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Research Article

ENHANCED PRODUCTION OF ETHANOL FROM RED POTATOES GROWN IN HILLY REGIONS OF NEPAL USING VARIOUS NITROGEN SOURCES

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Abstract

Ammonium sulphate, ammonium phosphate, sodium nitrate, urea and glycine were the five different commonly available nitrogen sources used at different concentration ranging from 0.5 to 4% w/v to produce ethanol in batch culture. Potato paste made from red potatoes grown in hilly regions of Nepal was used as carbon source. Prior to fermentation all carbon sources were saccharified enzymatically using α- amylase at pH 5 and temperature 55ºC. Maximum yield of ethanol 5.2% was obtained at a temperature of 30ºC and pH 5.0 without exogeneous supply of nitrogen. There is slight decrease in concentration when temperature is decreased to 25ºC but a drastic decrease in concentration when temperature is increased beyond optimum. All the exogeneously supplied nitrogen sources found to enhance ethanol production and cell viability when yeast strain *Saccharomyces cerevisiae* isolated from brewer's yeast was used. Ammonium sulphate was found as best nitrogen supplement among them. Maximum ethanol percentage of 8.3 was observed at pH 5.0 and temperature 30ºC with Ammonium sulphate concentration of 2%.

Key words: Ethanol, Red potatoes, Saccharification, *Saccharomyces cereviceae*, fermentation.

Introduction

During industrial fermentation, carbohydrates are not the only important group of compounds in the medium to be supplimented. It has been suggested that an appropriate amount and diversity of nitrogenous compounds are important for the successful completion of industrial fermentation process and product quality (Casey *et al.*, 1984; Stewart *et al.*, 1998). Nitrogen deficiency has been identified as one of the main causes for sluggish fermentation. It is important to select any sources that are not only rapidly and efficiently utilized but that they should ensure an efficient fermentation. In case of fuel ethanol production with cell reuse, it is important for yeast viability and viability maintenance between cycles (Alexandre *et al.*, 1998; Bisson, 1999). The sugary substrates available are comparatively expensive but can be easily used for ethanol production. On the other hand cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is expensive. The starchy substrates are promising due to their economic viability and availability. (Szambelan *et al.*, 2004; Shigechi *et al.*, 2004). The world over production of potatoes in 2007 was 325.3 million tones showing this as a promising crop but is being used for production of ethanol in some countries (Kobayashi *et al.*, 1998). It is semi-perishable food which can be stored only for considerable period without spoilage. So there is a need to explore the possibility of ethanol production from potato.

The yeast *saccharomyces cereviceae* still remains the major industrial ethanol producer. Efficient industrial production requires a rapid fermentation leading to high ethanol concentration, therefore a yeast strain must have a good specific growth rate and specific ethanol production rate at high osmotic pressure and ethanol concentration. This goal is not easy to achieve since many parameters during batch fermentation can cause the decrease of specific rate of growth and inhibition can be caused either by product or substrate (Bai. *et al.*, 2008). Many strategies have been explored to improve ethanol production.

Considering that Carbon and Nitrogen ratio are the main nutrients in industrial fermentation media. This would suggest that the mutual interactions of these nutrients may play an important role in yeast metabolism as suggested by Peter *et al.*, 2006 who describe the regulation of amino acid permeases by carbon catabolite repression.

It has also been reported that brewer's and baker's yeast differ in their ability to ferment galactose depending on the structural complexity of nitrogen source and yeast catabolite repression response to the fermentable sugar (Cruz *et al.*, 2003).

In this study, the effect of various defined nitrogen sources on metabolism of industrial yeast by taking four different

nitrogen sources and producing ethanol from Himalayan red potatoes at optimized condition of temperature, pH and agitation was investigated.

