SEMINARS DELIVERED

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1. Delivered a seminar at 19th West Bengal State Science & Technology Congress-2012 on **"Improvement of activities of alcohol dehydrogenase and aldehyde dehydrogenase during acetic acid production in presence of some complex nutrients by an ethanol resistant strain *Saccharomyces cerevisiae* AB_{100} ".**

2. Delivered a seminar at 18th West Bengal State Science & Technology Congress-2011 on **"Effect of different trace elements on alcohol dehydrogenase and aldehyde dehydrogenase during the production of acetic acid by an ethanol resistant strain *Saccharomyces cerevisiae* AB_{100} ".**

3. Delivered a seminar at 16th West Bengal State Science & Technology Congress-2009 on **"Effect of different carbon sources on alcohol dehydrogenase and aldehyde dehydrogenase during the production of acetic acid by an ethanol resistant strain *Saccharomyces cerevisiae* AB_{100} ".**

4. Delivered a seminar at 15th West Bengal State Science & Technology Congress-2008 on **"Development of alcohol resistant strain of *Saccharomyces cerevisiae* and production of acetic acid ".**

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Effect of physical parameters on alcohol dehydrogenase and aldehyde dehydrogenase during the production of acetic acid from ethanol by an ethanol resistant strain *Saccharomyces cerevisiae* AB_{100}

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Abstract : An ethanol resistant strain of *Saccharomyces cerevisiae* AB_{100} was examined to ascertain its acetic acid production. The influence of physical parameters on acetic acid production and activities of alcohol dehydrogenase (alcohol : NAD^+ oxidoreductase; E.C. 1.1.1.1) and aldehyde dehydrogenase (aldehyde : NAD^+ (P^+) oxidoreductase; E.C. 1.2.1.5) were also observed. The optimal conditions finally found were , initial pH = 4.5, incubation temperature = 30 °C, incubation time = 48 h, volume of medium = 85 ml, age of inoculum = 48 h and cell density = 10.25×10^5 These optimal conditions were also optimum for the activities of the two main regulatory enzymes i.e. alcohol dehydrogenase and aldehyde dehydrogenase.

Keywords : Acetic acid, physical parameters, alcohol dehydrogenase, aldehyde dehydrogenase.

Introduction

Acetic acid as an industrial chemical is produced using yeast by the three step fermentation process The first step is the production of ethanol from a carbohydrate source, such as glucose. The second step is the production of acetaldehyde by oxidation of ethanol and the third step is the oxidation of acetaldehyde to acetic acid[1-3].

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2$$

$$2CH_3CH_2OH + O_2 \rightarrow 2CH_3COOH + 2H_2O$$

Two enzymes such as, alcohol dehydrogenase (alcohol NAD^+ oxidoreductase; E.C. 1.1.1.1) and aldehyde dehydrogenase (aldehyde : NAD^+ (P^+) oxidoreductase; E.C. 1.2.1.5) are responsible for the production of acetic acid from ethanol[3-7].

$$CH_3CH_2OH + NAD^+ \leftrightarrow CH_3CHO + NADH + H^+$$

$$CH_3CHO + NAD^+ + H_2O \rightarrow CH_3COOH + NADH + H^+$$

Cultivation of microorganism require favourable environment, like - appropriate temperature, pH, incubation period, volume of medium, age of inoculum and cell density. These essential factors in optimum amount work together as a building material for the multiplication of microorganism. Thus the main objective of the present work was to investigate the optimum cultural conditions for the growth of *Saccharomyces cerevisiae* AB_{100} which gave the maximum activity of the two enzymes to give the maximum production of acetic acid from ethanol.

Experimental

(i) Microorganism used : *Saccharomyces cerevisiae* AB_{100}, a newly isolated ethanol resistant strain in our laboratory, have been used in these studies[8].

