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INHIBITORY EFFECT OF ACIDIC BY PRODUCTS ON ETHANOL PRODUCTION BY *SACCHAROMYCES CEREVISIAE* AB910X1

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ABSTRACT

Ethanol is the principal product by *Saccharomyces cerevisiae* AB910X1 when grown on sugar containing medium anaerobically. But some acidic by products namely acetic acid and citric acid produced during the ethanol fermentation inhibits ethanol production (11.2 to 9.2%) and final pH also reduced to 5.0 to 3.5 after 48 hours of incubation.

INTRODUCTION

In India, ethanol is produced mainly by fermentation using dilute cane molasses by different yeasts at ambient temperature. During fermentation, in addition to ethanol production, yeasts produce a variety of acidic by products like acetic acid, citric acid, glycerols and different other alcohols. The quantity of the products produced depends on the media compositions, strains employed and the fermentation temperature. 1,2

Several reviews are available on the effect of different metabolic by products on different alcohol fermentation. 3-12

Considering all these reviews, the main objective of this present study was to investigate the effect of some acidic by products on ethanol fermentation by *Saccharomyces cerevisiae* AB910X1.

MATERIALS AND METHODS

Microorganism: Ethanol, temperature resistant strain *Saccharomyces cerevisiae* AB910X1 was developed from its parent strain (*Saccharomyces cerevisiae* isolated from North Bengal Pineapple waste disposal materials, West Bengal, India) and subsequently employed in this present investigation.

Medium and Cultural conditions: The yeast *Saccharomyces cerevisiae* AB910X1 an ethanol and temperature resistant strain was maintained on YPD agar medium (1% yeast extract, 2% peptone, 2% dextrose and 4% agar, pH 5.0) stored at 40°C. The medium used for fermentation was composed of: sucrose, 20%; $(\text{NH}_4)_2\text{SO}_4$, 0.7%; KH_2PO_4 , 0.15%; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05%; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 15 µg/ml, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 1 µg/ml, pyredoxine-hydrochloride 0.3 µg/ml; calcium pantothenate, 0.5 µg/ml; pH 5.0. Sucrose was sterilized separately and then employed to the medium before incubation. The cell density of yeast was adjusted 2.4×10^7 cells/ml of the suspension. Surface culture fermentation was conducted using 500 ml Erlenmeyer Cincical flask, each containing 150 ml medium. The flask was then incubated at 30°C for 0-72 hours. The content of the flasks were analyzed at regular intervals to indicate the changes in medium composition in relation to final pH, total acid, citric acid, acetic acid and ethanol production (with and without adjustment).

Determination of ethanol: Ethanol concentration was estimated gas chromatographically¹¹.

Determination of invertase activity: The invertase activity was determined by the method as proposed by Haq and Ali (2005)¹².

Determination of cellular growth: The cellular growth was measured spectroscopically as proposed by Haq and Ali (2005)¹².

Determination of acidic by products: During ethanol fermentation, total acid, acetic acid and citric acid produced were estimated by the method of hinsvark *et al.* (1954) and Macdonald and Waterbury (1959)^{13,14}.