Materials and methods

Materials

Red potatoes grown at an altitude of 1625 meters were brought from local sellers. Active dry yeast (Victoria instant yeast, made in Belgium) was used as industrial yeast source. Different organic and inorganic chemicals used in this experiment were collected from Merk, Glaxo and Sigma companies. Components for growth media were collected from Difco laboratories. Heat stable α-amylase were purchased from Genie with the activity of 392 AGU/g (AGU: amyloglucosidase unit is the amount of enzyme that hydrolyzes 1 μM of maltose per minute under specified condition) at 25ºC for saccharification of potatoes.

Methods

Industrial yeast was isolated from active dry yeast using YEPD (Yeast extract, peptone, dextrose) agar. The isolated yeast was maintained on agar slant containing YEPD. The cultured yeast on agar slant was kept at 30ºC for 72 h. and then stored at 4ºC for further use (Pramanic *et al.*, 2003).

Inoculum preparation

Inocula were grown aerobically in 250 ml Erlenmeyer flasks containing YEPD at 30ºC in a shaker (Remi Scientific) at 200 rpm for 24 h. pH adjusted to 4.5 using Sulphuric acid.

Preparation of fermentation media

Potatoes were peeled and cooked. It was analyzed for different components by standard methods (AOAC, 1990). 50 gms of peeled potato mashed to the slurry with 100 ml distilled water in 250 ml flask with or without the addition of nitrogen suppliments. pH adjusted using 0.1 M sulphuric acid and 0.1M sodium hydroxide.

Saccharification and fermentation

250 mg of α-amylase were added to each flasks at pH 5 and saccharified at 55ºC for 4 h. The hydrolysis was performed in flasks in a thermostated water bath with shaking at 150 rpm. After saccharification each flasks were adjusted to pH ranges from 4 to 6, then inoculated all the flasks with 10 ml inocula and incubated.

To determine optimized temperature, temperature ranges were adjusted from 20 to 35ºC at optimized pH.

To investigate efficiency of nitrogen source types on fermentation process, ammonium sulphate, ammonium phosphate, Sodium nitrate, urea and glycine were used at different concentration ranging from 0.5 to 4% w/v.

Analytical methods

At specified time during fermentation, cell suspension was withdrawn, centrifuged at 1200 rpm for 20 min and supernatant taken for subsequent analysis. Total and viable cell counts were determined by direct microscopic observation (Inverted microscope, Nikkon Eclipse, Japan) with an improved Neubauer counting chamber. Samples were diluted and 0.1% methylene blue was used to stain cells. Cells that stained blue were considered to be viable.

Reducing sugar concentration as glucose was analysed using Millers, 1959 method.

The measurement of ethanol concentration was done by spectrophotometrically at 600 nm (Pramanik *et al.*, 2003)

Reproducibility

The results presented in this study were the average of a minimum of three independent experiments having 3% experimental error.

Results and Discussion

Hilly red potato contained about 77% moisture, 21% starch, 2.0% proteins. This result showed that red potatoes contained more starch than the potato used by Rani *et al.*, 2010 which contains only 20% starch. Maximum ethanol concentration of 5.2% was obtained at pH 5 (Fig. 1). This optimum pH was used for further study.

