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# Effect of some micronutrients on the production of bioethanol from water hyacinth by *Saccharomyces cerevisiae* $AB_{810}$

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The study was done to investigate the role of various micronutrients on the production of bioethanol by an ethanol and temperature resistant strain of *Saccharomyces cerevisiae* $AB_{810}$ as a suitable fermentation medium. Microelements like $Zn^{2+}$, $Mn^{2+}$ and $Fe^{3+}$ were effectively required for the ethanol and temperature resistant strain to increase ethanol production. The optimum amount was required for the maximum production of bioethanol from water hyacinth was $Zn^{2+}$: 5 μg/ml, $Mn^{2+}$: 10 μg/ml and $Fe^{3+}$: 15 μg/mL respectively. The other microelements mainly $Cu^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Fe^{2+}$ and $Mo^{6+}$ had shown an adverse effect on bioethanol production by this strain. This study shows that the addition of specific essential microelements into the fermentation medium helped in increase the bioethanol amount from 10.4% to 12.2% (v/v) compared to unsupplied micronutrients.

Keywords: *Saccharomyces cerevisiae*, micronutrients, bioethanol.

## Introduction

*Material and methods:*

Yeast like *Saccharomyces cerevisiae* microorganisms requires specific minerals for cell growth and its metabolic activities. The requirement of these specific nutrients varies with the type of microorganisms used as well as the nature of the basal medium[1]. For favouring the multiplication and successive function of the organisms, some specific conditions were employed in the conventional method in order to increase the productivity of ethanol[2]. Every enzyme has a different reaction path and selective nutrient requirement for optimum performance. In this studies different micronutrients and their great practical importance in ethanol production were optimised[3]. It had been found that $Zn^{2+}$ plays a major role in yeast cells[4]. The $Zn^{2+}$ had promotes and provides optimum growth of yeast at a concentration of 0.2 ppm. Several studies were made on the requirements of microelements for the growth of yeast and the production of ethanol[5]. Christopher White and Geoffrey M. Gadd[6] had studied the uptake and cellular distributions of $Zn^{2+}$ in *Saccharomyces cerevisiae*. They had examined and characterized external concentration which was probably of the utmost physiological importance and it had also studied the effect of toxicity an acceptance at higher $Zn^{2+}$ concentration. Micronutrients 0.1 g/L $MnSO_4$ and 0.024 g/L $FeSO_4$ was detrimental to yeast growth[7]. Yoshinori Ohumi *et al.*[8] had described the effect of $Cu^{2+}$ causing specific changes in the permeability of intact *Saccharomyces cerevisiae* cells. They also found that 100 μM $CuCl_2$ was added to cell suspension in a buffer of low ionic strength, the permeability barrier of the plasma membranes of the cells was lost within 2 min at 25°C. Considering all these aspects, an extensive study had been made to find out the microelement requirements for the selection of a suitable medium for bioethanol production from alkali hydrolysed water hyacinth by *Saccharomyces cerevisiae* $AB_{810}$.

*Microorganism used:*

*Saccharomyces cerevisiae* $AB_{810}$, a newly isolated ethanol and temperature resistant strain develop in the laboratory, has been used in these studies[9].

*Medium and cultured condition:*

The alcohol and temperature resistant strain of *Saccharomyces cerevisiae* $AB_{810}$ was maintained in YPD agar me-

dium containing yeast extract 1%, peptone 2%, dextrose 2%, agar 4%, and pH was adjusted to 5. The micro-organism was maintained at 28°C for 48 h. Surface culture fermentation was carried out using 500 ml conical flasks each containing 200 ml of the medium. Then the fermentation medium was placed in an autoclaved maintained at a temperature of 120°C in 15 lb/inch$^2$ pressure for 15 min, and to make it sterile. The yeast cells were harvested by washing the slant with sterilized distilled water and filtering the resulting cell suspension through several layers of absorption cotton. The cell density was adjusted to $3.6\times10^7$ cell/ml of the suspension and 5 ml of inoculums was added to it during the production of alcohol.The flasks were then incubated at 28°C for 48 h. The fermentation medium used for alcohol production contained glucose 25% (obtained from hydrolysed water hyacinth), $KH_2PO_4$ 0.1%, $NaNO_3$ 0.3%, $MgSO_4.7H_2O$ 0.05%, yeast extract 1% and pH 5.

