

Production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232 exposed to 4-hydroxy-6-methylcoumarin

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Abstract: The efficacy of 4-hydroxy-6-methylcoumarin on production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232 has been assessed. It has been found that the coumarin, i.e., 4-hydroxy-6-methylcoumarin under trial has stimulatory effect on production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232 and enhances the yield of ethanol to an extent of 6.93548% higher in comparison to control fermentor flasks, i.e., 6.20 ml/100mL in 50 hrs of incubation period, 4.25 pH and 30°C temperature with 18% (w/v) molasses solution.

(Key words : Molasses, ethanol, 4-hydroxy-6-methylcoumarin, *Saccharomyces cerevisiae* NCIM-2232)

Introduction

Coumarin is found naturally in many plants, notably in high concentration in the tonka bean (*Dipteryx odorata*). It also occurs in vanilla grass (*Anthoxanthum odoratum*), sweet woodruff (*Galium odoratum*), sweet grass (*Hierochloa odorata*) and sweet-clover (genus *Melilotus*), which are named for the sweet (i.e., pleasant) smell of the compound.

Other plants with substantial coumarin content are cassia cinnamon (*Cinnamomum cassia*; not to be confused with true cinnamon, *Cinnamomum verum*, or Ceylon cinnamon *Cinnamomum zeylanicum*, which contain little coumarin),¹ deertongue (*Carpheophorus odoratissimus*),² mullein (genus *Verbascum*), and in many cherry blossom tree varieties (of the genus *Prunus*).³ Coumarin is also found in extracts of *Justicia pectoralis*.^{4,5} Related compounds are found

in some but not all specimens of genus *Glycyrrhiza*, from which the root and flavour licorice derives.⁶

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.⁷⁻¹¹ The chemical name for coumarin is benzopyrone. 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 treating 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 sometimes used in combination with other blood thinners for medical treatment¹²⁻²⁰.

Literature survey reveals that a little work has been done on production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232 exposed to coumarins, therefore, the authors have employed 4-hydroxy-6-methylcoumarin on ethanol production by yeast *Saccharomyces cerevisiae* NCIM-2232.

Table - 1
Production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232
exposed to 4-hydroxy-6-methylcoumarin

Concentration of coumarin used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of ethanol*in ml/100ml	Molasses* left unfermented in g/100 ml	% Diff. in the yield of ethanol in 50 hrs
Control	45	5.65	3.22769	-
(-) Coumarin	50	6.20	2.44524	-
	55	6.05	2.00918	-
1.0×10^{-5} M	45	5.74	3.13779	+1.59292
(+) Coumarin	50	6.31	2.33536	+1.77419
	55	6.15	2.05932	+1.65289
2.0×10^{-5} M	45	5.76	3.11780	+1.94690
(+) Coumarin	50	6.38	2.26539	+2.90322
	55	6.21	1.99942	+2.64462
3.0×10^{-5} M	45	5.80	3.07789	+2.65486
(+) Coumarin	50	6.44	2.20536	+3.87096
	55	6.26	1.94932	+3.47107
4.0×10^{-5} M	45	5.86	3.01785	+3.71681
(+) Coumarin	50	6.50	2.14536	+4.83870
	55	6.33	1.94932	+4.62809
5.0×10^{-5} M	45	5.89	3.015621	+4.24778
(+) Coumarin	50	6.53	2.12539	+5.32258
	55	6.32	1.88793	+4.46280
6.0×10^{-5} M	45	5.91	2.96770	+4.60176
(+) Coumarin	50	6.55	2.0958	+5.64516
	55	6.34	1.86925	+4.79338
7.0×10^{-5} M**	45	5.97	2.90764	+5.66371
(+) Coumarin	50	6.63***	2.01528	+6.93548
	55	6.40	1.80927	+5.78512
8.0×10^{-5} M	45	5.85	3.02783	+3.53982
(+) Coumarin	50	6.50	2.14532	+4.83870
	55	6.27	1.93926	+3.63636
9.0×10^{-5} M	45	5.79	3.08783	+2.47787
(+) Coumarin	50	6.38	2.26547	+2.90322
	55	6.21	1.99941	+2.64462
10.0×10^{-5} M	45	5.69	3.18768	+0.70796
(+) Coumarin	50	6.31	2.33556	+1.77419
	55	6.14	2.06932	+1.48760

* Each value represents mean of three trials.