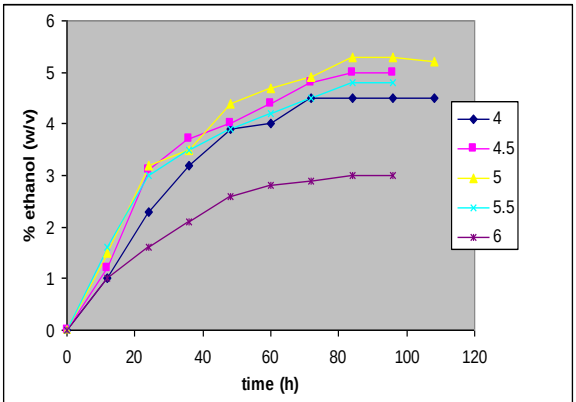

Fig.1: Effect of pH on ethanol production

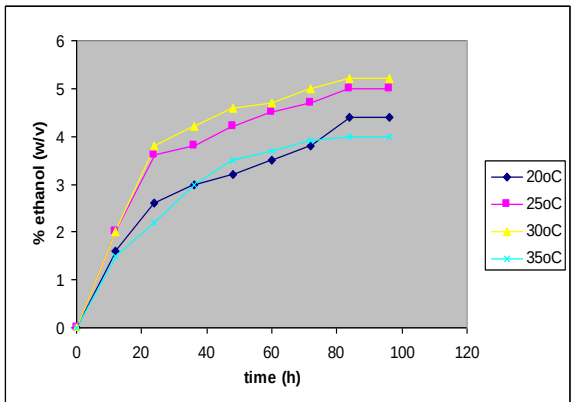

Fig.2: Effct of temperature on ethanol production

For each temperature the yield of ethanol were found and are shown in Fig.2. Maximum yield of ethanol 5.2% was obtained at a temperature of 30ºC. There is slight decrease in concentration when temperature is decreased to 25ºC but a drastic decrease in concentration when temperature is increased.

The result presented in Fig 3 clearly showed that potato mash is deficient in assimilable nitrogen and that a fast rate of fermentation requires the supply of nitrogen source provided exogenously or produced in situ through the hydrolysis of potatoes protein. It is not that all N- sources have the same effect in promoting the growth of yeast.

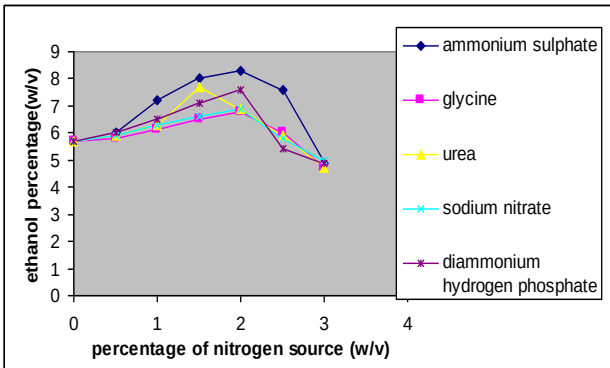

Fig.3: Effect of Nitrogen sources on ethanol production

Generally the addition of Nitrogen sources contributed to the achievement of higher ethanol concentration when compared without nitrogen sources along with maintenance of higher yeast stability. This result contradicts the finding of Rani *et al.*, 2010. Which suggests that, supplementation of nitrogen sources to potato flour did not contribute significantly to ethanol yield (Fig 4).

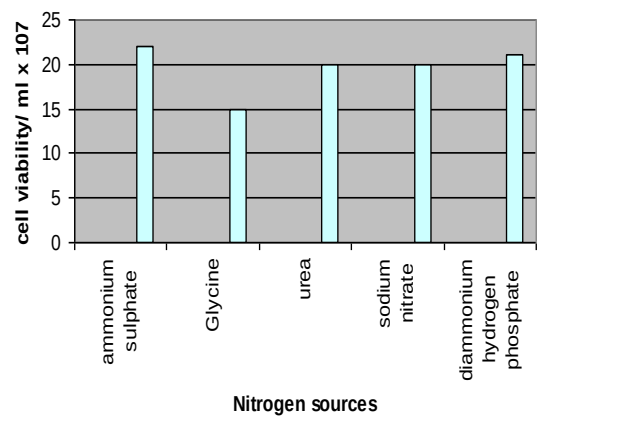

Fig.4: Effect of Nitrogen sources on cell viability

Conclusion

Among all tested nitrogen sources at optimized condition, the most efficient in improving ethanol productivity in fermentation of enzymatically hydrolyzed hilly red potatoes by *Saccharomyces cerevisiae*, isolated from brewer's yeast was 2% of ammonium sulphate. Positive effects were obtained with all added nitrogen sources indicating that exogeneous nitrogen source is necessary for improved viability of yeast cells and thus the productivity rate of ethanol.

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