Kinetics Study of Ethanol Fermentation Process by *Saccharomyces Cerevisiae*

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ABSTRACT

The depletion of fossil fuel reserves, the unstable panorama of the petrol prices and more recently, increasing environmental and political pressures has increased industrial focus toward alternative fuel sources, and encouraged the search of products originated from biomass, as renewable sources of energy. In this context, fermentative processes stand out, where microbial metabolism is used for the transformation of simple raw materials in products with high aggregate value. Among these, ethanol is one of the best examples of how fermentation can match market needs satisfactorily. Ethanol is used in various segments of industries, for e.g., medical, recreational and the most important, fuel industry as an additive. Hence vast research in this segment needs to be done. The study investigates the fermentation kinetics for ethanol production through the yeast Saccharomyces Cerevisiae. The study will help in various aspects of fermentor design and in setting the parameters of fermentation to carry out the fermentation smoothly and get maximum yields. The following study has been done by using glucose as the substrate. The yeast estimation, ethanol estimation as well as the sugar estimation has been carried out. Optical density method has been used for yeast estimation while Cole's method has been used for sugar estimation. For the ethanol estimation alcoholmeter has been used. The specific rate constant (μ) has been calculated to be 0.818 hours^{-1}.

Keywords: - *Fermentation, Kinetics, Specific rate constant*

1. INTRODUCTION

1.1 Fermentation Process

A metabolic process that converts sugar to different products like acids gases or alcohol is known as fermentation. Metabolism is the set of life –sustaining chemical transformations within the cells of living organisms. These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments [1].

Although the central component of the system is obviously the fermentor itself, in which the organism is grown under conditions optimum for product formation, one must not lose sight of operations upstream and downstream of the fermentor. Before the fermentation is started the medium must be formulated and sterilized, the fermentor sterilized, and a starter culture must be available in sufficient quantity and in the correct physiological state to inoculate the production fermentor. Downstream of the fermentor the product has to be purified and further processed and the effluents produced by the process have to be treated. Regardless of the type of the fermentation, an established process may be divided into six basic component parts:

1. The formulation of media to be used in culturing the process organism during the development of the inoculums and in the fermentor.
2. The sterilization of the medium, fermentor and ancillary equipment.
3. The production of an active, pure, culture in sufficient quantity to inoculate the production vessel.
4. The growth of an organism in the production fermentor under optimum conditions for product formation.
5. The extraction of the product and its purification.
6. The disposal of the effluent produced by the process.[2]

The "batch culture" fermentation is also known as "closed culture" system. In this system, in the beginning, the nutrients and other additives are added in required amounts. There is no refill of nutrients once the fermentation process has started and the product is recovered at the end of the process. Once the process is completed, then, the fermentation vessel is cleaned properly, sterilized before it use for another batch process. In the beginning, microorganisms grow at a rapid rate due to availability of excess nutrients. As time pass, they increase in number with rapid use of the nutrients and simultaneously produce toxic metabolites. [3]

Due to production of toxic metabolites the growth of organisms slows down during the later stages of the fermentation process. Once the sterile medium is inoculated with seed culture of interest, it passes through a number of phases of growth as below:

1. Lag phase
2. Log phase
3. Stationary phase
4. Death phase

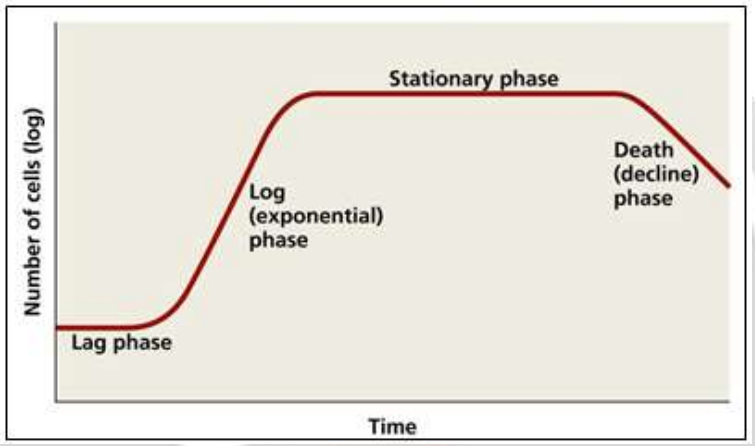

Fig -1: The pattern of typical microbial culture growing in a batch culture conditions.

