Kinetics Study of Citric Acid Fermentation by Aspergillus niger Using Sugar Cane Bagasse

Tushar Patel[1], Hiral Pandya[2]

1. Student, Chemical Engineering Department, L.D. college of Engineering, Gujarat, India

2. Assistant Professor, Chemical Engineering Department, L.D. college of Engineering, Gujarat, India

ABSTRACT

*The study investigates the fermentation kinetics for citric acid production through the fugs A. niger. 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 sugar cane bagasse as the substrate. The A. niger estimation, citric acid estimation as well as the sugar estimation has been carried out. Spore culture of Aspergillus niger and moisture contain was regular maintained which played very important in fermentation process. The sugar from the sample can be estimated using dinitrosalicylic acid colorimetric method and Anthrone colorimetric method. The produced citric acid estimated by acetic anhydride and pyridine. The specific rate constant (μ) has been calculated to be **0.0436** hours-1.*

Keywords: - *citric acid Fermentation, Kinetics study, Specific rate constant for Aspergillus niger, sugar cane bagasse*

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 downst ream 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 uses 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

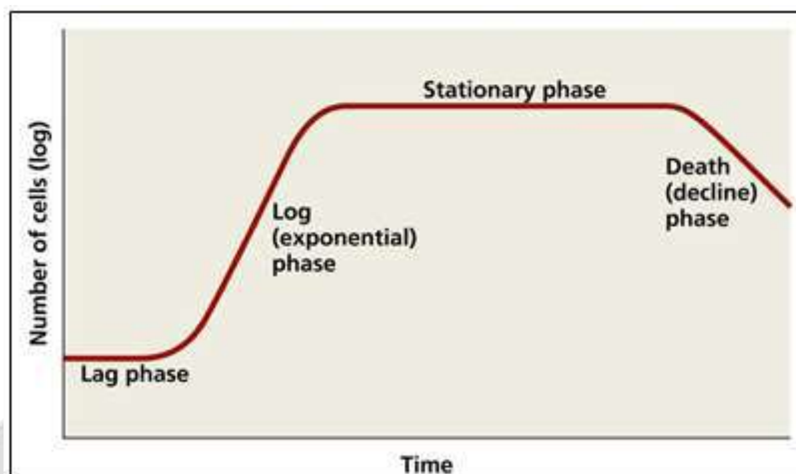

Fig -1: The pattern of typical microbial culture growing in a batch culture conditions.

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 analyse 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 bend enveloped 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 pro cesses 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. Micro-Organism

A citric acid producing strain of *Aspergillus niger* 1055, was procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory Pune. It was maintained on Sabouraud Dextrose Ager (SDA) slants and subculture periodically which were stored at 4 ℃.

2.2. Media Preparation

Bagasse was sun dried, cut into small pieces, grounded and screened to collect two fractions of different particle sizes 0.85mm to 1.40 mm. Bagasse (3 g) of sired particle size was taken in 250 ml Erlenmeyer flasks and

moistened with medium (20% sucrose, 0.25%, NH_4NO_3, 0.1%, KH_2PO_4, 0.025%, $MgSO_4$ and 0.004%, $CuSO_4$, pH 4.0) to set the 75% moisture level. Media were sterilised at 121 °C for 60 min to provide proper cooking of the substrate and to increase its susceptibility to microbial attack.

2.3. Inoculum

Activated culture was inoculated in sterilized SDA broth and incubated at 120 rpm for 48 hr. which was then used as inoculums for SSF. Spore count in activated culture was calculated used counting chamber and find spore count (1×10^7) was maintained.

2.4. Solid State Fermentation

Each flask, containing medium was inoculated with 1 ml of spore suspension followed by mixing and incubation at 30±2 °C, inside the humidity-controlled incubator. 4% (w/v) Methanol was added to the medium before inoculation. One flask was harvested every day for the estimation of citric acid produced and sugar consumed, till whole sugar consumed. Sampling was continued until all the sugar of the medium was consumed.

2.5. Analytical Methods

There are two methods used for sugar estimation calorimetric determination of sugar by the 3,5-dinitrosalicylic acid method [6] and citric acid estimation by acetic anhydride and pyridine method [7].

3. RESULTS AND DISCUSSION:

3.1. *Aspergillus niger* Estimation

Chart 1 shows the amount of *Aspergillus niger* 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 **0.0436** per hours.