(ii) Medium and cultural conditions : The maintanance media consisted of 1% D-glucose, 0.5% peptone, 0.5% yeast extract and 4% agar agar powder pH was adjusted to 5.0 Organism was maintained at 30 °C for 48 h Inoculum was harvested by washing the slant with sterile distilled water, adjusting the cell density to 2.05×10^5 per ml

The medium for fermentation consisted of 10% D-glucose, 0.1% KH_2PO_4, 0.5% $(NH_4)_2SO_4$, 0.05% $MgSO_4.7H_2O$, 0.1% yeast extract. pH was adjusted to 4.5. Both medium were sterilized at 121 °C and 15 lb/inch^2 pressure for 15 min.

250 ml conical flasks containing the fermentation medium were inoculated with 5 ml of inoculum and incubated at 30 °C for 96 h.

After fermentation, the cell was separated by centrifugation and the supernatant was used for analysis of acetic

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Effect of minerals (macro and micro) on alcohol dehydrogenase and aldehyde dehydrogenase activity during acetic acid production by an ethanol resistant strain of *Saccharomyces cerevisiae* AB_{100}

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Abstract : To overcome the limitation of low productivity, some conditions are to be established for the control of micro organism activity, so that their multiplication and successive functioning can be favored. During the growth period, mineral supplements are necessary to enhance the activity of different enzymes. Addition of right mixture of minerals (both macro and micro), can make yeast to perform better than ever. In this study, it has been found that, 0.1% NaCl, 0.01% $CaCl_2$, 0.125% KH_2PO_4, 0.125% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 1% $Na_2HPO_4.2H_2O$, 1 mg/L boric acid, 10 µg/ml each of Fe^{2+}, Mn^{2+} and Zn^{2+} is the optimum requirement for our organism to produce the maximum amount of acetic acid i.e. 10.678 g/L.

Keywords : Acetic acid, *Saccharomyces cerevisiae*, macro and micro elements.

Introduction

Microorganisms require specific minerals for growth and metabolic activity. The requirement varies with the type of organism as well as the nature of the basal medium used

Yeast has a complex nutritional requirement[1]. To overcome the limitation of low productivity of acetic acid, some conditions are to be established to favor the multiplication and successive functioning of the organisms[2] Each enzyme catalyzes a different reaction and also has specific nutrient requirement for optimum performance. So the studies of the optimized combinations of minerals are thus of great practical importance[1] It has been reported that Zn^{2+} is an important element for yeast cells[3] At a concentration of 0 2 ppm, Zn^{2+} promotes and provides optimal growth of yeast Considerable studies have been made in the requirement of minerals for growth of yeast It has been reported that when phosphate was restricted, the phosphorus pool decreased before growth rate decreased and was accompanied by changes in respiration and metabolite production[4]. The threshold phosphorus concentration for yeast growth is 0 7 ppm 0 1 g/L $MnSO_4$ and 0.024 g/L $FeSO_4$ were detrimental to yeast growth and NaCl at 0.228 g/L limited both growth rate and the total yield of yeast cells[5]

The present study was undertaken to examine the effect of minerals, both macro and micro, on alcohol dehydrogenase and aldehyde dehydrogenase activity of *Saccharomyces cerevisiae* AB_{100} during the production of acetic acid

Materials and methods

(i) Microorganism used

Saccharomyces cerevisiae AB_{100}, a newly isolated ethanol resistant strain in our laboratory have been used in these studies[6]

(ii) Medium and cultural condition

The maintenance media consisted of 1% D-glucose, 0 5% peptone, 0 5% yeast extract and 4% agar agar powder. pH was adjusted to 5 0 Organism was maintained at 30 °C for 48 h Inoculum was harvested by washing the slant with sterile distilled water, the cell density to 2 05 $\times 10^5$ per ml The medium for fermentation consisted of 10% D-glucose, 0.125% KH_2PO_4, 0.5% $(NH_4)SO_4$, 0 05% $MgSO_4.7H_2O$ pH was adjusted to 4 5 Both me-

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Role of complex nutrients, vitamins and amino acids on alcohol dehydrogenase and aldehyde dehydrogenase activity during acetic acid production by an ethanol resistant strain of *Saccharomyces cerevisiae* AB_{100}