*Addition of micronutrients to the basal medium:*

Primarily the basal medium did not contain the microelements to be inspected. The impurities existed in the inorganic salts which were further decontaminated by the method of Majumder and Bose[10]. The solutions of all micronutrients were prepared in triple glass distilled water, sterilized in an autoclave at 15 lb/inch$^2$ pressure for 15 min and added separately to the medium to reach the necessary concentration.

*Determination of ethanol concentration:*

After alcohol fermentation, the concentration of ethanol in the solution was determined by a Exactive™ GC Orbitrap™ GC-MS System (Thermo Scientific) attached with a flame ionization detector (FID). A non-polar column DB-5MS Wax (0.25 mm×30.0 m×0.25 μm) was employed for the analysis in presence of helium as carrier gas (flow rate 1.51 ml/min). The GC was programmed at 70°C for 4 min then increased to 300°C at a constant heating rate of 5°C min$^{-1}$ and hold there for 30 min. The column temperature and detector temperatures are 190°C and 230°C respectively. The oven was programmed to hold at 35°C for 5 min and then ramped up at a constant heating rate of 20°C min$^{-1}$ to 250°C and maintained it for another 15 min. In each case 1 μL sample was injected and data obtained in a scan mode in the mass range of 30–120 *m/z*[11]. Fragmentations were used for identification and quantification 31 *m/z* and 50 *m/z*. A calibration curve was obtained from 0.1, 0.2, 0.3 up to 1% (v/v) ethanol in HPLC grade water and their peak areas. The quantitative calculation of ethanol concentration was made by measuring the peak areas of the sample in calibration relative to the interval standard ethanol used as an internal standard[12]. The components of the ethanol at different retention times after GC-MS analysis were determined from the NIST library that came as a reference.

*Determination of cellulose, hemicellulose, lignin and reducing sugar:*

Cellulose, hemicelluloses and lignin content in the given water hyacinth were estimated by the method of Goering and Von Soest[13]. Calculation of total reducing sugars after hydrolysis in different methods hydrolyzing biomass was done by DNS methods[14]. Hydrolysis (%) was estimated by the produced total reducing sugars TRS (mg/L)[15]:

$$\text{Hydrolysis (\%)} = \frac{\text{Formed TRS}\times0.9\times100}{\text{Cellulose and hemicellulose contents of substrate}} \quad (1)$$

*Hydrolysis efficiency calculation:*

The alkali hydrolysis efficiency was calculated using the following equation:

