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Parametric Determination for Bioenergetic Transformation of Molasses Pollutant to Ethanol by Saccharomyces Cerevisiae NCIM- 2086

Dr. Ravi Ranjan
TGT, Department of Physical Sciences, S.R.P.S. Govt. +2
School Gardanibagh, Road no-16, Patna, Bihar, India

ABSTRACT

The present investigation deals with the optimization of right conditions for alcoholic fermentation using molasses as the basal fermentation media by *SACCHAROMYCES CEREVISIAE* NCIM- 2086. This strain was subjected to parametric studies. Major effects were caused due to pH value, incubation temperature, incubation period and concentration of molasses. It has been reported that optimum values for alcoholic fermentation has bee found when molasses 16% (w/v) is allowed to ferment for 46 hours at 32°C by maintaining the pH value of the fermentation medium at 4.8 in the presence of yeast Saceharomces cerevisiae NCIM-2086.

Keywords :- Alcoholic fermentation. Saceharomces cerevisiae NCIM-2086.Molasses.

I. INTRODUCTION

Alcohol fermentation is done by yeast and some kinds of bacteria. Production of ethanol from sugary materials is one of the oldest known microbiological processes. These microorganisms convert sugars in ethyl alcohol and carbon dioxide. Alcoholic fermentation begins after glucose enters the cell. The glucose is broken down into pyruvic acid. This pyruvic acid is then converted to CO_2, ethanol, and energy for the cell. Humans have long taken advantage of this process in making bread, beer, and wine. In these three product the same microorganism is used: the common yeast or *Saccharomyces cerevisae*

Parametric studies of different strains causing alcoholic fermertation is done either by classical or statstical techniques[1-7]Fermantation media for alcoholic fermatation should consist of substract necessary for the growth of microorganisms, primarily the carbon, nitrogen and phosphorus sources. Moreover, water and air can be inculded as fermentation substract sources[8-9]. The basic substrates for alcoholic fermentration using submerged technique of fermentation are beet or cane-molasses. The present investigation deals with parametric studies of alcoholic fermentation. Blackstrap molasses ws employed as the basal fermentration media in the fermentor flasks under the submerged fermentation conditions. The study reveales the nutritional status of the organisms and basic fermartation parameters.

II. EXPERIMENTAL

Medium :

The composition of production medium for bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 is prepared as under :

Molasses : 16%; Malt extract : 1.25%; Yeast extract : 1.25%; Peptone : 1.25%; $(NH_4)_2HPO_4$: 1.25%, pH : 4.8

Culture medium

Saccharomyces cerevisiae -2086 was periodically cultured on malt-agar media. The fresh culture media was prepared every fortnight as follows :

Sucrose	: 8%,
Malt extract	: 0.30g
Yeast extract	: 0.30g
Peptone	: 0.40 g
Agar-Agar	: 0.30g
Distilled water	: 100 ml
pH	: 4.8

Sterilization :

The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 30 min.

Strain : *Saccharomyces cerevisiae* - 2086 was used in the present study. The strain was procured from NCL, Pune, India.

Assay methods:

Evalution of ethanol formed and molasses left unfermented was made colorimetrically[134-135]

Age of the inoculum: 46 hours old.

Quantum of the inoculum: 0.5 ml yeast suspension of *Saccharomyces cerevisiae* NCIM-2086

Molasses concentration : 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, 20% and 2%.

Temperature (in ^{0}C) : 15, 20, 30, 32, 35, 38, 40, 45, 50 and 55^{0}C

Incubation period: 10, 15, 30, 35, 40, 46, 50, 55, 60 and 65 hours

pH : 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 4.8, 5.0, 5.2 and 5.6.

III. RESULT AND DISCUSSION

The results obtained from optimization of different parameters show that optimum values for bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 proceeds best when 16% (w/v) molasses solution is allowed to ferment for 46 hours at 32°C by maintaining the pH value of the fermentation medium at 4.8 in the presence of strain of yeast *Saccharomyces cerevisiae* NCIM-2086.

The results of bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 in the present investigation support the view that in general ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086 becomes more easier as the size, molecular weight and the structure complexity of the sugar molecules decreases.It is obvious that the simplest sugars glucose and fructose both are easily fermentable because both the monosaccharide sugars are easily phosphorelated. Galactose fermentability is also much similar to glucose and fructose while arabinose, rhamnose and sorbose were found to be least fermentable.

Sucrose is made from glucose and fructose units. Sucrose a disaccharide sugar has been found suitable for production of ethanol while lactose was found to be unsuitable. However, maltose was fermented to some extent on the basis of total sugar taken. In the group of polysaccharides, starch was found to be least

fermentable and unsuitable for ethanolic fermentation

However, raffinose, inulin and dextrin produced no ethanol. Polyethanol mannitol also could not produce any ethanol by fermentation. On the basis of above observation it is concluded that glucose amongst monosaccharides and sucrose amongst disaccharides are most suitable and useful for ethanolic fermentation.

