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INFLUENCE OF TEMPERATURE AND AGITATION SPEED ON FERMENTATION PROCESS DURING PRODUCTION OF BIOETHANOL FUEL FROM CASSAVA

MUREGI, Muhammad Abubakar[1*], M. S. Abolarin[1], O. J. Okegbile[1], and Eterigho, E. J.[2]

[1]Department of Mechanical Engineering, School of Infrastructure, Process Engineering and Technology, Federal University of Technology
[2]Department of Chemical Engineering, School of Infrastructure, Process Engineering and Technology,
Federal University of Technology, Minna, Niger State, Nigeria

ABSTRACT: *This study investigate the influence of temperature and agitation speed on fermentation process during the production of bioethanol from cassava using sabourand dextrose agar. The fermentation was carried out under various temperatures of 28^{o}C, 30^{o}C, 35^{o}C and 37^{o}C and agitation speed of 100, 150, 200, and 300rpm in a 250ml flask. The yield of bioethanol were 12%, 18%, 37% and 22% respectively. The glucose was also used to energize the agar sample and broth to keep the inoculum in liquid state for production of ethanol. Temperature has a remarkable influence on bioethanol production, suitable temperature in fermentation is good condition for organisms and fungal to grow allowing yeast to react properly. From the results it was observed that high temperature kills yeast while low temperature slows down yeast reaction. Agitation speed on the other hand is imperative for adequate mixing, heat and mass transfer. It determines mass transfer between phases in culturing and also maintains homogenous chemical and physical condition in culturing by continues mixing. Agitation creates shear force, which affects microorganisms, causing morphological changes, variation in their growth and product formation, also damage cell structure. This study thus, revealed that temperature of 35^{o}C and agitation speed of 200rpm with pH of 4.5 prove more suitable for fermentation process resulting in higher bioethanol yield of 37%.*

KEYWORDS: Fermentation, Culturing, Agitation speed and Temperature.

INTRODUCTION

The oldest way for production of bioethanol is through fermentation, and it is also the traditional way of making alcoholic beverages [1]. Production of bioethanol from cassava starch involves two processes which are enzymatic hydrolysis and fermentation with the latter being adopted for this work. The objective of this paper is to investigate the influences of temperature and agitation speed on the production of bioethanol from cassava starch using sabourand dextrose. Fermentation: This is the enzymatic breakdown of carbohydrates in the absence of air, it could also be refer to as

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chemical transformation of organic substance into simpler compounds by the action of enzymes. In industrial practice, fermentation implies any process by which raw materials are transformed by the controlled action of carefully selected strains of organisms into definite products [2]. Louis Pasteur used the term in a narrower sense to describe changes brought about by micro-organisms growing in the absence of air. However, biologically, in the fermentation process of ethanol production, the reaction of fermentation is caused by yeast or bacteria which feed on simple sugars. The glucose produced from the hydrolysis described above is fermented with yeast to produce ethanol.

Carbon-dioxide is also produced as glucose is consumed

$2C_2H_5OH + 2CO_2$ ---------- $C_6H_{12}O_6$ (Equation 1)

Microorganisms play a significant role in fermentation process, thus, selection of suitable strain is essential for the individual process. Although, microorganism has been found yet to meet all these requirements, that is, (1) process water economy, (2) tolerance in inhibitor, (3) product yield (ethanol) and (4) ethanol productivity (specifications). Out of these, the one of particular importance is the tolerance of the fermenting organism to fermentation inhibitors formed during fractionation/pretreatment and hydrolysis of the raw material, which necessitates the use of robust industrial strain background. Though. a wide varieties of microorganisms including yeasts, bacteria and fungi have been exploited offering different advantages and disadvantages [3]. The most frequently used microbe has been yeast and among the yeasts, sabourand dextrose which tolerates ethanol concentration as high as 20% which is the preferred strain. Some species of bacteria such as Zymomonas mobilis and the genetically engineered to produce ethanol at higher yields, but they are less resistant to the end product (ethanol) and other compounds present in the hydrolyzates when compared to other yeast [4].