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Abstract : Complex nutrients are composed of different vitamins, amino acids and minerals. Addition of such components in the production media of acetic acid can increase the yield affecting the two main regulatory enzymes alcohol dehydrogenase and aldehyde dehydrogenase and also makes it cost effective due to easy availability. In the present study soyabean meal has proved its maximum effectivity by increasing the acetic acid production upto 13.46 g/L and cell weight upto 4.48 g/L. Vitamins like – nicotinic acid and D-biotin and amino acids such as – L-tryptophan and L-glutamic acid also showed their effectivity. Other vitamins and amino acids had negligible effect on both acetic acid production and activity of alcohol dehydrogenase and aldehyde dehydrogenase.

Keywords : Acetic acid, *Saccharomyces cerevisiae*, complex nutrients, vitamins, amino acids.

Introduction

Acetic acid is an important feedstock for many chemicals, such as – sodium acetate is used as an acidulant and as a meat spray to inhibit microbial growth. Calcium magnesium acetate (CMA) has been identified by the US Federal Highway Administration as an environmentally safe and non corrosive deicer for use of roads in winter and potassium acetate was identified as a heat exchange fluid[1-3]. At present these products are made from petroleum derived acetic acid at a cost of about $ 650 per ton. Fermentation is potentially a cost effective alternative for acetic acid production. Production of acetic acid via fermentation using renewable biomass feedstock has been studied extensively since late 1970s[1,3-7].

However, one obstacle to the successful commercialization of this process is the expensive nutrients required in this medium. If a low cost medium can supply all the nutritional requirements (like, nitrogen sources, vitamins, amino acids and trace elements) to sustain viability and productivity, then it would be economically feasible to produce acetates by fermentation[8].

The present study has been designed to show the effect of the complex nutrients from both plant and animal origin, vitamins and amino acids on the activity of the two main enzymes of acetic acid production i.e. alcohol dehydrogenase and aldehyde dehydrogenase, because these are the cheap sources of different vitamins, amino acids and minerals, highly required for satisfactory growth and productivity of our desired organism.

Materials and methods

Microorganism used

Saccharomyces cerevisiae AB_{100}, a newly isolated ethanol resistant strain in our laboratory have been used in these studies[9,10].

Medium and cultural condition :

The maintenance media consisted of 1% D-glucose, 0.5% peptone, 0.5% yeast extract and 4% agar agar powder, pH was adjusted to 5.0. Organism was maintained at 30 °C for 48 h. Inoculum was harvested by washing the slant with sterile distilled water, the cell density to 2.05×10^5 per ml. The medium for fermentation consisted of 10% D-glucose, 0.125% KH_2PO_4, 0.5% $(NH_4)_2SO_4$, 0.05% $MgSO_4.7H_2O$ and 10 µg/ml of each of $FeSO_4.7H_2O$, $MnSO_4.H_2O$ and $ZnSO_4.7H_2O$. pH was adjusted to 4.5. Both medium were sterilized at 121 °C

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Development of high yielding ethanol resistant strain of *Saccharomyces cerevisiae* for ethanol production

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Abstract *Saccharomyces cerevisiae* AB, an ethanol producing strain (4.0%) was treated with 10%, 15% and 20% ethanol for 30, 60 and 180 min. Only 5.5% colonies isolated by 30 min incubation in 10% ethanol gave higher alcohol production (7.5%) than the parent strains. These strains when incubated in 15% alcohol for 60 min then only 4.5% alcohol resistant strains gave higher alcohol production (9.5%) than the parent strains. On further exposure to 20% ethanol, there was a decrease in alcohol production.

Keywords *Saccharomyces cerevisiae* AB, alcohol resistance.

