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Bioenergetic Transformation of Molasses Pollutant to Ethanol by Saccharomyces Cerevisiae Ncim-2086 Exposed to Dihydrocoumarin

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ABSTRACT

The efficacy of dihydrocoumarin on bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086 has been assessed. It has been found that the coumarin i.e dihydrocoumarin under trial has stimulatory effect on bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086 and enhances the yield of ethanol to an extent of 7.93103% higher in comparison to control fermentor flasks i.e, 5.35ml/100ml in 46 hours of optimum incubation period 4.8 pH and 320C temperature with 16% (W/V) molasses solution.

Keywords : Molasses, coumarin, alcoholic fermentation, dihydrocoumarin and Saccharomyces cerevisiae NCIM- 2086

I. INTRODUCTION

Coumarins owe their class name to 'coumarou', the vernacular name of the tonka bean (Dipteryx odorata Wild, Fabaceae); From Which coumarin itself was isolated in 1820. Coumarin is classified as a member of the benzopyrone family of compounds, all of which consist of a benzene ring joined to a pyrone ring. The benzopyrones can be subdivided into the benzo-a-pyrones to which the coumarins belong and the benzo-g-pyrones, of which the flavonoids are principal members. Coumarin is a chemical compound which is found naturally in some plants; although it can be synthetically produced as well. It has a distinctive odour. Which has led people to use it as a food additive and ingredient in perfume. Due to concerns about coumarin as a potential liver and kidney toxin; its use as a food additive is heavily restricted, although it is perfectly safe to eat foods which naturally contain the compound.1-5

The distinctive sweet odour reminds many people of freshly cut grass or hay and it has been used in perfumes since the late 1800s. In a pure form, this compound has a crystalline structure, and it is said to taste faintly like vanilla. When ingested, it acts as a blood thinner and it also appears to be effective in trelating some tumors.

Coumarin has fungicidal properties as well. However other much safer substances can be used for all of these purposes although the compound is also used in combination with other blood thinners for medical treatment. 6-10

Their application is in agriculture as ecofriendly pesticides and weed control agents. Naturally occurring coumarins have shown biological and allelopathic potential on a broad range of organisms. Coumarin inhibits seed germination, root growth, histology, water uptake, respiration, photosynthesis, cell elongation, cell division and differentiation.

Literature Survey reveals that a little work has been done on ethyl fermentation with yeast exposed to coumarins, therefore, the authors have employed dihydrocoumarin on bioenergetic transformation of molasses pollutant to ethanol by Sacchoromyces cerevisiae NCIM-2086.

II. EXPERIMENTAL

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

Molasses	:	16 % (w/v)
Malt-Extract	:	1.25%
Yeast-Extract	:	1.25%
Peptone	:	1.25%
Distilled water	:	To make up 100 ml
pH	:	4.8

Distilled water was added to make up the volume up to '100 ml'.

The pH of the medium was adjusted to 4.8 by adding requisite amount of lactic acid and this pH was also ascertained by a pH meter.

Now, the same production medium for bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 fermentor-flasks. The remaining 9 fermentor-flasks out of 99 fermentor-flasks were kept as control and these were also rearranged in 3 subsets each consisting of 3 fermentor flasks.

After preparing the above sets of fermentor flasks, M/1000 solution of dihydrocoumarin was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ml of this solution was added to the fermentor-flasks of first 10 sets respectively. The control fermentor-flask contained no dihydrocoumarin. Now total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of dihydrocoumarin in 1^{st},2^{nd},3^{rd},4^{th}, 5^{th}, 6^{th}, 7^{th}, 8^{th}, 9^{th} and 10^{th} subsets were approximately as given below :

$A \times 10^{-x}M$,
1.0×10^{-5} M,
2.0×10^{-5} M,
3.0×10^{-5} M,
4.0×10^{-5} M,
5.0×10^{-5} M,
6.0×10^{-5} M,
7.0×10^{-5} M,
8.0×10^{-5} M,
9.0×10^{-5} M, and
10.0×10^{-5} M respectively.

