Optimization of Carbon, Nitrogen Concentration and pH value for High Ethanol Production by Yeast

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Abstract: **- The purpose of this study was to yield high ethanol production by yeast under optimized condition. Glucose Yeast Extract medium was used for isolation, growth and preservation of yeast. A sequential study has been done by consecutive pH levels of 3, 4, 5, 6, 7, 8, 9, 10 and 11. The best ethanol production was obtained at pH 5.0. The various sucrose concentrations examined were 2.5%, 5%, 7.5%, 10%, 12.5%, 15%, 17.5% and 20%. The maximum ethanol production was at 10% (36g/L). In case of yeast extract various concentrations examined were 0.25%, 0.75%, 0.5%, 1.0%, 1.25%, 1.5%, 1.75% and 2.0%. The highest specific ethanol production after 48hr was observed in flask containing 1% yeast extract concentration which was 47g/L.**

***Key Words*: Ethanol, Yeast, Sucrose**

I. INTRODUCTION

Alcohol is an organic compound that have hydroxyl group (-OH) bound with carbon atom. It is a colorless, volatile, flammable liquid compound (Edgard and Arnaud, 2005). Alcohol has various types of uses, the most common alcohol is ethanol know as ethyl alcohol. It is the same alcohol found in alcoholic beverages (Debajit *et al*., 2011). Alcohol production is a metabolic product of yeast which is produced by utilizing reducing sugars like glucose, sucrose, fructose, xylose and mannose (Lange and Simatupang, 1994). Alcohol can be produced by using synthetic medium in lab by submerged fermentation process using yeast.

Fermenting yeast species are being isolated from the various natural sources like flowers, fruits for over decades and is being used in various fermentation processes. (Spencer *et al.*, 1997; Li *et al.*, 2008). But yeast as being a sugar-loving organism, it is usually isolated from sugar rich materials. Fruits like grapes, banana, contain high sugar concentration and hence naturally present on these and can be also easily isolated from fruits (Spencer *et al*., 1997). Fermentation is allowed to proceed by inoculation with must successfully fermented yeast which isolated from fruits. Once fermentation sets in; the rap production of carbon dioxide maintains anaerobic conditions, which prevents the growth of undesirable aerobic organisms, such as bacteria and moulds. The temperature of fermentation is usually 30°C (Macmillan, 1972).

To utilize the existing or converted fermentable sugars into high ethanol production, it is also important to improve the efficiency of the fermentation system(Zacchi and Axelsson, 1989). Many parameters influence the ethanol production rate, such as pH, sugar concentration and nitrogen concentration. Alcohol fermentation is greatly influenced by these factors. The specific rate of fermentation and the sugar uptake rate are all directly related to the desired medium condition (Holzberg *et al*; 1967).

Alcohol have large application in varies industries. Ethanol is very pure form of alcohol and it also sold for other uses than beverages. It use in food industries, pharmaceutical industries, cosmetic industries and as a fuel. Pure alcohol used in industries and medical or hospital fields as cleansing and sanitizing agent. Industrial ethanol is mainly produced petrochemically through the acid-catalyzed hydration of ethylene.

The purpose of this research was to obtain high ethanol production with high productivity. The effect of pH value, initial sucrose concentration and yeast extract concentration on the production of ethanol fermentation performance.

II. MATERIALS AND METHODS

Media

Glucose Yeast Extracts media: Glucose, Yeast extract, Peptone.

Reagents

Production medium: $(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$, $CaCl_2$,Sucrose, Sodium thiosulphate, Potassium iodide, Potassium dichromate, Sulfuric acid, Starch.

All chemicals used in the experiment were of highest purity.

Method

Isolation and Identification of Yeast

Fruit samples (banana, grapes and orange) were obtained from local market. Fruit samples were kept in tube of sterile distilled water over night. Next day this sample was plated on the GYE plate by streak pate method. Then incubate the plate at 30° for 48hr. After incubation period individual colonies were picked from these and microscopic examination was done. After morphological and microscopic examination identified isolated colonies were transferred to GYE stock slants. After 42hr colonies were used as inoculum. During this

study the stock cultures were transferred every two weeks (Zahida *et al*., 2014).

Sterilization of Culture Media

After preparing the media (GYE broth), the flask was covered with cotton plugs. The medium was then sterilized by autoclaving at 121°C at 15lbs pressure for 15 minutes (Zahida *et al*., 2014).

Inoculation Preparation

A loop full of colonies from GYE plate were inoculated in GYE broth and incubated at 30°C for 48hr under static condition. After incubation time period O.D of broth was taken at 600nm and 10% inoculum was used for alcohol production.

Ethanol production

The production medium for alcohol production was composed of $(NH_4)_2SO_4$: 0.5%, Yeast extract: 0.4%, KH_2PO_4: 0.5%, $MgSO_4$: 0.1%, $CaCl_2$: 0.01% and sucrose: 7%. Sterilized the medium and inoculate with 10% inoculum to production medium. Incubate flask at 30°C for 48hr under static condition. After incubation period observed the flask for alcohol presences (Mohmoud and Asmaa, 2014).

