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Research Paper

Determination of bioethanol production efficiency using native Saccharomyces cerevisiae and Pichiia fermentans in glucose inhibition concentrations

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Abstract

Today, the use of biofuels has become a point of interest due to the limited fossil fuels resources. The objective of the present study was to investigate the production of Saccharomyces cerevisiae (D99), and ethanol and Pichiia fermentans (PTCC No:5296) at glucose inhibition concentrations. Samples were cultured in fermentation mediums containing concentrations of 100, 150, and 200 g/l glucose at 25 ° C. The amount of produced ethanol was measured by potassium dichromate method. The results showed that the ethanol production efficiency was achieved at 150 g/l concentration of glucose for both yeasts. In addition, the concentration of 200 g/l limited the production of ethanol.

Keywords: Glucose; Saccharomyces Cerevisiae; Pichiia Fermentans; Bioethanol; Efficiency

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1. Introduction

biofuels can be produced from biomass of forestry and agricultural wastes [1]. Biofuels can be divided into four groups including biodiesel, biogas, bioethanol, and biohydrogen. Biofuels can be derived from different sources including starch-rich products for bioethanol, vegetable oils for biodiesel, organic wastes for biogas and cellulose for bioethanol [2]. Starch and cellulose are among the main materials for producing bioethanol [12]. Bioethanol can be divided into three generations based on the raw materials: the first generation is starchy substances, cereals and sucrose-rich materials such as sugarcane. The second generation mainly consists of lignocellulosic materials such as wheat straw, corn grass, and stalk, and the third generation includes Algaes such as seaweed [1, 5, 9]. Using bioethanol comes with a number of benefits; 1. Ethanol is a sustainable and renewable fuel; 2. It is a suitable alternative to fossil fuels 3. It reduces greenhouse gases with many environmental benefits 4. It provides clean and safe fuel for the future [14]. Currently, more than 98% of the world's ethanol is produced using the sugar fermentation method. The sugar used in this method can be extracted from various sources such as starch, sugar, agricultural, industrial wastewater and lignocellulosic resources [3].

One of the most important effects of sugar concentration is its direct effect on the fermentation rate and growth of microorganism cells. Generally, the fermentation increases with the increase in the concentration of sugar up to a certain level [8]. Fast fermentation and high levels of alcohol are beneficial for minimizing the major costs and distillation energy, while good returns are needed for economic processes [10]. The efficiency of ethanol production processes depends on a number of factors including the simplicity of the operation, the increase in the concentration of the product in fermentation, and the improvement of the product. Circa 80% of the global alcohol supply is produced by fermentation of products including

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sugar and starch, as well as manufacturing industry by-products [15]. One of the main limitations in obtaining a higher rate of ethanol production is the inhibition of yeast metabolism by a high concentration of sugar and the final product [4]. Saccharomyces cerevisiae cells face different stresses in industrial applications. Using high concentrations of initial sugar and high temperatures can help increase the final concentration of alcohol and reduce the concentration of remaining sugar in the fermenter. Using the mediums with high concentrations of sugar and microorganisms that can tolerate high osmotic pressure is one of the ways to obtain high levels of ethanol during fermentation [7].

In the present study, the efficiency of bioethanol production by Saccharomyces cerevisiae and Pichiia fermentans at glucose inhibition concentrations was compared.

2. Materials and methods

The native strain of Saccharomyces cerevisiae (D99) and Pichiia fermentans PTCC:5296 were used. The yeasts were grown separately in Potato Dextrose Agar medium. The preculture medium was prepared with 10 g/l yeast extract, 0.12 g/l ammonium sulfate, 0.6 ammonium phosphate, and 30 g/l glucose, and adjusted at pH 5.5 using HCl (1M). Fermentation mediums were prepared similar to the preculture mediums and glucose levels of 100, 150 and 200 g/l was obtained [6-13]. One loop of the microorganisms were separately inoculated into the preculture medium and incubated for 20 hours in an incubator with a shaker at 150 rpm at 25 °C temperature for aeration. The inoculation from the fermentation medium was conducted with a ratio of 1/10 and equivalent to 1x 10^7 Cfu / mL. The fermentation cultured was first incubated for 7 hours in an aerobic condition (150 rpm) and then incubated under the anaerobic condition for 40 hours at 25 ° C temperature. After the incubation period, the solid phase was isolated from the liquid phase. For this purpose, a centrifuge with 4000 rpm was utilized. Then, the liquid phase was distilled and the obtained ethanol was evaluated using potassium dichromate method at 590 nm wavelength. The sugar in the culture medium was also evaluated using a glucose assay kit (Pars Test company), and the ethanol production efficiency was calculated [6]. It should be noted that all experiments were conducted with three replications.

3. Evaluation of ethanol production efficiency

In order to evaluate ethanol production efficiency, the following formula was used [11]:

$$\text{Ethanol production efficiency (\%)} = \frac{\text{Ethanol concentration } \left(\frac{V}{V}\right)}{\text{The concentration of used sugar } \left(\frac{g}{l}\right)} \times 100$$

Ethanol concentration: The ethanol concentration obtained in the fermentation process of glucose (V / V) using the potassium dichromate method

Glucose concentration: Concentration of the primary glucose- Concentration of the residual glucose (g / L)

4. Results

Glucose inhibitory effect on the ethanol production efficacy by Saccharomyces cerevisiae and Pichiia fermentans yeasts were investigated. Glucose levels were determined to 100, 150 and 200 g / l and the amount of ethanol produced by yeasts in these three concentrations was investigated.

Comparison of the ethanol production efficiency by Pichiia fermentans and Saccharomyces yeast was done under anaerobic conditions and the error bar resulted from the production of ethanol in three replicates.

The results showed that the efficiency of Saccharomyces cerevisiae ethanol was higher than Pichiia fermentans by 19% and 28% at concentrations of 100 and 150 g/l, respectively.

5. Conclusion

The results from the experiments showed that both yeasts had the highest level of ethanol production at the concentration of 150 g / l of glucose. The production trend of ethanol was increasing for both yeasts up to 150 g/l concentration, and then it was declined in the concentrations above 150 g/l. Saccharomyces cerevisiae had better performance and efficacy compared to Pichiia fermentans at high concentrations of glucose.

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