrate. The range of uses for Eps has been further expanded by the creation of real-time techniques for tracking enzyme activity in complex systems (Bourlieu *et al.,* 2020).

To design a bioreactor for the saccharification process, it is necessary to regulate specific parameters and variables, particularly temperature, pH, and agitation. Controlling temperature and pH is crucial to maintain optimal conditions for cellulolytic enzyme activity (Van Dyk and Pletschke, 2012; García-Aguirre et al., 2009). Furthermore, proper mixing is essential to ensure effective interaction between cellulases and the substrate, promoting enzyme adsorption and synergism, thereby enhancing the hydrolysis

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efficiency of cellulose (Volynets et al., 2016). In addition, the design of an enzymatic hydrolysis bioreactor must account for the control of mass and heat transfer conditions (Benz, 2011), as well as the assessment of the biological system's shear sensitivity defined as its susceptibility to shear forces under excessive agitation which can result in decreased enzyme activity (García-Aguirre et al., 2009). Therefore, this study investigates the effect of enzymes on bioethanol production within a bioreactor system.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Kitchen Waste

The kitchen waste samples were prepared as stated in Edenseting et al., (2025). Slurry obtained was pretreated using NaOH, transferred to 1000ml beaker then the pH was checked and adjusted to 5. alpha amylase and cellulase enzyme were added for hydrolysis simultaneously then S. cerevisiae baker yeast was added for formation following the method state by Shen *et al.,* (2012), The slurry was stirred for 1 hour for homogeneity then transferred to water bath for saccharification and fermentation. The substrate was allowed to undergo hydrolysis and fermentation for 60 hours. Temperature, pH, and rate of bioethanol production were monitored and recorded. The experiment was repeated at temperatures between 33 and 37 while the pH was monitored between 3.5 and 6. The bioreactor was monitored every ten hours. Distillation was carried out to obtain bioethanol at varying temperatures. Effect of enzyme on the yield of bioethanol in the bioreactor was studied at various enzyme concentration of 0.1g/L to 0.7g/L at constant pH, Temperature and substrate concentration to ascertain their effect on the yield of the produced bioethanol.

The pilot-scale bioreactor, situated at the Chemical engineering reaction laboratory of the University of Uyo, fabricated by Edensetting (2024), it was designed with stainless steel to prevent feedstock contamination (Figure 1). The pilot-scale bioreactor was designed with a working volume of twenty-one liters at the mixer and twenty-two litres in the bioreactor, facilitating a controlled environment for saccharification and fermentation processes.

**Figure 1: Fabricated Bioreactor**

A distillation pilot-unit in the reactor, operating at 78°C, was utilized to recover the produced bioethanol. The entire pilot plant operation was closely monitored and controlled using a Programmable Teliswitch Controller.

## 3. RESULTS

The study of the effect of enzyme on bioethanol generation was carried out and the results are presented in this section. Figure 2 shows the yield of the bioethanol at diferent enzyme concentration and also the rate of convertion of bioethanol with time under different enzyme concentration. It is observed that as the enzyme concentration increases from 0.1g/L to 0.7g/L, there is an increase in the yield of the bioethanol. This is because an increasing enzyme concentration can increase the rate of reaction, as there are more enzyme molecules available to catalyse the reaction, leading to more frequent enzyme-substrate interactions. However, this may rarely occur as the substrate concentration becomes the limiting factor leading to first a plateau in reaction rate and, when all the substrate molecules are consumed, an eventual zero reaction rate as seen in the shape of the curve. Also, the increase in enzyme concentration is observed to reduce the fermentation time as more of the substrates are converted to bioethanol in a shorter period of time. Enzyme concentration directly affects the rate of reaction. More enzyme molecules mean more active sites for substrate binding, which leads to an increase in reaction rate in the presence of enough substrate. However, if the amount of substrate is limited, increasing enzyme concentration beyond a certain point will have little to no effect on enzyme activity, since there will be a surplus of unnecessary enzyme molecules. This is depicted in the plot shown in Figure 2. it can be seen that the yield of bioethanol was highest 80.4% at the highest enzyme concentraion of 0.7g/L. But at 50hrs a slight decrease in yield was observed, this may be due to the depletion of the substrate. This is similar to the report of song et al., (2019)