Introduction

In view of increasing importance of alcohol as an alternative resource for chemicals and liquid fuel, a great deal of research interest in ethanol fermentation has been generated in recent years[1-5] The major areas of research interests in ethanol fermentation have been : (i) increased ethanol concentration and specific ethanol production rate, (ii) improvement of ethanol tolerance of yeast[6-9] and (iii) development of continuous ethanol fermentation process using high density cell culture[10] In India, due to rising cost of petroleum and the rate of depletion of fluid fossil fuel resources, it is necessary to produce more and more ethanol from agricultural carbohydrate products to satisfy the fuel consumption demand and other chemical industries need[11-17]

The present paper deals with the development of high yielding ethanol resistant strains of *Saccharomyces cerevisiae* to increase the rate of production of ethanol from carbohydrate materials

Results and discussion

(A) Isolation of 10% ethanol resistant strains of Saccharomyces cerevisiae AB The cell suspension of the parent strain, containing 2 4 × 10^7 cells/ml, was then treated with 10% ethanol for 30, 60 and 180 min of incubation at 30 °C After incubation for 30, 60 and 180 min, the cell suspension in each case was diluted and plated out on YPD agar medium 570 isolates were selected from different stages of treatment with 10% ethanol for ethanol production It was observed that the *Saccharomyces cerevisiae* AB 510, gave higher yield of ethanol (7 5%) than the parent strain (4 0%) in medium I The results are shown in Fig. 1 and Table 1

A total of 570 colonies were isolated during the incubation in 10% ethanol It appears from Table 1 and Fig 1 that the maximum number of *Saccharomyces cerevisiae* AB is killed in 10% ethanol during 180 min incubation Only 5 5% of 10% ethanol resistant strains obtained during 30 min incubation gives more ethanol production (7 5%) than the parent strain (40%) whereas maximum killing takes place in 180 min incubation So there is no correlation between the maximum killing and the isolation of more positive variants for higher ethanol production than the parent strain

(B) Isolation of 15% ethanol resistant strains of Saccharomyces cerevisiae AB 510 . The cell suspension of

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EFFECT OF METABOLIC INHIBITORS ON ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE OF AN ETHANOL & TEMPERATURE RESISTANT STRAIN OF *SACCHAROMYCES CEREVISIAE* AB_{100}

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Keywords:

Metabolic inhibitors, *Saccharomyces cerevisiae*, acetic acid

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ABSTRACT

Acetic acid is an important feedstock for many chemicals Several chemical factors are known to cause inhibitory influence on microbial growth which can hamper the acetic acid production also. There are several chemicals that can prevent the growth of *Saccharomyces cerevisiae* as well as acetic acid production, such as- 2,4 DNP, 2-thio uracil, sodium arsenate, sodium arsenite, malonic acid etc. These inhibitors effect the growth & acetic acid production in several ways.

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KINETICS OF ETHANOL PRODUCTION BY AN ETHANOL & THERMO-TOLERANT STRAIN OF *SACCHAROMYCES CEREVISIAE* ISOLATED FROM PINEAPPLE WASTE

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Keywords:

Saccharomyces cerevisiae, Ethanol, Ethanol resistance, Temperature resistance

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ABSTRACT

Ethanol is the main product produced by *Saccharomyces cerevisiae* when grown on sugar containing medium anaerobically. But ethanol itself inhibits the growth of yeast & its own production by feedback inhibition process The exothermic nature of ethanol producing mechanism also inhibits the growth of yeast. So, in this study, we have isolated an ethanol & temperature resistant strain of *Saccharomyces cerevisiae* Y_B, which was originally isolated from North Bengal Pineapple waste disposal material We also studied that, the isolated *Saccharomyces cerevisiae* Y_{B23} strain produces maximum amount of ethanol (4.7%) at pH 4.5, 30°C temperature, 48 hours of incubation period & 10.25×10^5 cells/ml cell density

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STUDIES OF ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE ACTIVITIES DURING ACETIC ACID PRODUCTION BY AN ETHANOL RESISTANT IMMOBILIZED STRAIN OF *SACCHAROMYCES CEREVISIAE* AB_{100}