1.2 Kinetics of a Fermentation Process

Studies of fermentation processes in nearly all development programs involve periodic observations of growth, carbohydrate utilization, and product formation throughout the course of the fermentation. The fermentation literature abounds with such data for a large number of processes, and often also for a wide variety of operating conditions for a particular process. Kinetic analysis is the interpretation of these data and the factors which influence them, to shed light on proposed reaction schemes or fermentation patterns. Analyses carried out to date have followed mainly three avenues of approach-phenomenological, thermodynamic, and kinetic. [4]

In order to effectively analyze and subsequently optimize a biological process, the kinetics of the process needs to be understood and quantified. The use of kinetic models to describe the behaviour of biological systems has been acknowledged to be important because it can reduce the number of experiments needed to eliminate extreme possibilities and provide mathematical expressions that can quantitatively describe the mechanism of the process as required for optimization and control. Though a lot of kinetic models have been developed for the growth of cells in both batch and continuous processes, unstructured models still give the most basic understanding of metabolism of microbiological processes. These unstructured models fairly approximate the dynamic behaviour of these processes for non-steady state cases. The crux of any kinetic analysis lies in determining how the rate of product formation and its stoichiometric coefficient vary with respect to the chemical and physical factors that influence them. [5]

2. MATERIALS AND METHODS

2.1 Glucose, Yeast and Nitrogen Sources

Glucose, a monosaccharide is used as a sugar substrate for fermentation process. The yeast *saccharomyces cerevisiae* contains enzymes which will break the sugar molecules and produce ethanol. The nitrogen sources like peptone, yeast extract and $(NH_4)_2SO_4$. Other mineral sources used are $MgSO_4$ and KH_2PO_4.

2.2 Media Compostion for Yeast Growth

The media is useful for the growth of micro-organisms. The components of the media provide the necessary conditions and requirements for the successful growth of yeast. The media that is to be prepared for micro-organism growth is called as GYe (glucose, yeast extract). The composition for the media is Glucose (10 gm/ lit), Peptone (10gm/ lit), Yeast extract (5gm/lit) and Agar (30gm/lit).

2.3 Media Composition for Production Medium

The steps to be followed for preparing the media for the production are the same only the composition varies. The composition is Glucose (76.5 gm/lit), (NH4)2SO4 (1 gm/ lit), KH2PO4 (1 gm/lit), MgSO4 (5 gm/lit), Yeast extract (4 gm/lit) and Peptone (10 gm/lit).

2.4 Procedure for Yeast Estimation

The yeast estimation is done with the help of a UV Spectrophotometer. The wavelength used for the analysis is 600 nm. Water is use as a blank for taking the reference reading. Optical density as 1 means that there are $3x10^6$ cells in the sample.

2.5 Procedure for Sugar Estimation

Sugars having free –CO or –CHO groups when heated in alkaline solution, their keto or aldehyde group is converted into the form enediol. This has more reducing power and reduces $K_3Fe(CN)_6^{3-}$ to $K_4Fe(CN)_6^{4-}$ and sugars are oxidized to complex mixture of acids . The amount of ferricyanide reduced depends upon the conc. of sugar in the given sample. Ferricyanide ions (yellow solution) are reduced to ferrocyanide ions (colorless solution). Potassium ferricyanide solution is treated with alkali and boiled. The mixture is titrated with sugar solution. When the yellow color has nearly disappeared a drop of methylene blue is added. The titration is continued until the color is discharged. Both methylene blue and ferricyanide are reduced by sugar in hot alkaline solution but the indicator is not affected until the whole ferricyanide has been reduced. The end point is reached when the solution is decolorized. [6]

2.6 Procedure for Ethanol Estimation

For alcohol estimation, the supernatant collected after centrifuge needs to be distilled. A distillation unit is set up at 81^0C. Now the distillate collected is used for alcohol estimation. A device called as alcoholmeter is used for alcohol estimation. The device belongs to the series of hydrometer family, which works on the concept of specific gravity. We need to simply dip the alcoholmeter in the solution. After letting the instrument get stable we note down the reading on the meter which has a vol % scale for showing the alcohol percentage. A Gay Lussacs Alcoholmeter is used in the procedure.