Fermentation process can be performed in batches, fed batch or continuous process. The choice of most suitable process will depend upon type of starch and properties of microorganisms [5]. There are many factors or parameters to be considered in fermentation process such as temperature, agitation speed, pH value, dissolved oxygen and nutrient. The influence of temperature and agitation speed during fermentation process in the production of ethanol is important for successful yield and concentration of final products.

Influence of Temperature: Temperature has a marked influence on fermentation process during the production of ethanol [6] Suitable temperature in fermentation process is the good condition for the yeast to react. Low temperature slows down yeast activity and too high temperature kills yeast, Thus, to keep a specific range of temperature is important; in this study , four different temperatures were considered.[7]

Agitation Speed: Agitation is key for adequate mixing for homogeneity, as well as for heat and mass transfer. It assists mass transfer between the different phases present in the culture, also maintains physical and homogeneous chemical conditions in the culture by continuous mixing.

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Agitation creates shear forces, which affect microorganisms, causing morphological changes, variation in their growth, damaging the cell structure and product formation [8].

METHODOLOGY

The inoculum were produced using sabourand dextrose agar, commercial yeast baker, sugar, Saccharomyces cerevisiae (Zymaflore VL1) (Bordeaux, France) and Saccharomyces cerevisiae (Uvaferm CM) (Lallemand, France.)

Culture preparation (Inoculum):

(a) Agar preparation: 16g of sabourand dextrose agar was thoroughly mixed with one (1) liter of de-ionized water in a pipette, another sample of 65g of commercial Baker's Yeast one (1) liter of water was also prepared

(b) Auto cleave: The two samples and an empty pettish were auto cleaved at a temperature of 101^{o}C for 15mintes to remove possible impurities. The samples were then remove and allowed to cool to room temperature before the agar was poured into the pettish plates and allow to solidify to jelly form to accommodate/sustain fungi and propagation of organisms.

(c) The yeast samples were injected into the jelly formed agar samples that was poured into the pettish dish that was also auto cleaved employing the process of pour plating (wire loop). The sample was then incubated at a temperature of 30^{o}C for 48h for bacterial growth. Another sample of agar (Saccharomyces cerevisiae) was prepared using broth to prepare a liquid inoculum and glucose was added for energizing, the yeast cultures were transferred into the broth sample and incubated on a shaker for 24 hours at 28^{o}C after which they were ready to be used as inoculums for cassava hydrolyses.

Fermentation Process (Starch production): Freshly harvested cassava roots were used for this experiment. 5kg of the cassava were properly peeled and washed thoroughly to remove the soil particles, then grated with a mechanical commercial grater. The resultant pulp was weighed and mixed with 10litres of water. It was sieved with a screen of 25 mesh, the starch pulp milk was left overnight to sediment before decanting. The resultant thick starch cake at the bottom of the bowl was then pressed to remove the remaining water, the bright white coloured starch cake obtained was sun-dried for 48 hours to increase the surface area of the cassava starch for gelatinization and scarifications.

Starch Composition:

(1) Moisture content - this was determined to ascertain the water content in the starch. It was carried out by measuring a 2g of the starch and placed in the oven at a temperature of 105 ^{o}C over a period of 24 hour, thus, reading was taken intermittently.

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(2) Ash content: 2g of powered starch was measured and place in a muffle furnace at a temperature of 580-600 °C for a period of 2 hours to burn off the organic matters, then allow to cool before re-weighing.