$$E_p\,(\%) = {}^{\Delta S_p}\!/_{\text{TCF}} \times 100 \quad (2)$$

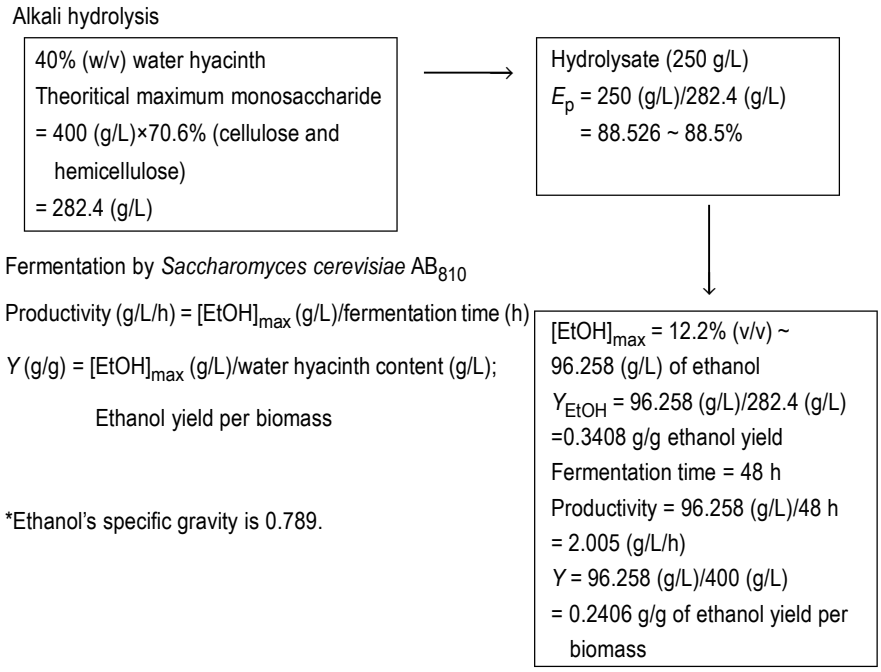
where $E_p$ was saccharification efficiency using high-pressure hydrolysis (%), $\Delta S_p$ the monosaccharide after alkali hydrolysis (g/L), and TCF total carbohydrate and fiber (g/L).

*Ethanol yield coefficient calculation:*

The ethanol yield coefficient calculation was calculated using the following eq.:

$$Y_{\text{EtOH}} = {}^{[\text{EtOH}]_{\max}}\!/_{[\text{Monosaccharide}]_{\text{ini}}} \quad (3)$$

where $Y_{\text{EtOH}}$ was ethanol yield (g/g), $[\text{EtOH}]_{\max}$ the maximum ethanol concentration gained during fermentation (g/L), and $[\text{Monosaccharide}]_{\text{ini}}$ was the total initial fermentable sugar (glucose) concentration (g/L).




Mass balance flow chart of bioethanol production from bioethanol

*Statistical analysis:*

All data were expressed as mean ±SEM, where n = 6. The data were analysed by one way ANOVA followed by Dennett's posthoc multiple comparison testusing "Prism 4.0" software (Graph pad Ind., USA). A '*p*' value of less than 0.05 was considered significant and considered highly significant less than 0.01.

## Results and discussion

The effect of different trace elements on ethanol production by *Saccharomyces cerevisiae* $AB_{810}$ was depicted in Tables 1 to 3.

Among them $Zn^{2+}$, $Mn^{2+}$, $Fe^{3+}$ showed a positive effect on ethanol production. $Zn^{2+}$ 5.0 µg/ml, $Mn^{2+}$ 10.0 µg/ml and $Fe^{3+}$ 15.0 µg/ml were proved to the optimum concentration for ethanol production by *Saccharomyces cerevisiae* $AB_{810}$. Other elements studied namely $Cu^{2+}$, $Ni^{2+}$, $Co^{2+}$, $Fe^{2+}$ and $Mo^{6+}$ had an adverse effect on ethanol production. Martin and Daniel[16], Hughes and Poole[17] was suggested that those ions probably acted as either activator or inhibitor of some enzymes involved in synthetic steps of metabolites. Hughes and Poole also claimed that even though some toxic metals like $Cu^{2+}$ and $Ni^{2+}$ had a detrimental effect on growth and metabolism of microorganisms including yeast. Besides, in

**Table 1.** Effect of nickel (added as $NiSO_4.7H_2O$), copper (added as $CuSO_4.5H_2O$) and zinc (added as $ZnSO_4.7H_2O$) on ethanol production by *Saccharomyces cerevisiae* $AB_{810}$*

| Concentration (µg/ml) | $NiSO_4.7H_2O$ Ethanol production (%) | $CuSO_4.5H_2O$ Ethanol production (%) | $ZnSO_4.7H_2O$ Ethanol production (%) |
|---|---|---|---|
| 0 | 10.4±0.098 | 10.4±0.035 | 10.4±0.065 |
| 1 | 9.5±0.095 | 9.0±0.078 | 10.6±0.119 |
| 5 | 8.6±0.054 | 8.2±0.065 | 11.0±0.132 |
| 10 | 8.0±0.058 | 7.1±0.047 | 10.7±0.098 |
| 15 | 7.2±0.125 | 6.1±0.084 | 10.2±0.056 |
| 20 | 6.5±0.105 | 5.2±0.071 | 9.5±0.092 |

*All values of ethanol production are significant at the level $p < 0.05$.