Fig -2: Alcoholmeter

3. RESULTS AND DISCUSSION

3.1 Yeast Estimation

Chart 1 shows the amount of yeast cells produced during the fermentation. The y-axis features the natural logarithm of the amount of cells produced and the x-axis shows the days. The graph shows that the biomass increases exponentially. The slope of the graph here is 0.818 which the specific rate constant (μ). Hence the value of μ is 0.818 per hours.

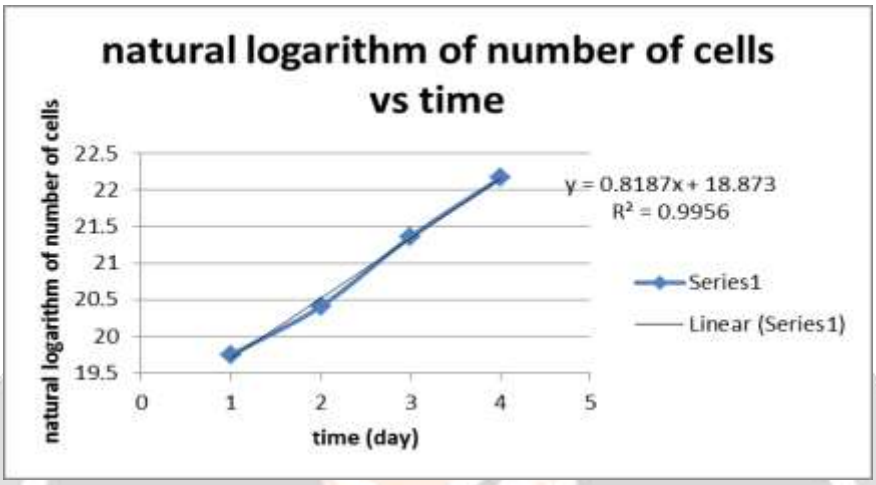

Chart -1: Graph for Yeast Estimation

3.2 Glucose Estimation

Chart 2 shows the amount of glucose present tin the solution after consumption by the yeast. The graph has an R^2 value of 0.953. The amount of glucose will never become zero as the yeast will not be able to consume all the sugar because of the inhibition by the ethanol produced.