Where

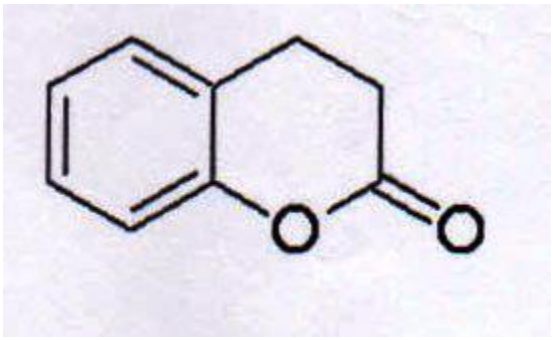
A= amount of dihydrocoumarin in ml, ie;
from1.0 ml _ _ _ _ to 10.0 ml.
x = molarity of the dihydrocoumarin solution.

The above fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 32^0C and analysed colorimetrically after 40, 46, and 50 hours for ethanol formed and molasses sugars left unfermented.

III. RESULTS AND DISCUSSION

The influence of dihydrocoumarin on bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086

DIHYDROCOUMARIN



The data given in the table-1 shows that the coumarin , i.e ; dihydrocoumarin has also been found stimulatory for the bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086. From the data given in the table - 1, it is obvious that dihydrocoumarin influences the ethanolic fermentation process in different phases. The main characteristics of the effect of dihydrocoumarin on bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086 is as under :

(i) Dihydrocoumarin is stimulatory at its all molar concentrations used during the course of the bioenergetic transformation of molasses pollutant to ethanol by *Saccharomyces cerevisiae* NCIM- 2086 , i.e; from 1.0 x 10^{-5} M to 10.0 x 10^{-5} M.

(ii) The molar concentration of the coumarin, i.e; dihydrocoumarin from 1.0 x 10^{-5} M to 7.0 x 10^{-5} M influences the yield of ethanol significanlty and enhances the production of ethanol regularly.

(iii) The other molar concentrations of dihydrocoumarin i.e; from 8.0 x 10^{-5} M, to 10.0 $\times 10^{-5}$M influences the yield of ethanol in almost decreasing manner and could give the production of ethanol approximately with slight differnce, i.e., 5.68995% , 3.96551% and 1.55172% respectively. However, the maximum yield of ethanol has been found at 7.0 $\times$ 10^{-5}M concentration of dihydrocoumarin, i.e., 6.26 ml/100 ml which is 7.93103% higher in comparison to control fermentor flasks.

It has been observed further that after optimum concentration, i.e, 7.0 x 10^{-5}M the addition of the same coumarin i.e, dihydrocoumarin to the production medium causes fall in the yield of ethanol gradually and reached to 0.53956%. However, at all the experimental concentration of coumarin, i.e, dihydrocoumarin used, the yield of ethanol by the *Saccharomyces cerevisiae* NCIM- 2086 has been found higher in comparison to control fermentor flasks. It was interesting to note that at molar concentrations 8.0 x 10^{-5}M and onwards the production of ethanol has been found insignificant.

Table-1. Bioenergetic transformation of molasses pollutant to ethanol by Saccharomyces cerevisiae NCIM- 2086 exposed to dihydrocoumarin

Concentration Of coumarin Used A X 10* M	Incubation Period in hours	Yield of ethanol* in ml/100 ml	Molasses Sugars* left unfermented in g/100 ml	% Difference in yield of ethanol in 46 hours
Control (-) Coumarin	46	5.80	2.33886	-
1.0 x10^{-5}M (+) Coumarin	46	5.85	2.28894	+ 0.86206
2.0 x10^{-5}M (+) Coumarin	46	5.91	2.22896	+ 1.89655

3.0 x10^{-5}M (+) Coumarin	**46**	5.96	2.17891	+ 2.75862
4.0 x10^{-5}M (+) Coumarin	**46**	6.08	2.05895	+ 4.82758
5.0 x10^{-5}M (+) Coumarin	**46**	6.14	1.99888	+ 5.86206
6.0 x10^{-5}M (+) Coumarin	**46**	6.20	1.93887	+ 6.89655
7.0 x10^{-5}M (+) Coumarin	**46**	6.26***	1.87888	+ 7.93103
8.0 x10^{-5}M (+) Coumarin	**46**	6.13	2.00890	+ 5.68965
9.0 x10^{-5}M (+) Coumarin	**46**	6.03	2.10893	+ 3.96551
10.0 x10^{-5}M (+) Coumarin	**46**	5.89	2.24895	+ 1.55172

* Each value represents mean of three trials.

** Optimum concentration of the chemical coumarin used.

*** Optimum yield of ethanol in 46 hours.

(+)Values indicate % increase in the yield of ethanol in comparison to control.

Experimental deviation (+) 1.5–3%.

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