Table – 1. Effect of different carbohydrates on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086

S.No.	Carbohydrates	Yield of ethanol * in ml/100 ml
1	Arabihose	1.39
2	Rhamnose	1.05
3	Xylose	0.75
4	Glucose	5.90
5	Fructose	5.08
6	Galactose	4.10
7	Sorbose	1.55
8	Lactose	3.45
9	Sucrose	5.90
10	Maltose	1.45
11	Starch	0.95
12	Inulin	-
13	Dextrine	-
14	Raffinose	-
15	Mannitol	-
16	Molasses	5.85

It was interesting to note that in the case of molasses 5.85 ml of ethanol was produced from 20% solution of molasses. Since it is economical, cheapest and richest source of sugar substrate it has been employed as starting raw material for ethanolic fermentation during the course of present investigation. In the present investigation different concentrations of molasses, i. e., from 2% to 25% was employed for ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086 and it was observed that 16% molasses solution (w/v) was found to be most suitable for ethanolic fermentation. Different concentrations of molasses and yields of ethanol has been recorded in the Table-2. It has been observed that lower concentrations of molasses has been found insignificant and therefore, production of ethanol is negligible. On the other hand it has been observed that higher concentrations of molasses interferes with the enzymes activities of *Saccharomyces cerevisiae* NCIM-2086 and therefore, retards the bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086

Hydrogen ion concentrations of the production medium also plays vital role in the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086. The results of the influence of hydrogen ion concentrations (pH) are recorded in table-2. It was observed that production of ethanol at the pH values 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 4.8 was found to be in increasing order. It was further observed that at pH value of 4.8 production of ethanol, i.e., 5.76ml/100 ml was recorded which is maximum. Therefore, it is clear from the table - 2 that the pH of 4.8 is optimum for the production of ethanol (5.76 ml/100 ml) using 16% molasses as a starting material. It was interesting to note that there was a gradual fall in the production of ethanol with the increase of hydrogen ion concentrations from 5.0 and onwards. It was thus, concluded that hydrogen ion concentrations of 4.8 (pH) was most effective and suitable for the optimum (maximum) ethanolic fermentation of molasses and therefore, the pH 4.8 was selected and maintained in the production medium for bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 .

Ethanolic fermentation is also greatly influenced with the temperature. The results recorded in the

table - 2 show that ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086 increases with the increase in temperature from 15°C to 32°C. The fermentative yields of ethanol at lower temperature was found to be minimum, i.e., 3.10 ml/100 ml at 15°C, while the maximum yield of ethanol, 5.80 ml/100 ml was recorded at 32°C. The fermentative yields of ethanol gradually decrease with increase in temperature from 35°C onwards. However, higher temperature, i.e., 38°C onwards were found to be insignificant for production of ethanol. It was thus concluded that the temperature 32°C was most suitable and effective for maximum ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086 and therefore, this temperature was selected and maintained throughout in the present investigation.

In fermentative processes incubation period also plays vital role because it is directly related to the great economy of industry. The results recorded in the table -2 shows that the yields of ethanol increases with the increase in incubation period from 10 hours to 46 hours and then yields of ethanol gradually falls (from 50 hours to 65 hours of incubation period).

The study of the influence of different incubation periods on yields of ethanol from 16% molasses reveals that it proceeds in different phase .The very first phase completes in 10-15 hours where slow molasses consumption is accompanied by poor yields of ethanol. The next second phase occurs during 30 hours and 46 hours of incubation period where molasses consumption and yields of ethanol follows the first phase with slight improvement in the yield of ethanol. After 40 hours of incubation period that the 3rd important and effective last phase begins and the ethanol yields are maximum in this very phase, i.e., in 46 hours. In this way 46 hours of incubation period gives the maximum yield of ethanol, that is 5.70 ml/100 ml. Thus, ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086 was optimized using 16% molasses, 4.8 pH, 32°C temperature and 46 hours of incubation period alongwith some other necessary growth ingredients.

Table -2

Effect of concentration of molasses, pH, temperature and incubation period on bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086

% of Molasses	pH	Temp. in (°C)	Incubation period in hours	Corresponding yield of ethanol* in ml/100ml			
2	2.0	15	10	0.35	2.05	3.10	2.50
4	2.5	20	15	1.32	2.10	3.80	2.80
6	3.0	30	30	1.90	2.45	5.20	4.15
8	3.5	32**	35	2.70	3.00	5.80***	4.80
10	4.0	35	40	3.50	4.80	5.20	5.10
12	4.5	38	46**	4.15	5.76***	****	5.70***
14	4.8**	40	50	4.90	5.60	****	5.40
16**	5.0	45	55	5.82***	****	****	5.08
20	5.2	50	60	5.70	****	****	4.10
25	5.6	55	65	5.60	****	****	4.05

* Each value represents mean of three trials

** Optimum values of molasses solution, pH, temp. and incubation period.

*** Optimum yield of ethanol.

**** Insignificant value.

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