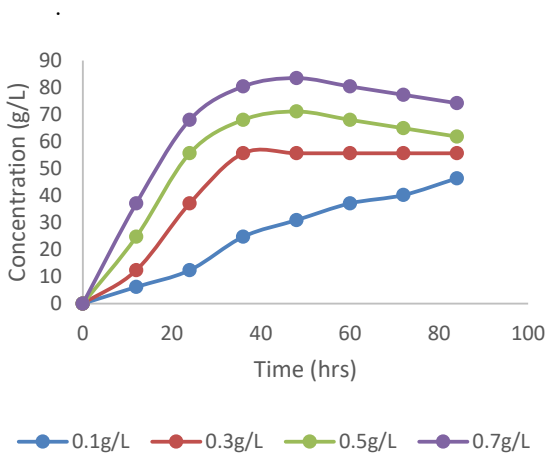


**Figure 2: Plot of bioethanol yield against time at different enzyme concentration.**

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Higher substrate and Batch fermentation were performed with various initial glucose concentrations to produce bioethanol. The initial glucose concentrations used were 20, 40, 80, 160 and 350 g/L tested at 35°C and a pH of 5. Figure 3 and 4, shows the percentage yield at different initial concentration after 72 hours incubation at 35°C. More so, the available data illustrates that higher initial glucose concentration may decrease the percentage conversion of glucose to bioethanol. The maximum yield of bioethanol after 72 hours incubation was observed to be 76.4%, 80.4%, 59.1%, 26.9% and 9.2% for 20, 40, 80, 160 and 320 g/L of glucose respectively. The highest yield being 80.4%.

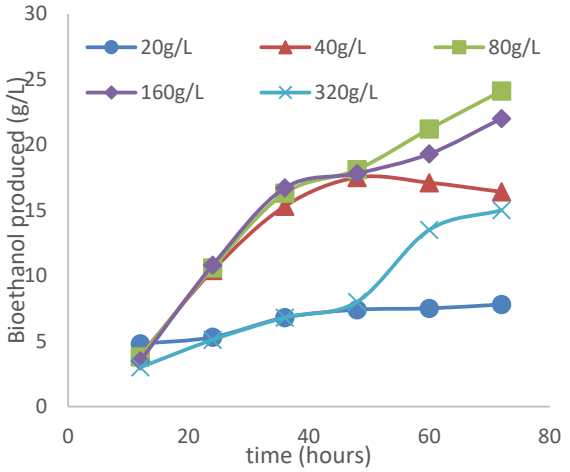


Figure 3: Effect of initial concentration and time on bioethanol production

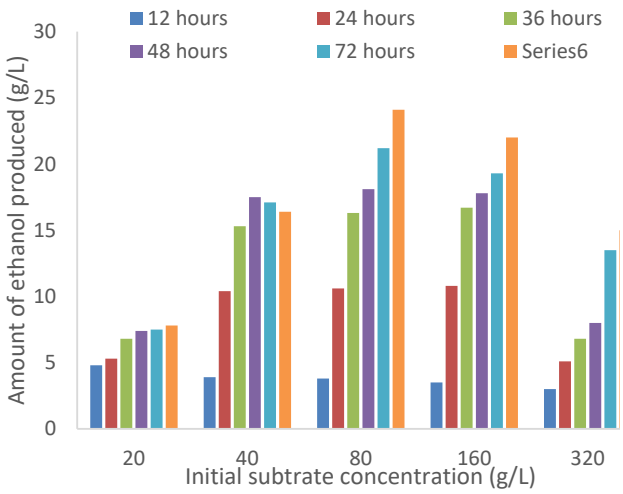


**Figure 4: Bioethanol produced against initial substrate concentration**

Figure 5 shows the effect of temperature on the rate of bioethanol production in a bioreactor system the highest bioethanol concentration of 15.4g/L was obtained at 35ºC, from the result 25ºC yielded about 6g/L of bioethanol. As the temperature increases, the bioethanol production increased. After 35ºC, the produced bioethanol concentration dropped from 15.4g/L as seen in figure 5. Bioethanol production is seen to be effective at 35ºC. When the temperature exceeds 37ºC productivity starts to decrease with toxic effects on yeast causing yeast inhibition (Salihu *et al.,* 2022).