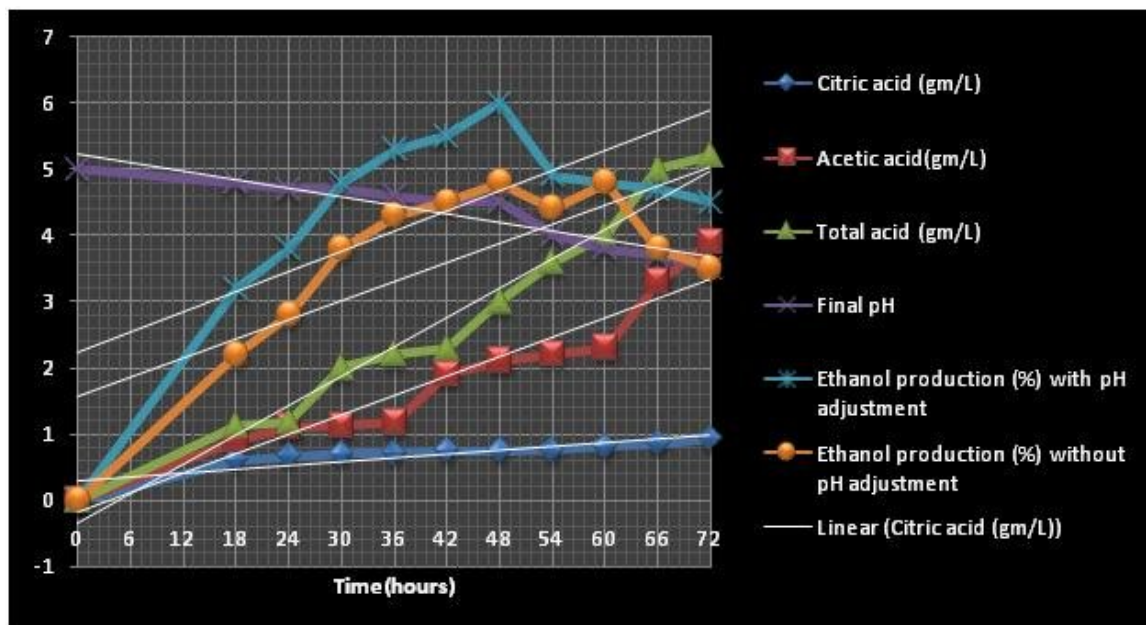


Fig.1. INHIBITORY EFFECT OF ACIDIC BY PRODUCTS ON ETHANOL PRODUCTION BY *Saccharomyces cerevisiae* AB910X1

RESULTS AND DISCUSSION

Fig.1 revealed that during ethanol fermentation, the final pH of the fermentation broth decreases rapidly from 5.0 to 3.5 with progress of the

fermentation as acidic by products increases gradually which increases total acidity. At 48 hours of incubation, maximum yield of ethanol was achieved (11.7% with adjustment of pH and 9.2%

without adjustment of pH). From 18 to 48 hours of fermentation, the amount of total acidity of the fermentation broth changes from 1.2 gm/L to 5.2 gm/L respectively. Acetic acid (0.8 to 3.8 gm/L) was the major by product which might have toxic effect on *Saccharomyces cerevisiae* AB910X1. This fig also suggests that the acidic by products inhibited the ethanol yield markedly without adjustment of pH.

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REFERENCES

1. Moyer A.J., Effect of alcohols on the mycological production of citric acid in surface and submerged culture, *Applied Microbiology*, 1953, vol.1(1):1-7.
2. Byers F.M., Goodrich R.D. and Meiske J.G., Influence of acetic acid, Lactic acid and ethanol on the fermentation of corn silage, *Journal of animal science*, 1982, Vol.54(3):640.
3. Pampultha M.E. and dias M.C.L., combined effect of acetic acid, pH and ethanol on intracellular pH of fermenting yeast, *Applied*

Microbiology and Biotechnology, 1989, vol.31(5):547-550.

4. Acharya K.T., Alcoholic fermentation and its products in ancient India, *Indian Journal of History of Science*, 1991, vol.26(2) :123-129.

5. Guldfieldt L.U. and Arnebor N., Measurement of the effects of acetic acid and extracellular pH of non-fermenting individual *Saccharomyces cerevisiae* cells by Fluorescence Microscopy, *Applied Environmental microbiology*, 1998, vol.64(2):530-534.

6. Torija M.J., Beltran G., Novo M., Poblet M., Rozes N., Mas A. and Guillamon J.M., Effect of organic acids and nitrogen sources on alcoholic fermentation: study of their buffering capacity, *Journal of Agricultural Food Chemistry*, 2003, vol.51(4):916-922.

7. Mairelia B., Blanch H.W. and Wilke C.R., By product inhibition effects on ethanol fermentation by *Saccharomyces cerevisiae* *Biotechnology and Bioengineering*, 2004, vol.25(1):103-121.

8. Lu F., He P.J., Shao L.M. and Chen H.H., Effect of pH value on fermentation pathways of Biodegradable organic waste, *Huan Jing ke Xue*, 2006, vol.27(5):991-997.

9. Bischoff K.M., Liu S., Leathers T.D., Worthington R.E. and Rich J.O., Modeling Bacterial contamination of fuel ethanol fermentation, *Biochemistry and Bioengineering*, 2009, vol.103(1); 117-122.
10. Casey E., Sedlak M., Ho N.W., Moiser N.S., Effect of acetic acid and pH on the cofermentation of glucose and xylose to ethanol by a genetically engineered strain *Saccharomyces cerevisiae*, *FEMS yeast research*, 2010, vol.10(4):385-393.
11. Coldwell B.B., Solomonraj G., Trenholm H.L. and Wiberg G.S., The gas chromatographic estimation of ethanol, acetaldehyde and acetone in ethanol metabolism studies, *Clinical Toxicology*, 1971, vol.4(1):99-113.
12. Haq I.U. and Ali S., Invertase production from a hyperproducing *Saccharomyces cerevisiae* strain isolated from dates, *Pakistan Journal of Botany*, 2005, Vol.37(3):749-759.
13. Hinsvark O.N., Houff W.H., Wittwer S.H. and Sell H.M., The extraction and colorimetric estimation of indole-3-acetic acid and its esters in Developing corn kernels, *Plant Physiology*, 1959, vol.29 (1) : 107 -108.
14. Macdonald R.E. and Waterbury W.E., Colorimetric estimation of citric acid, *Nature*, 1959, vol. 184 : 988-989.