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ABSTRACT

The wine yeast *Saccharomyces cerevisiae* was immobilized in sodium alginate beads for production of acetic acid. In order to optimize immobilization conditions, a study was conducted using various concentrations of alginate, $CaCl_2$, cell loading, bead diameter etc. The optimized parameters were alginate concentration 4% (w/v), $CaCl_2$ concentration 0.3 (M), cell: alginate ratio 5·4, storage period 24 hrs. and cell bead diameter of 3 mm. In comparison to free cells (1.0678 gm/100ml), the rate of fermentation by immobilized cell proved to be greater (1.395 gm/100ml), showing suitability for acetic acid production.

BT-4

Improvement of Activities of Alcohol Dehydrogenase and Aldehyde Dehydrogenase during Acetic Acid Production in Presence of Some Complex Nutrients by an Ethanol Resistant Strain of *Saccharomyces cerevisiae* AB $_{100}$

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Alcohol Dehydrogenase and Aldehyde Dehydrogenase are oxidoreductases present widely in animal tissues, plants and microorganisms. *Saccharomyces cerevisiae* AB_{100} requires sequential activity of these two enzymes-alcohol dehydrogenase and aldehyde dehydrogenase, respectively, for production of acetic acid from a natural source However, one obstacle to the successful commercialization of this process is the expensive nutrients required in this medium. If a low cost medium can supply all the nutritional requirements (like-nitrogen sources, vitamins, amino acids and trace elements) to sustain viability and productivity, then it would be economically feasible to produce acetic acid by fermentation. Complex nutrients, composed of different vitamins, amino acids and minerals, can increase the yield of acetic acid affecting the two main regulatory enzymes alcohol dehydrogenase and aldehyde dehydrogenase and also makes it cost effective due to easy availability.

In the present study, among various complex nutrients, soybean meal has proved its maximum effectivity by increasing the acetic acid production up to 13.46 gm/L and cell weight up to 4 48 gm/L. Vitamins like- nicotinic acid and D-biotin and amino acids, such as- L-tryptophan and L-glutamic acid also showed their effectivity. Other vitamins, amino acids and complex nutrients had negligible effect on both acetic acid production and activities of alcohol dehydrogenase and aldehyde dehydrogenase

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2BT(10)

Effect of Chemical Nutrients on Alcohol Dehydrogenase and Aldehyde Dehydrogenase activity during Acetic Acid production by an Ethanol resistant strain of *Saccharomyces cerevisiae*

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Abstract :

Alcohol Dehydrogenase and Aldehyde Dehydrogenase are oxidoreductases present widely in animal tissues, plants and microorganism. *Saccharomyces cerevisiae* requires sequential activity of these two enzymes—Alcohol Dehydrogenase and Aldehyde Dehydrogenase respectively, for production of acetic acid from a natural source Alcohol Dehydrogenase acts initially to produce acetaldehyde and then comes Aldehyde Dehydrogenase to produce our desired product i.e. acetic acid. Present study was done in order to assess whether changes in nutrient conditions resulted in any alteration of the enzyme's activity.

Among these nutrients, there are some macro & some micro elements. 10% glucose & 0.5% $(NH_4)_2$, SO_4 were studied as the best carbon and nitrogen source respectively (0.2719 gm/100ml) KH_2PO_4 (0.125%) and $MgSO_4$, $7H_2O$ (0.05%) stimulated the enzyme activities as well as acetic acid production (0.2941 gm/100ml).

Among the micro nutrients, Fe^{3+}, Mn^{2+} and Zn^{2+} (10 µg/ml of each) had maximum positive effect on the activities of both enzymes and therefore maximum production of acetic acid occurred (1.0678 gm/100ml). Cu^{2+} exerted its toxic effect on our organism and thus decreased both the enzyme activities as well as acetic acid production Other trace elements, like- Ni^{2+}, Co^{2+}, V^{3+} and Mo^{6+} showed very insignificant effect.