** Optimum concentration of the chemical coumarin used.

*** Optimum yield of ethanol in 50 hours.

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

Experimental deviation (+) 1.5–3%.

Experimental

The composition of production medium for the production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232 is prepared as follows :
Molasses : 18 % (w/v), Malt-Extract : 1.25% Yeast-Extract : 1.25%, Peptone: 1.25%, Distilled water : To make up 100 ml pH: 4.25.

The pH of the medium was adjusted to 4.25 by adding requisite amount of lactic acid.

The same production medium for ethanol fermentation with yeast *Saccharomyces cerevisiae* NCIM-2232 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.

Now, M/1000 solution of 4-hydroxy-6-methylcoumarin 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 to 10th sets respectively. The control fermentor-flask contained no coumarin. The total volume in each fermentor-flask was made upto '100 ml' by adding requisite amount of distilled water.

Thus, the concentration of 4-hydroxy-6-methylcoumarin in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

A × 10^{-x}M,
1.0 × 10⁻⁵ M, 6.0 × 10⁻⁵ M,
2.0 × 10⁻⁵ M, 7.0 × 10⁻⁵ M,
3.0 × 10⁻⁵ M, 8.0 × 10⁻⁵ M,
4.0 × 10⁻⁵ M, 9.0 × 10⁻⁵ M,
5.0 × 10⁻⁵ M, 10.0 × 10⁻⁵ M

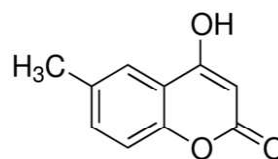
Where, A = amount of coumarins in ml, ie;
from 1.0 ml to 10.0 ml.

and x = molarity of the solution respectively.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30^o C and analysed colorimetrically after 45, 50, and 55 hours for ethanol²¹ formed and molasses sugars²² left unfermented.

Results and Discussion

The influence of 4-hydroxy-6-methylcoumarin on production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232.



4-hydroxy-6-methylcoumarin

The data given in the table-1 shows that the coumarin, i.e., 4-hydroxy-6-methylcoumarin has also been found stimulatory for the ethanol production by yeast *Saccharomyces cerevisiae* NCIM-2232. From the data given in the table- 1, it is obvious that 4-hydroxy-6-methylcoumarin influences the ethanolic fermentation process in different phases. The main characteristics of the effect of 4-hydroxy-6-methylcoumarin on ethanol production by yeast *Saccharomyces cerevisiae* NCIM-2232 is as under.

- (i) 4-hydroxy-6-methylcoumarin is stimulatory at its all molar concentrations used during the course of the production of ethanol by yeast *Saccharomyces cerevisiae* NCIM-2232, i.e; from 1.0 × 10⁻⁵ M to 10.0 × 10⁻⁵ M.
- (ii) The molar concentration of the coumarin, i.e; 4-hydroxy-6-methylcoumarin from 1.0 × 10⁻⁵ M to 7.0 × 10⁻⁵ M influences the yield of ethanol significantly and enhances the production of ethanol regularly.
- (iii) The other molar concentrations of 4-hydroxy-6-methylcoumarin i.e; from 8.0 × 10⁻⁵ M, to 10.0 × 10⁻⁵ M influences the yield of ethanol in

almost decreasing manner and could give the production of ethanol approximately with slight difference, i.e., 4.83870%, 2.90322%, and 1.77419% respectively. However, the maximum yield of ethanol has been found at 7.0×10^{-5} M concentration of 4-hydroxy-6-methylcoumarin,

i.e., 6.63 ml/100 ml which is 6.93548% higher in comparison to control 6.20 ml/100 ml fermentor flasks.

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