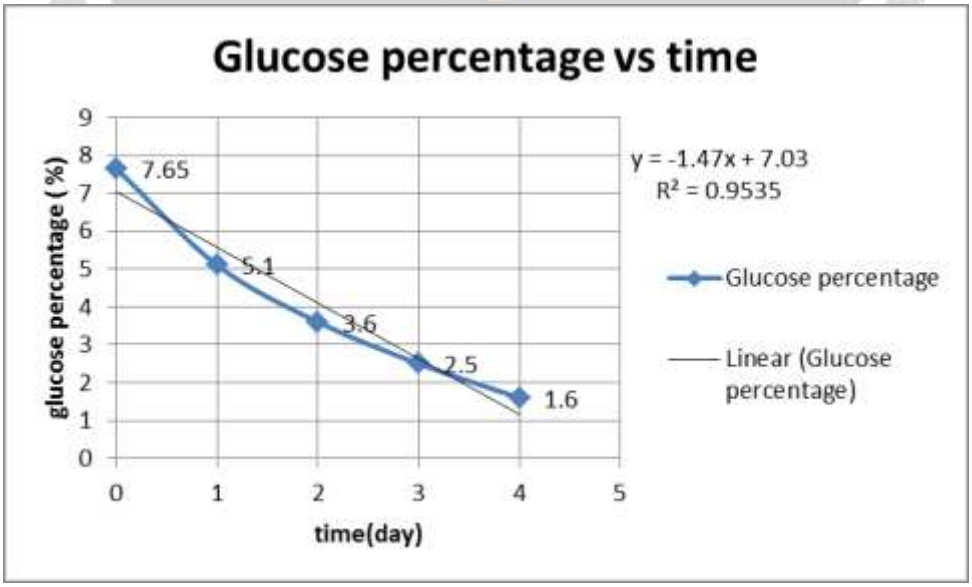

Chart -2: Graph for Glucose Estimation

3.3 Alcohol Estimation

Chart 3 shows the alcohol that has been produced by the yeast as the days progress. The amount of ethanol produced itself becomes the inhibition for the yeast to consume the sugar and hence we always get low yields of ethanol. The maximum alcohol produced here is 12.5 %.

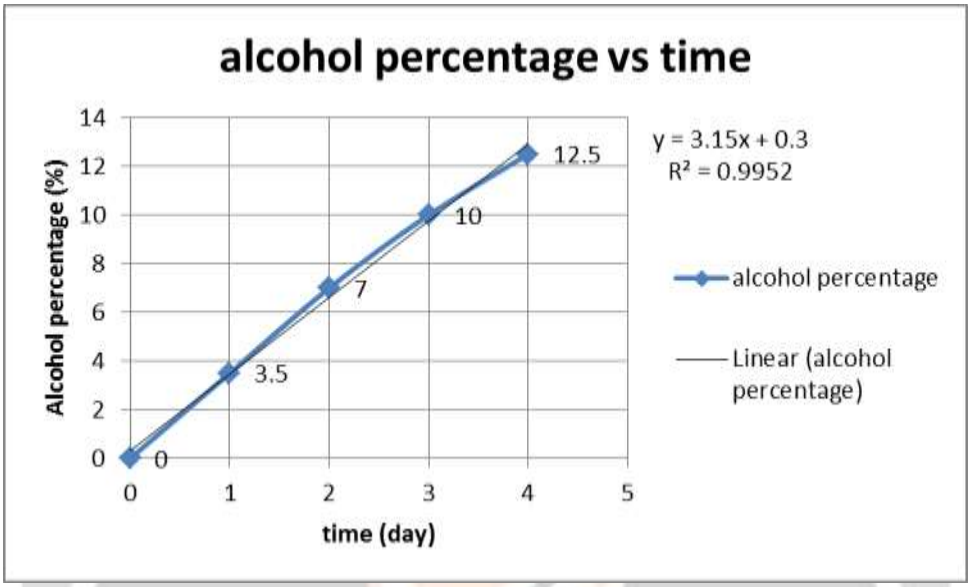

Chart -3: Graph for Alcohol Estimation

4. CONCLUSION

From the experiments the specific rate constant has been found to be 0.818 per hours and this result will assist in the fermentor design for ethanol production using glucose as a substrate. This will also help in sizing the fermentor. The maximum amount of alcohol produced was 12.5%. Similar analysis for the kinetics study of different substrate can be done.

5. REFERENCES

[1]. Klein, Donald W, Lansing M, Harley and John, "Microbiology*",* New York: McGraw-Hill, 2006
[2]. Stanbury P. F., A.Whitekar, Hall S. J., "*Principles of Fermentation Technology"*, 2nd ed. Aditya Books (P) Ltd., New Delhi, (1997) 9-15
[3]. http://www.elearnmicrobiology.com/theory_project.php?id=28&type=theory&chapter=4, <batch fermentation>
[4]. Elmer L. Gaden, Jr., "*Fermentation Process Kinetics*", Biochemical and Microbiological Technology and Engineering, 1 (1959) 413-429
[5].Amenaghawon, N.A, Okieimen, C.O and Ogbeide, S.E, "*Kinetic Modelling of Ethanol Inhibition during Alcohol fermentation of Corn Stover using Saccharomyces Cerevisiae*", International Journal of Engineering Research and Applications, 2 (2012) 798-803
[6]. Jagshish patel, Darshan Patel, *"Experimental Biology",* 2 (2012) 140-141