গবেষণাপত্রের ক্রমিক সংখ্যা BIOT - 14

অ্যালকোহল সহনশীল স্যাকারোমাইসি সেরেভিসি এবি$_{১০০}$ দ্বারা অ্যাসেটিক অ্যাসিড প্রস্তুতি চলাকালীন অ্যালকোহল ডিহাইড্রোজিনেজ ও অ্যাল্ডিহাইড ডিহাইড্রোজিনেজ উৎসেচক দুটির উপর বিভিন্ন কার্বন উৎসের প্রভাব

মধুরিমা চক্রবর্তী, রঞ্জনকুমার বসু, অজিত কুমার বণিক

কেমিক্যাল ইঞ্জিনিয়ারিং বিভাগ, বায়োকেমিক্যাল ইঞ্জিনিয়ারিং উপশাখা
কলিকাতা বিশ্ববিদ্যালয়, ৯২, এ পি সি রোড, কলকাতা-৭০০ ০০৯

শিল্পক্ষেত্রে অ্যাসেটিক অ্যাসিড প্রস্তুতিতে স্যাকারোমাইসিস্ সেরেভিসি এবি$_{১০০}$-এর গুরুত্ব নির্ধারণের জন্য দুটি মুখ্য উৎসেচক অ্যালকোহল ডিহাইড্রোজিনেজ ও অ্যাল্ডিহাইড ডিহাইড্রোজিনেজ-এর উপর বিভিন্ন সুলভ কার্বন উৎসের প্রভাব পরীক্ষা করা হল। এক্ষেত্রে আটটি বিভিন্ন কার্বন উৎস ব্যবহার করা হয়েছে, যেমন— গ্লুকোজ, গ্যালাক্টোজ, অ্যারাবিনোজ, জাইলোজ, মল্টোজ, ল্যাকটোজ, সুক্রোজ এবং স্টার্চ। এদের মধ্যে গ্লুকোজ, প্রস্তাবিত অণুজীবটির নির্ধারিত কার্বন উৎস হিসেবে সর্বাধিক ব্যবহৃত হয়ে থাকে। অবশিষ্ট কার্বন উৎসগুলির বিরল ব্যবহার পরিলক্ষিত হয়। ১০০ গ্রাম/লিটার পর্যন্ত গ্লুকোজের ক্রমবর্ধমান ঘনত্বের সঙ্গে সঙ্গে উৎপাদিত অ্যাসেটিক অ্যাসিডের ঘনত্বও বৃদ্ধি পায় এবং তারপর এটি ক্রমাগত হ্রাস পেতে থাকে। সর্বাধিক উৎপাদিত অ্যাসেটিক অ্যাসিডের ঘনত্ব এবং কোষভর যথাক্রমে ৩.৯৭৮ গ্রাম/লিটার এবং ৩.৬৯ গ্রাম/লিটার। অ্যালকোহল ডিহাইড্রোজিনেজ (২৪ ঘন্টায়) ও অ্যাল্ডিহাইড ডিহাইড্রোজিনেজ (৪৮ ঘন্টায়) উৎসেচক দুটির সর্বাধিক কার্যকারিতাও প্রস্তাবিত গ্লুকোজ ঘনত্বে পরিলক্ষিত হয়।

Effect of Different Carbon Sources on Alcohol Dehydrogenase and Aldehyde Dehydrogenase during the production of Acetic Acid by an Ethanol Resistant strain *Saccharomyces cerevisiae* AB$_{100}$

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The influence of low cost carbon sources on the two major enzymes of acetiq acid *A* production, alcohol dehydrogenase and aldehyde Dehydrogenase, of *Saccharomyces cerevisiae* AB$_{100}$ was studied to ascertain the industrial importance of this organism Eight different carbon sources were used in this study, like - glucose, galactose, arabinose, xylose, maltose, lactose, sucrose and starch , in which the organism used glucose mostly as the desired carbon source. Rest of the sugars were utilized rarely. The produced acetic acid concentration increased with increasing concentration of glucose upto 100 gm/L and after that, it decreases gradually The maximum acetic acid production and cell weight was 3 978 gm/L and 3 69 gm/L respectively

Maximum activity of alcohol dehydrogenase (at 24 hours) and aldehyde dehydrogenase (at 48 hours) was also achieved at this concentration of glucose.

