A STUDY ON ETHANOIC FERMENTATION BY THE YEAST EXPOSED TO COUMARIN

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ABSTRACT

The coumarin compounds present in the citrus peel oils are essentially non-volatile. Coumarins is a water-insoluble crystallized powder which has an odour that is pleasant, soft and warm, evoking cut grass or new mown hay, but it's more complex than that; it sometimes even veers into a smell of fresh paint. Coumarins have shown some evidence of many biological activities, although they are approved for few medical uses as pharmaceuticals. In this article, author studies the effect of 7-methoxycoumarin on ethanoic fermentation by the yeast *Saccharomyces cerevisiae* NCIM-2012.

Key-words:- Coumarin, ethanoic fermentation, Saccharomyces cerevisiae NCIM-2012

INTRODUCTION

Ethanol fermentation is a form of anaerobic respiration. Anaerobic respiration used primarily by yeasts when oxygen is not present in sufficient quantity for normal cellular respiration.

The chemical equations below summarize the fermentation of sucrose, whose chemical formula is $C_{12}H_{22}O_{11}$. One mole of sucrose is converted into four moles of ethanol and four moles of carbon-di-oxide.

$$C_{12}H_{22}O_{11} + H_2O + \text{invertase} \rightarrow 2C_6H_{12}O_6$$

$$C_6H_{12}O_6 + \text{Zymase} \rightarrow 2C_6H_{12}O_6$$

C_2H_5OH is the chemical formula for ethanol. Before fermentation takes place, one glucose molecule is broken down into two pyruvate molecules. This process is known as glycolysis. Glycolysis is summarized by the chemical equation:

$$C_6H_{22}O_6 + 2ADP + 2Pi + 2NAD \rightarrow 2CH_3COCOO^- + 2ATP + 2NADH + 2H_2O + 2H^+$$

The chemical formula of pyruvate is $CH_3CO\ COO^-$. Pi stands for the inorganic phosphate. As shown in the reaction, glycolysis causes the reduction of two molecules of NAD^+ to NADH. Two ADP molecules are also converted into two ATP and two water molecules via substrate – level physphorylation.

COUMARINS

Coumarin (2H-1-benzopyran 2 one) is a crystalline white solid when seen pure, with a hay-like, sweet aromatic creamy odour with certain nutty shadings, much used in synthetic form as a fragrance chemical for perfumes and for fragranced soaps and detergents. Coumarin has a widespread occurrence in natural products too and is a representative of the lactones (where a lactone is an ester group integrated into a carbon ring system).

Structure

Chemically, Coumarin is a benzopyrone (1-benzopyran-2-one) which, apart from tonka beans, also occurs naturally in vanilla grass (Anthoxanthum odoratum), sweet woodruff (Galium odoratum), sweet clover (Meliotus L.), sweet grass (Hierochioe odorata) and cassia cinnamon (Cinnamomum aromaticum) among other species. In short, it's rather sweet, and evokes cut grasses.

The Odour Profile of Coumarin

Coumarin is a water-insoluble crystallized powder which has an odour that is pleasant, soft and warm, evoking cut grass or new mown hay, but it's more complex than that, it sometimes even veers into a smell of fresh point! This is what gives Jicky its bracing almost "Petrol" opening which alienates some people. Originally biosynthesized via hydroxylation, glycolysis and cinnamic acid cyclization nowadays coumarin is produced via more sophisticated techniques.

In dilution, coumarin projects with soft hazelnut or almond facets underneath the hay, even licorice; smell Lolita Lempicka (1997). But, in higher concentration it also has spicy fresh and herbaceous facets, no doubt reminiscent of its primary role in different grasses. In combination with Vanilla and bergamot, we're veering into chypre territory. Elixir des Merveilles is a no man's land with its chypre tonalities and gourmand facets.

Effect of 7-Methoxy Coumarin on Ethanol Formation by Yeast

In view of the importance and good physiological activities of the coumarins, the author has employed 7-Methoxycoumarin on ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012. The structure of 7-Methoxycoumarin

Structure

The composition of production medium for the ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012 is prepared as follows:

Molasses	:	22% (w/v)
Malt – Extract	:	1.50%
Yeast Extract	:	1.50%
Peptone	:	1.50%
Distilled water	:	To make 100ml
pH	:	5.0

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

The pH of the medium was adjusted to S.O. by adding requisite amount of lactic acid.

Now, the same production medium to ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012 was prepared for ……. Fermentor-flasks i.e., each containing 100ml of production medium. These formentor-flasks, the remaining 9 fermentor-flasks out of …….. fermentor-flasks were kept as control and

these were also rearranged in …… subsets each containing consisting of 3 fermentor flasks.