Optimization of fermentation medium

The fermentation media was optimized by varying initial sucrose concentration (5, 7.5, 10, 12.5, 15, 17.5 and 20g/100ml) and 0.25g, 0.75g, 0.5g, 1.0g, 1.25g, 1.5g, 1.75g and 2.0g in100ml concentration were used for yeast extract. Various pH of medium were 3 to 10 adjusted. Inoculum size 10% was added to medium and incubated at 30°C for 48hr under static condition. Analyses were made after 48hr interval (Nayuum *et al*; 2013).

Analytical method for Ethanol estimation

After incubation period samples were collected from flask and centrifuged at 5000rpm for 15 min. The supernatants were used for analyses of alcohol production. Concentration of produced alcohol in flask was estimated by dichromate method after 48hr (Mohamed *et al*; 2013).

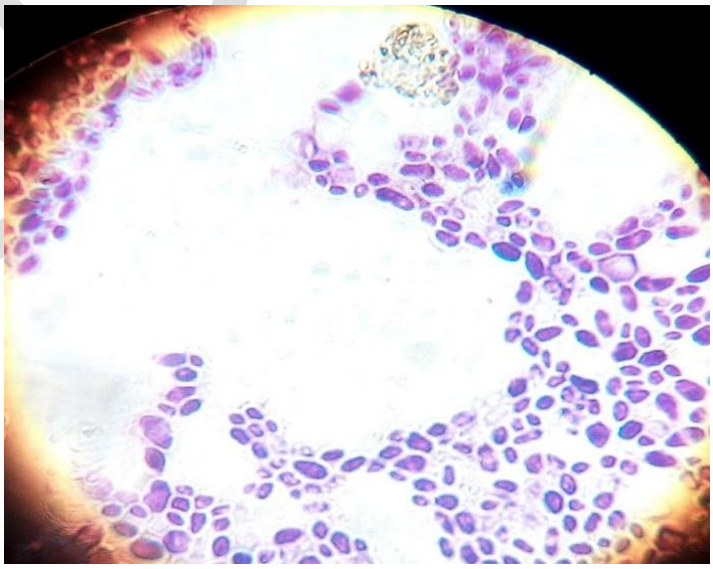
III. RESULT AND DISCUSSION

Identification of Yeast

After incubation period white to creamy colored colonies were observed on GYE plate, which were large in size, round in shape and have smooth surface with entire margin. By microscopic examination the cells were observed with budding characteristic. The cells were oval in shape and occurred mostly singular or in pairs. According Zahida *et al* (2014) to above characteristics were similar to yeast so it was confirmed that isolated colony was of yeast.

Fig.1 Morphological characteristic of Yeast

Fig.2 Microscopic examination of yeast



Estimation of ethanol

After incubation period CO_2 and foam formation was observed in alcohol containing flask. Samples were collected from flask and centrifuged at 5000rpm for 15min. The supernatants were used for estimation of alcohol by potassium dichromate method. Sea-green color at the end of the titration indicates the presence of alcohol.

Fig.3 CO_2 & Foam Formation

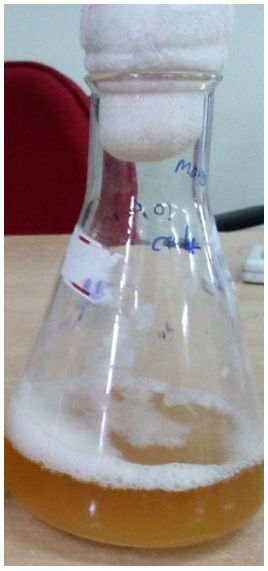
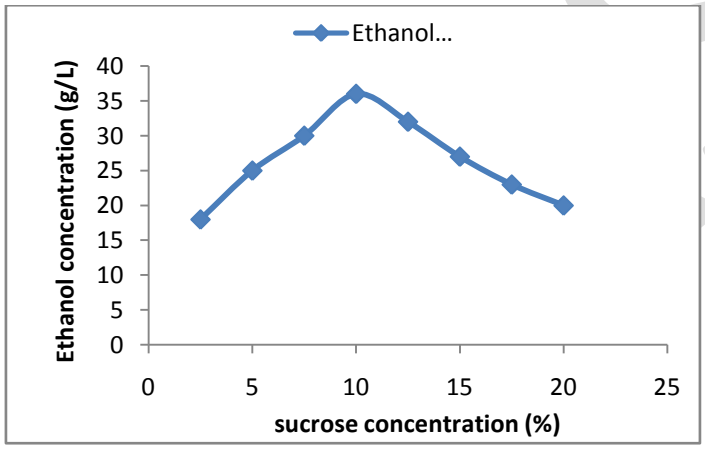