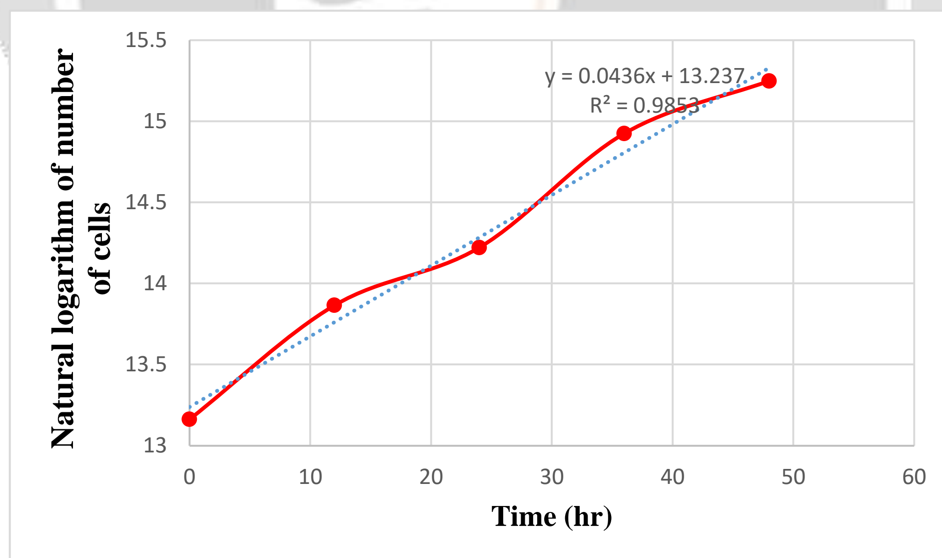

Chart 1: Exponential phase growth rate estimation for *Aspergillus niger* (particle size 0.85 mm to 1.40 mm)

3.2 Sugar Estimation by the 3,5-dinitrosalicylic acid method

Chart 2 shows the amount of glucose present tin the solution after consumption by the *Aspergillus niger*. The amount of sugar will never become zero as the A. niger will not be able to consume all the sugar because of the inhibition by the citric acid produced.

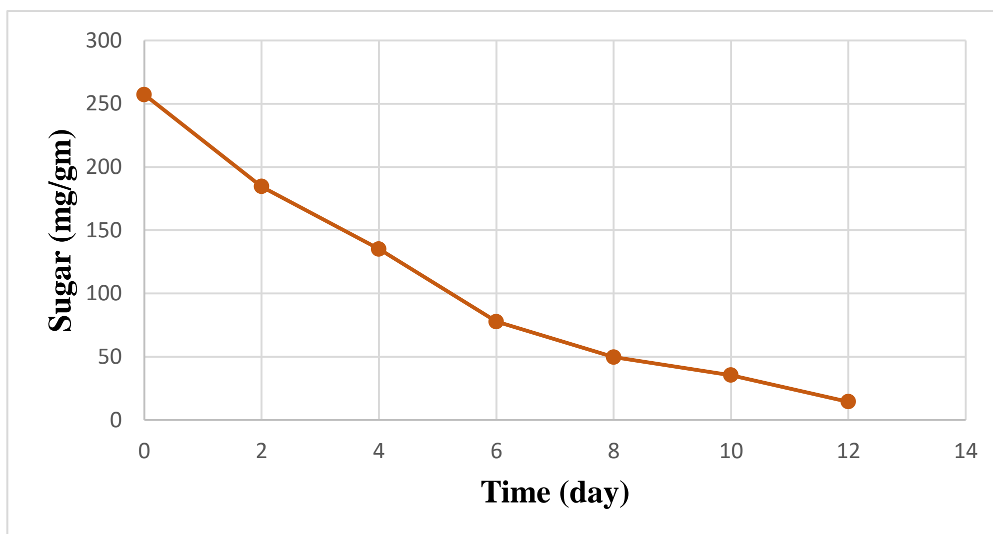

Chart 2: Sugar estimation for DNS method (particle size 0.85 mm to 1.40 mm)

3.3. Citric acid production

Chart 3 shows the alcohol that has been produced by the A. niger as the days progress. The amount of citric acid produced itself becomes the inhibition for the yeast to consume the sugar and hence we always get low yields of ethanol. The maximum citric acid produced here is 150 mg/gm.

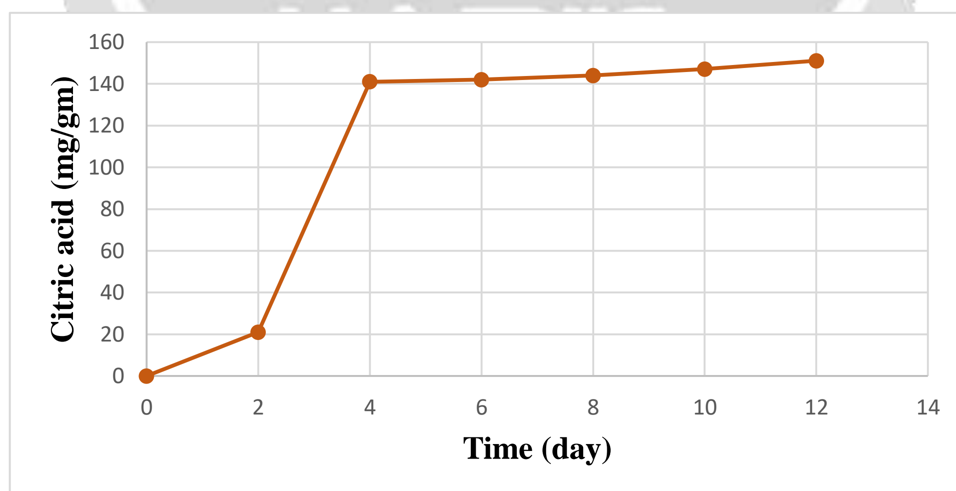

Chart 3: Citric acid production (particle size 0.85 mm to 1.40 mm)

4. CONCLUSION

From the experiments the specific rate constant has been found to be 0.0436 per hours and this result will assist in the fermentor design for citric acid production using sugar cane bagasse as a substrate. This will also help in sizing the fermentor. The maximum amount of alcohol produced was 150 mg/gm. Similar analysis for the kinetics study of different substrate partial size can be done.

5. REFERENCES

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