Now, …… solution of …… coumarin 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 …… flask contained no coumarin. The total amount in each fermentor-flask was made upto 100ml by adding requisite amount of distilled water.

Thus, the concentration of …….. coumarin in first, second, third, fourth, fifth, sixth, seventh, eighth and ninth and tenth …… were approximately as given below

A	×	$10^{-\times}$ 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
10.0	×	10^{-5} M respectively

Where, A = amount of mutagens in ml, i.e.; from 1.0 ml to 10.0 ml
M=Molarity of solution

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 25^{O}C and analysed colorimetrically after 50,52 and 54 hours for ethanol formed and molasses sugars left unfermented.

Result and Discussion

The results obtained in the study of the influence of 7-methoxycoumarin under trial for ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012 are tabulated in the table given below.

Ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012 exposed to 7-methoxy coumarin

Concentration of mutagen used A×10^{-x}M	Incubation Period of hours	Yield of ethanol* in ml/100 ml	Molasses sugars* left unfermented (g/100ml)	% Difference in yield of ethanol in comparison to control
Control (–) Coumarin	50	6.90	3.01863	-
	52	7.20	2.77681	-
	54	7.00	2.58185	-
1.0×10^{-5} M (+)Coumarin	50	6.95	3.01361	+0.72463
	52	7.25	2.72180	+0.69444
	54	7.05	2.50105	+0.71428
2.0×10^{-5} M (+)Coumarin	50	7.00	2.99854	+1.44927
	52	7.30	2.69362	+1.38888
	54	7.10	2.45462	+1.42857
3.0×10^{-5} M (+)Coumarin	50	7.05	2.94632	+2.17391
	52	7.38	2.64843	+2.50000
	54	7.15	2.39985	+2.14285
4.0×10^{-5} M (+)Coumarin	50	7.10	2.91508	+2.89855
	52	7.45	2.59301	+3.47222
	54	7.20	2.33418	+2.85714
5.0×10^{-5} M (+)Coumarin	50	7.15	2.89375	+3.62318
	52	7.56	2.52416	+5.00000
	54	7.25	2.28182	+3.57142
6.0×10^{-5} M (+)Coumarin	50	7.20	2.82789	+4.34782
	52	7.62	2.49803	+5.83333
	54	7.30	2.22303	+4.28571
7.0×10^{-5} M** (+)Coumarin	50	7.14	2.87412	+3.47826
	52	7.55	2.54693	+4.86111
	54	7.26	2.29982	+3.71428
8.0×10^{-5} M (+)Coumarin	50	7.08	2.93720	+2.60869
	52	7.51	2.56982	+4.30555
	54	7.12	2.31886	+1.71428
9.0×10^{-5} M (+)Coumarin	50	6.98	2.96419	+1.15942
	52	7.43	2.58312	+3.18444
	54	7.08	2.35018	+1.14285
10.0×10^{-5} M (+)Coumarin	50	6.93	3.11562	+0.43478
	52	7.29	2.70413	+1.25000
	54	7.02	2.53186	+0.28571

*Each value represents mean of three trials

**Optimum concentration of the chemical mutagen used

***Optimum yield of ethanol in 52 hours (+) values indicate % increase in the yield of ethanol in comparison to control.

Experimental deviation (±) 1.5-3%

The influence of 7-methoxycoumarin on ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012.

The data recorded in the above table shows that 7-methoxycoumarin has stimulatory effect on ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012.

The maximum yield of ethanol i.e., 7.62 ml/100ml in the presence of 7-methoxy coumarin was observed at 6.0×10^{-5} M molar concentration in 52 hours of optimum incubation period which is 5.83333% higher in comparison to control fermentor-flasks i.e. 7.20ml/100ml in the same times course and other same experimental parameters.

The higher molar concentrations of 7-methoxycoumarin were not much favourable for the ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012. So, the gradual addition of the coumarin, i.e. 7-methoxycoumarin after certain concentration were found not beneficial for the ethanol formation by the yeast *Saccharomyces Cerevisiae* NCIM-2012.

It has been observed that the molar concentraton of the coumarin, i.e. 7-methoxycoumarin from 1.0×10^{-5} M to 6.0×10^{-5} M enhances the yield of ethanol to a certain order being 0.69444%, 1.38888%, 2.50000%, 3.47222%, 5.000% and 5.83333 higher in comparison to control flasks.

It has been observed further that after optimum concentration, i.e. 6.0×10^{-5} M, the addition of the same coumarin, i.e., 7-methoxycoumarin to the production medium causes fall in the yield of ethanol gradually and reached to 1.25000%. However at all the experimental concentration of coumarin i.e. 7-methoxycoumarin used, the yield of ethanol by the yeast *Saccharomyces Cerevisiae* NCIM-2012 has been found higher in comparison to control fermentor flasks.

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