গবেষণাপত্রের ক্রমিক সংখ্যা ঃ এ - ১

স্যাকারোমাইসিস সেরেভিসি -এর অ্যালকোহল সহনশীল প্রজাতি প্রস্তুতি এবং অ্যাসেটিক অ্যাসিড উৎপাদন

মধুরিমা চক্রবর্ত্তী, রঞ্জন কুমার বসু, অজিত কুমার বণিক

কেমিক্যাল ইঞ্জিনিয়ারিং বিভাগ, কলিকাতা বিশ্ববিদ্যালয়

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স্যাকারোমাইসিস সেরেভিসি শিল্পক্ষেত্রে মুখ্য ইথানল উৎপাদক, কারণ - এটি একটি সর্বজনস্বীকৃত নিরাপদ অণুজীব। শিল্পক্ষেত্রে উৎপাদনকালে সঞ্চিত ইথানল ঈষ্ট কোষের বৃদ্ধি এবং উৎপাদনশীলতায় বৈপরীত্য সৃষ্টি করে। সেই কারণবশতঃ, ইথানল সহনশীল ঈষ্ট প্রজাতি খুবই প্রয়োজনীয়।

ইথানলের বিষক্রিয়া দূরীভূত করার জন্য আমাদের গবেষণাগারে *স্যাকারোমাইসিস সেরেভিসি* - র ইথানল সহনশীল প্রজাতি উদ্ভাবন করা হয়েছে। ১০ শতাংশ থেকে ২০ শতাংশ পর্যন্ত বিভিন্ন ঘনত্বের ইথানল ব্যবহার করে ক্রম লঘুকরণ ও পোর প্লেট পদ্ধতির দ্বারা সহনশীলতা প্রদান করা হয়। দেখা যায়, সাবমার্জড কালচার পদ্ধতিতে সহনশীল প্রজাতি, মাতৃ প্রজাতি অপেক্ষা অধিক ইথানল উৎপাদনে সক্ষম। অতঃপর, উৎপাদিত ইথানলকে শেক কালচার পদ্ধতিতে অ্যাসেটিক অ্যাসিডে রূপান্তরিত করা হয়। ১০ শতাংশ ইথানল সহনশীল প্রজাতিগুলোর মধ্যে (মোটপ্রজাতি ১৬ টি) একটি প্রজাতি সর্বাধিক অ্যাসেটিক অ্যাসিড (৫ গ্রাম/লিটার) উৎপাদনে সক্ষম বলে প্রমাণিত হয়। ১০ শতাংশ অধিক ইথানল সহনশীল প্রজাতিগুলো অপেক্ষাকৃত কম অ্যাসেটিক অ্যাসিড উৎপাদন করে।

Development of Alcohol Resistant strain of *Saccharomyces cerevisiae* and production of Acetic Acid

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Ethanol is used as biofuel for automobiles worldwide and Saccharomyces cerevisiae remains the major industrial ethanol producer, beacuse it is a "generally recognized as safe" (GRAS) microorganism During industrial production yeast cells are exposed to the stress due to the accumulation of ethanol which affects the cell growth, activity and productivity of target products. So the ethanol stress tolerant yeast strains are highly desired.

Alcohol resistant strains of Saccharomyces cerevisiae were developed in our laboratory to remove the toxic effects of ethanol. Resistance was done by serial dilution and pour plate method with alcohol concentration from 10% to 20%. By submerged culture method the resistant strain produces more alcohol than parent one. Then this alcohol is converted to acetic acid by shake culture method There we found that, one of the 10% alcohol resistant strains (total 16 strains) produces maximum acetic acid (5gm/ litre). Resistant strains above 10% alcohol concentration produce less acetic acid.