(3) pH A required quantity of 2g powdered starch was weighed and mix with the same quantity of distil water (1:1) and stirred continually for a period of 30 minutes, then measured using pH prop to obtain the pH

(4) Conductivity: Conductivity was determined through the same process that was used to determine the Ph

Finally, fermentation is carried out by adding inoculum to the filtrate and starch, then allow to ferment for a period of 36 hours at a temperature 35^{o}C then subsequent distillation was commenced for pure ethanol to be achieved

RESULTS AND DISCUSSION

The effect of fermentation temperature on funger growth during culturing, it was observed that no lag phase was noticed during the growth of either species at all temperatures. [9] An active dry yeast used as inoculum occurs from cultures grown exponentially in aerated media and short or apparent absence of a lag phase in yeast growth may be the result of the pre-adapted state of the cells used as inoculum. However, it should be considered that the samples in the present study were withdrawn at intervals and the lag phase could have already taken place before the sampling. At certain temperatures, a distinct stationary phase was observed following the exponential phase. Fermentation profiles of both yeast species were the same. By energizing the process using sugar, the bioethanol concentration rose to the maximum level, consequently the yeast growth stopped and the stationary phase started. It was observed that bioethanol accumulation in fermenters inhibits specific growth rate, cell viability, increases ethanol production rate, and substrate consumption [10] The yeasts were able to utilize sugar completely at both temperatures. As expected, fermentation was shorter at 35°C compared to 28°C. Fermentation was completed in a shorter time. Both sugar consumption and bioethanol formation rates were higher at 35°C compared to those at 28°C. The rate of yeast growth and alcoholic fermentation increases as temperature increases, with maximum rates occurring at temperatures between 28 and 35 °C as displayed in Table 1. However, the yield began to decrease with increase in temperature and agitation speed as shown in Figures 1 and 2.

Table1: Results of Fermentation yield

(%) Agitation speed	100	150	200	300
Temperatures	28^{O}C	30^{O}C	35^{O}C	**37^{O}C**
Fermentation yield (%)	12%	18%	37%	22%

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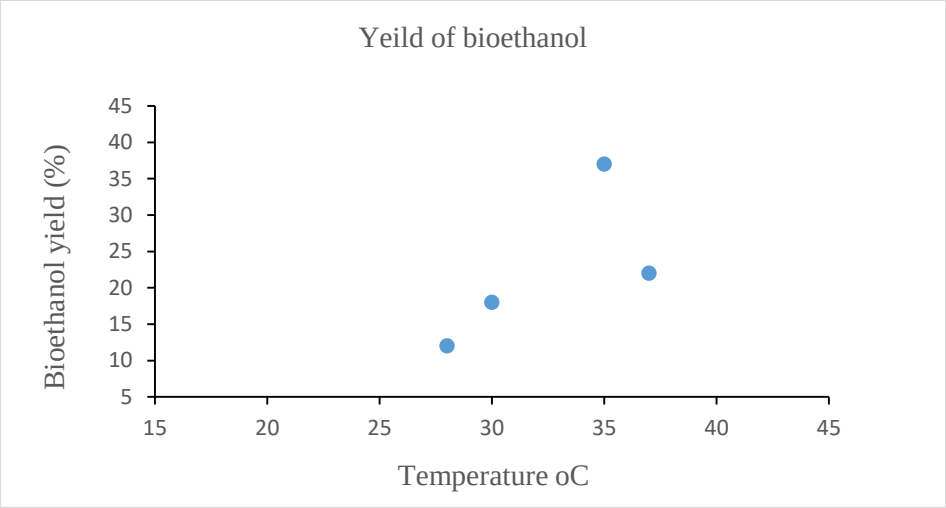

