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Comparison of alcohol production in batch culture using different substrates by *Saccharomyces cerevisiae*

AMIT K. DUTTA and ARPITA MUKHERJEE

Department of Biotechnology, Rungta College of Science & Technology, Durg (India).

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ABSTRACT

Ethanol is one of the most advanced liquid fuels because it is environmental friendly. It is a clear, colorless liquid with a characteristic odor. In dilute aqueous solution, it has a sweet flavor, but in more concentrated solutions it has a burning taste. *Saccharomyces cerevisiae* another well known microorganism plays a crucial role in the production of ethanol by fermentation of fruit juices. *Saccharomyces cerevisiae* contains two main enzymes Invertase and Zymase. Invertase converts sucrose present in the sample to glucose and fructose, while zymase converts it finally to ethanol and CO_2. A fixed volume of fruit extracts were fermented anaerobically by *Saccharomyces cerevisiae*. Sterilized extracts were then inoculated with 3% of activated yeast. Carbohydrate present in fruit juices are acted upon by yeast under anaerobic condition. Anaerobic condition was made up by sealing the extract containing containers. Catabolism of sugars is an oxidative process which results in the production of ethanol under anaerobic condition. Dichromate method was then used for the estimation of ethanol produced. Difference in production rate of ethanol from same volume of substrates is the main focus of this work. Dichromate method was used for the estimation of ethanol produced. Addition of acidified Potassium Dichromate converts Ethanol into Ethanal, an orange brown colour complex.

Key words: *Saccharomyces cerevisiae*, Extracts, Anaerobic Fermentation, Invertase, Zymase, Ethanol, Acidified Potassium Dichromate.

INTRODUCTION

Ethanol is one of the most advanced liquid fuels because it is environmental friendly. It is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, it has a sweet flavor, but in more concentrated solutions it has a burning taste[1]. It is an alcohol, a group of chemical compounds whose molecules contain an OH group, bonded to a carbon atom. It melts at -114.1°C, boils at 78.5°C and has a density of 0.789 g/ml at 20°C[2]. Ethanol is produced by fermentation: when certain species of yeast (notably *Saccharomyces cerevisiae*) metabolize sugar in the absence of oxygen, they produce ethanol and carbon dioxide. Ethanol is particularly useful in industrial applications because of its relatively high affinity for both water and organic compounds. It is a biofuel, which is produced from biomass and wastes. Bio-fuels provide an alternative to fossil fuel dependency and emit fewer pollutants[3]. Various processes have been developed for ethanol production but worldwide demand of ethanol is generally satisfied by biotechnological fermentation process. A number of organisms including fungi, yeast and bacteria have been screened for ethanol fermentation. Extensive studies have been carried out on the fermentation process of ethanol by these organisms, especially through yeast cells[4].

The increasing demand for ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives, has necessitated increased production of this alcohol. Ethanol production is usually accomplished by chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural products. Owing to the depleting reserves and competing industrial needs of petrochemical feed

stocks, there is global emphasis in ethanol production by fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology[5].

An ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appreciable osmotolerance, enhanced ethanol tolerance and good thermotolerance. Although no microbial strain has all these desirable qualities, few yeast strains have been found to possess appreciable characteristics for ethanol production [6, 7]. Ethanol along with CO_2 is produced by fermentation, when *Saccharomyces cerevisiae* metabolize sugar in the absence of oxygen. Catabolism of sugars is an oxidative process which results in the production of ethanol under anaerobic conditions when yeast acts upon different substrates, the enzyme Invertase secreted by *S. cerevisiae* convert sucrose present in the sam-ple into glucose and fructose. Glucose and fructose is then converted to ethanol and CO_2 by another enzyme Zymase, present in *Saccharomyces cere-visiae*.

Although fungi, bacteria, and yeast microorganisms can be used for fermentation, a specific yeast (*Saccharomyces cerevisiae* also known as Bakers' yeast, since it is commonly used in the baking industry) is frequently used to ferment glucose to ethanol. Theoretically, 100 grams of glucose will produce 51.4 g of ethanol and 48.8 g of carbon dioxide[8].

Ethanol was also determined with good precision by oxidation with acid dichromate solution. The ethanol in the known masses of the solution was oxidized to acetic acid using a known mass of standard potassium dichromate (0.1N) in the presence of sulfuric acid[9].

MATERIAL AND METHODS

Instruments

Incubator, Colorimeter, Distillation Unit, Single Pan Balance.