**Table 2.** Effect of manganese (added as $MnSO_4.4H_2O$), vanadium (added as $NaO_4V$) and ferrous ($Fe^{2+}$ added as $FeSO_4.7H_2O$) on ethanol production by *Saccharomyces cerevisiae* $AB_{810}$*

| Concentration | $MnSO_4.4H_2O$ | $NaO_4V$ | $FeSO_4.7H_2O$ |
|---|---|---|---|
| (μg/ml) | Ethanol production (%) | Ethanol production (%) | Ethanol production (%) |
| 0 | 11.0±0.116 | 11.4±0.125 | 11.4±0.058 |
| 1 | 11.1±0.148 | 11.0±0.110 | 11.3±0.108 |
| 5 | 11.2±0.101 | 10.2±0.075 | 11.0±0.115 |
| 10 | 11.4±0.085 | 9.6±0.105 | 10.4±0.075 |
| 15 | 11.0±0.102 | 8.4±0.068 | 9.6±0.110 |
| 20 | 10.7±0.098 | 7.2±0.052 | 9.0±0.055 |

*All values of ethanol production are significant at the level $p < 0.05$.

**Table 3.** Effect of ferric ($Fe^{3+}$ added as $Fe_2(SO_4)_3.H_2O$), cobalt (added as $CoCl_2.6H_2O$) and molybdenum (added as $Na_2MoO_4.2H_2O$) on ethanol production by *Saccharomyces cerevisiae* $AB_{810}$*

| Concentration | $Fe_2(SO_4)_3.H_2O$ | $CoCl_2.6H_2O$ | $Na_2MoO_4.2H_2O$ |
|---|---|---|---|
| (μg/ml) | Ethanol production (%) | Ethanol production (%) | Ethanol production (%) |
| 0 | 11.4±0.123 | 12.2±0.126 | 12.2±.128 |
| 1 | 11.5±0.110 | 11.4±0.098 | 12.0±0.098 |
| 5 | 11.8±0.065 | 10.0±0.095 | 11.6±0.125 |
| 10 | 12.0±0.043 | 9.6±0.085 | 11.0±0.075 |
| 15 | 12.2±0.084 | 9.2±0.098 | 10.2±0.095 |
| 20 | 11.9±0.059 | 8.7±0.114 | 9.2±0.125 |

*All values of ethanol production are significant at the level $p < 0.05$.

some cases it played some beneficial effect on microorganisms at lower concentrations. However, in our present study we observed that both $Cu^{2+}$ and $Ni^{2+}$ was showed a toxic effect even at the lower concentration on ethanol production by *Saccharomyces cerevisiae* $AB_{810}$.

The result of the present study show that the production of ethanol by *Saccharomyces cerevisiae* $AB_{810}$ had increased significantly ($p < 0.01$) after addition of required essential microelements (12.2%) compared to the production of ethanol (10.4 %) by this strain using minimal salt medium without any microelements. Thus, from this study, the following suitable medium was recommended for bioethanol production by *Saccharomyces cerevisiae* $AB_{810}$ with a composition of glucose 25.0%; $NaNO_3$ 0.3%; $KH_2PO_4$ 0.1%; $MgSO_4.7H_2O$ 0.05%, $ZnSO_4.7H_2O$ 5 μg/ml; $MnSO_4.H_2O$ 10 μg/ml; $Fe_2(SO_4)_3.H_2O$ 15 μg/ml; yeast extract 1.0% and pH 5.

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