Figure 1: The effect of temperature on the yield of bioethanol

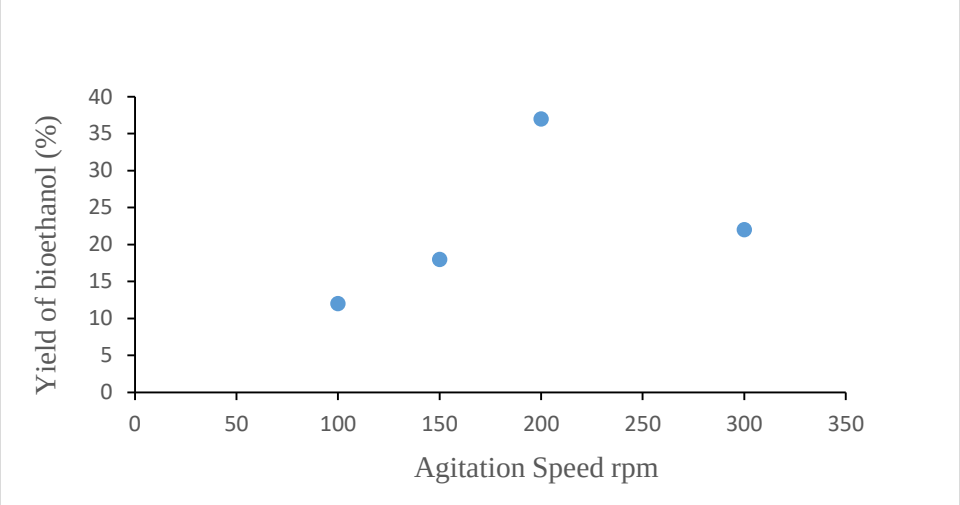

Figure 2: The effect of agitation speed on the bioethanol yield

The combine effect of agitation speed and temperature on the yield of bioethanol is clearly shown in Figure 3.

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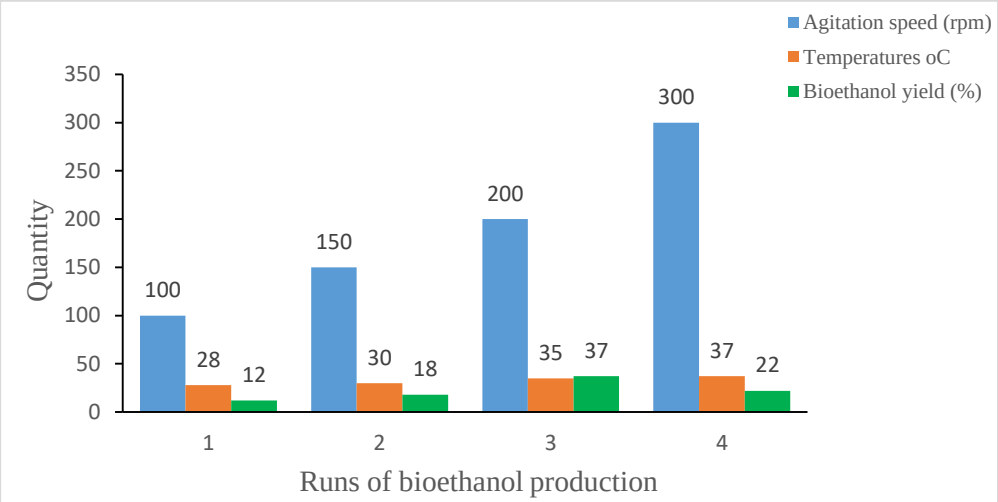

Figure 3: Effect of the interaction of the two parameters (agitation speed and temperature) on the yield

It was also observed from Table 1 that temperature affects fermentation in many ways. At low temperatures yeasts tend to be less sensitive to the toxic effects of high alcohol concentration. The growth rate of yeast cells wass strongly influenced by fermentation temperature. This is particularly evident during the exponential phase. At warmer temperatures (> 20 °C), yeast cells experience a rapid decline in viability at the end of fermentation. At cooler temperatures, cell growth was retarded, but viability is enhanced. Cool temperatures prolong the lag phase of fermentation and slow the rate of fermentation. Excessively high temperatures may disrupt enzyme and membrane functions, resulting in stuck fermentation. Although quick onset and completion of fermentation have advantages, the preferred temperature for kinetic period often less than the optimum for bioethanol production or yeast growth. This is because yeast strains differ in response to temperature, the optimum temperature for kinetic period can vary widely

CONCLUSIONS

The following conclusion was drawn from this study:

There seemed to be no lag phase during the growth of both yeast species at either temperature, at all temperatures tested, the growth rate and fermentation were faster at higher temperature than that of lower thereby achiving completed fermentation in a shorter time.

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