Chemicals

Dichromate solution, concentrated sulphuric acid, distilled water.

Preparation of substrates

Juicer was used for the extraction of extracts from apples, potatoes, carrots, spinach leaves.

Microorganism used

Saccharomyces cere-visiae.

Quantitative estimation of Ethanol production in the samples was carried out from the standard graph plotted.

Standard Preparation

Different concentration of ethanol was taken and the volume was making it up to 2ml with the help of distilled water. 1ml of Dichromate solution was added thereafter. Test tubes were kept in ice bath and 1ml of concentrated Sulphuric acid was added to it. Tubes were then transferred to room temperature and incubated for 10 minutes. Optical density was then measured.

Sample Preparation

100 ml of sterilized extracts were inoculated with 3% of activated *Saccharomyces cerevisiae* and incubated for 7 days at 37ºC anaerobically. Anaerobic condition was maintained by sealing the conical flask by using wax. After 7 days of incubation the sealing was open under sterile condition. 1ml of the extracts was taken which was making up to 2 ml with the help of Distilled Water. 1ml of Dichromate solution was added thereafter. Test tubes were kept in ice bath and 1ml of concentrated Sulphuric acid was added to it. Tubes were then transferred to room temperature and incubated for 10 minutes. Optical density was then measured.

The concentration of alcohol produced by different substrates was estimated from the standard graph of alcohol (plotted by taking different concentration of alcohol).

RESULTS AND DISCUSSION

The concentration of alcohol (ethanol) produced from 100ml of substrates which were incubated anaerobically at 37°C for 7 days was as given in the table:

Samples Taken	Concentration of Alcohol
Apple Extract	64.50 mg/ml
Carrot Extract	26.25 mg/ml
Potato Extract	59.25 mg/ml
Spinach Extract	28.50 mg/ml

Maximum ethanol production was obtain-ed from apple juice which has a concentration of 64.5 mg/ml. While minimum Ethanol production was observed in Spinach extract having a concentration of 28.5 mg/ml. Ethanal production is related to the accessibility of the carbohydrate degrading enzyme which in turn depends upon the type of carbohydrate molecule present in the substrate. The simpler the carbohydrate present, more actively the enzyme could act upon it and the amount of alcohol produced will be more. On the other hand, if the substrate has complex carbon source i.e., polysaccharide, then the amount of alcohol produced will be less. Polysaccharides are

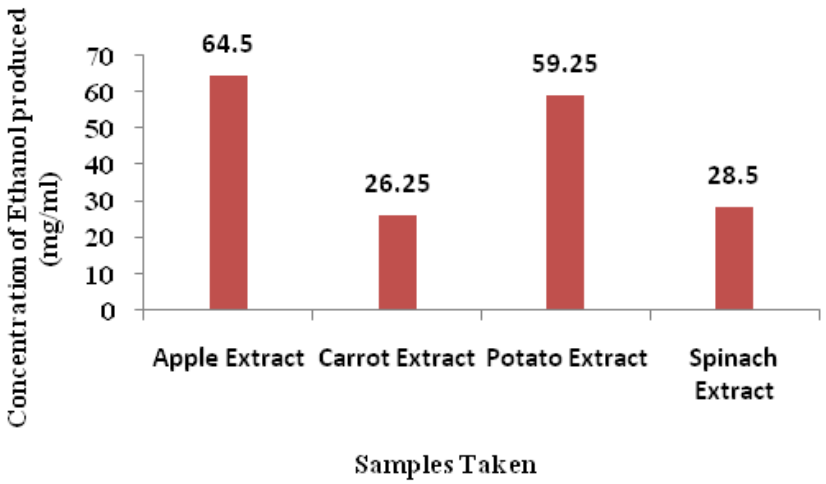

Fig. 1.

broken into simpler sugar and then the enzyme will act upon it, this result in less production of ethanol in the given time duration. Apple extracts contain simple carbohydrate thus exhibiting maximum concentration of alcohol while Spinach extracts have comparatively complex carbohydrate thus producing less amount of ethanol.

CONCLUSION

From our result, it can be concluded that the Ethanol production by anaerobic fermentation depends on the complexity of carbohydrate and the amount of carbohydrate present in the substrate. The order of complexity of carbohydrate among the sample taken was observed to be carrot extract (minimum) followed by Spinach extract then Potato extract and maximum in Apple extract.

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