Fig.4 Sea-green colored end point

Optimization of ethanol production

Effect of sucrose concentration on ethanol production

Fig.5

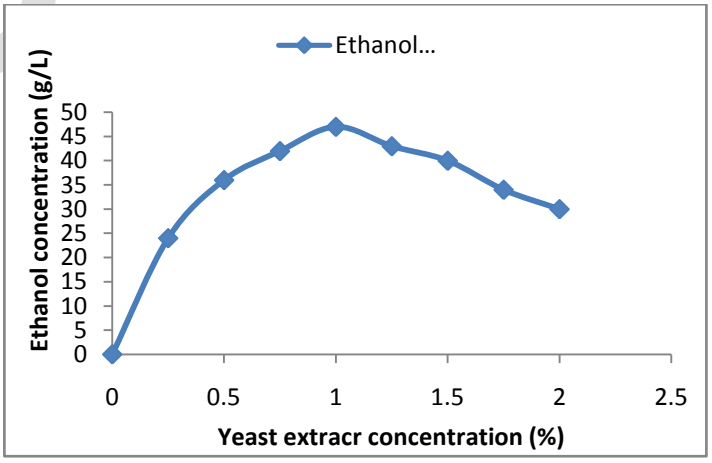


In order to enhance the production of ethanol, the fermentation process was carried out at different concentration of reducing sugars. For that purpose 2.5% to 20% (w/v) was used in this study. It was found that the maximum ethanol production occur at initial sugar concentration 10% after 48hrs. Ethanol production level was decreased due to increase or decrease in the initial sugar concentration from 10% (fig. 5). Ethanol production rate slightly increases when initial reducing sugar concentration increases from 5%-10% but after 10% production rate was decreases.

According to Nayuum *et al*. (2013), 5% to 10% sugar concentration for ethanol production was considered. They reported optimum sugar concentration at 6%. Ethanol production rate increases slightly when initial reducing sugar concentration increases from 5 to 6% but production decreases slightly at 8% and 10%.

Effect of yeast extracts concentration on ethanol production

Fig.6



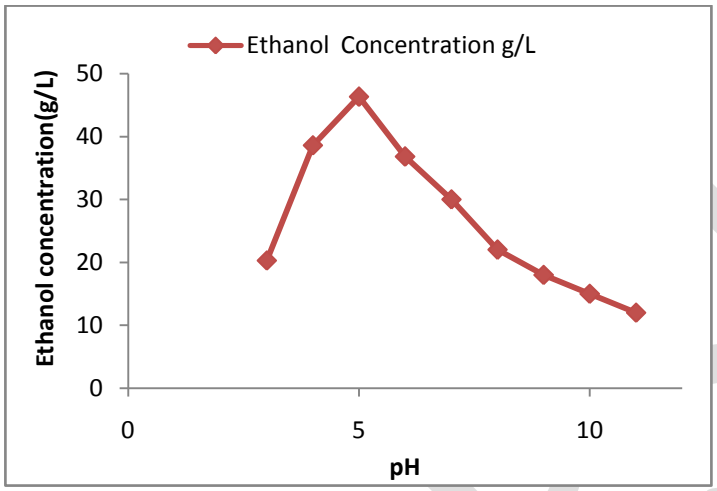
Very small amount of nitrogen source is requires for ethanol production. The maximum ethanol produced during fermentation is closely related to the amount of available nitrogen present in the culture medium. Yeast extract was used as nitrogen source which is considered as nutrient responsible for limiting the alcoholic fermentation by yeast when present more than adequate amount. Yeast extract when used in the range of 0.25% to 1% alcohol concentration was also increased, which was 24g/L and 47g/L respectively.

Above 1% alcohol production were decreases, which were 43g/L at 1.25% and 30g/L at 2%, it can be observed in (fig.6). This study illustrated that more than 1% yeast extract inhibitory for alcohol production so ethanol production was carried out with 1% yeast extract.

Acourene and Ammouche (2010), also reported similar concentration for yeast extract. In this study, they use different concentrations of ammonium phosphate, namely 0.25, 0.5, 1.0, 2.0 and 2.5 g/L. Thus, an improvement of the ethanol content with increasing ammonium phosphate concentration was observed to stabilize at a maximum of 131.0 g/L with a concentration greater than or equal to 1.0 g/L. Our sources of the study varied with the above study.

Effect of pH on ethanol production

Fig.7



In this study, influence of pH on ethanol production was investigated. Fig.7 shows the results of the effect of pH on ethanol production. When the pH was lower than 4.0, the concentration of ethanol lower. The highest ethanol production rate was achieved at pH-5.0 which was 46.3g/L.When the value of pH above 5.0, the quantity of ethanol production substantially decreased.

According to Yin *at el.* (2012) maximum specific alcohol produced at 5.5 –pH. They were reported that initial pH (5.5) of the fermentation media had great influence on ethanol production. Increases or decreases in the initial pH from 5.5 of the fermentation media have marked decreases in the ethanol yield.

IV. CONCLUSION

From the present study it was concluded that the isolated colonies were of yeast. The ethanol production using optimum sucrose and yeast extract concentration as carbon and nitrogen source respectively and optimum pH value at 5, gave high ethanol yield.

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