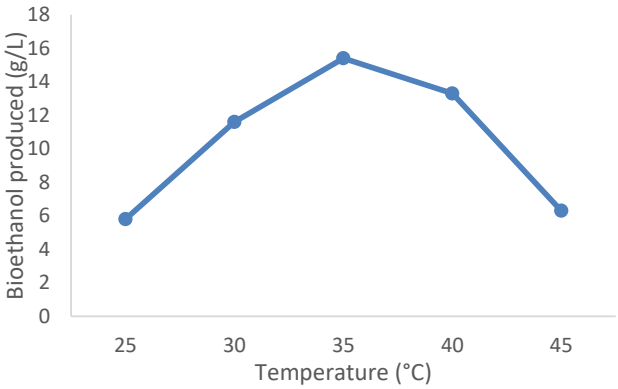


**Figure 5: Effect of Temperature on bioethanol production**

The effect of pH on bioethanol production is reflected in Figure 6. Higher amounts of bioethanol were produced at lower pH values (acidic conditions), whereas the highest amount produced was 15.4 g/L at pH of 5.0. this shows that as acidic conditions decreased, the amount of bioethanol produced decreased, the least amount of bioethanol produced was 7.1 g/L at pH of 6. When the pH is below 4.0, the incubation time for maximum bioethanol concentration was prolonged, but the maximum concentration was not very low this corresponds with the findings of Lin *et al.,* (2012). When the pH value is set above 5.0 the amount of bioethanol produced decreases substantially (Lin *et al.,* 2012). According to Salam *et al.* (2024), a pH range of 4.0 to 5.0 may be regarded as the best pH condition for anaerobic bioethanol production. This agrees with the results obtained from the bioreactor system. Again, pH decrease indicates that microorganisms lack nutrients and begin to consume the organic acids as the nutrient's sources (Salam *et al.,* 2024). The decrease in pH was caused by the formation of Carbon monoxide and other organic acid (Hernández *et al.,* 2015). Furthermore, the significance of pH in bioethanol production in a bioreactor system shows that lower bioethanol production at 3.5 – 4.0 represents less yeast activity because yeast, particularly S. cerevisiae is an acidophilic organism that thrives in acidic vicinity (Salihu *et al.,* 2023). Each enzymes performs optimally at its optimum pH, which is acidic.

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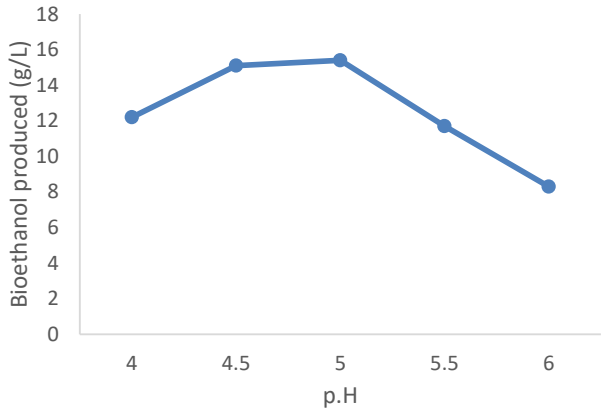


**Figure 6: Effect of pH on bioethanol production with experimental data**

Narendranath and Thomas (2001) further states that if the extracellular pH deviates too far from the optimal range, cell may find it difficult to maintain a steady intracellular pH and the enzymes may fail to function normally. Again, enzymes become deactivated, the yeast cell therefore will be unable to grow and produce bioethanol adequately.

## 4. CONCLUSION

The results shows that increase in enzyme concentration at its optimum condition will increase the yield of bioethanol production and shorten the time for the fermentation process, it shows that the increase will continue until there is no more. This show that increase in enzyme concentration has a positive effect on the yield of bioethanol. Also, it was established that the highest bioethanol yield of 15.4g/L was obtained at 35ºC therefore, Bioethanol production is seen to be effective at 35ºC. When the temperature exceeds 37ºC productivity starts to decrease with toxic effects on yeast causing yeast inhibition. Higher amounts of bioethanol were produced at lower pH values (acidic conditions). This show that bioethanol production is favoured by acidic medium.

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