

Biotic production of bioethanol exposed to 7-hydroxycoumarin-3-carboxylic acid

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Manuscript received online 26 March 2022, accepted on 24 June 2022

Abstract: In the current communication efficacy of some of the more effective steps has been taken for the significant bioethanol production exposed to some phytochemicals specially 7-hydroxycoumarin-3-carboxylic acid by the microbial strains such as *Thermoanaerobacter matheranii* NT-166, *pseduoethanolicus* NT-120, *T. pentosaceous* NT-132, *Zymomonas mobilis* NT-145 and *Saccharomyces cerevisiae* NT-138.

It has been found that the fungal strain of *S.cerevisiae* NT-138 has been found most successful bioethanol producer. It has been further reported that the phytochemical 7-hydroxycoumarin-3-carboxylic acid at its molar concentration 5.5×10^{-5} M has stimulatory effect on bioethanol production and increases the yield to an extent of 27.611% higher in comparison to control, i.e. 6.55ml/100 ml when 22.5% molasses solution is allowed to ferment for 55 hrs of optimum incubation period 4.8 pH, and 33.5°C temperature along with some other nutritional supplements required by the fungal strain under trial.

(Key words : Bioethanol; coumarin; Molasses; *S.cerevisiae* NT-138; 7-hydroxycoumarin-3-carboxylic acid.

Introduction

The coumarin (benzopyran-2-one, or chromen-2-one) ring system present in natural products such as toka beans warfarin and cloverleaf, displays interesting pharmacological properties. The parent molecule was first isolated by Vogel from tonka beans.

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 (*Hierochloe 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 (*Carphephorus 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 *S.cerevisiae* NT-138 exposed to coumarins, therefore, the authoress have employed 7-hydroxycoumarin-3-carboxylic acid on ethanol production by yeast *S.cerevisiae* NT-138

Experimental

The composition of production medium for the production of ethanol by yeast *S.cerevisiae* NT-138 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 *S.cerevisiae* NT-138 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 7-hydroxycoumarin-3-carboxylic acid 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 7-hydroxycoumarin-3-carboxylic acid in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$$A \times 10^{-x} M,$$

$1.0 \times 10^{-5} M,$	$6.0 \times 10^{-5} M,$
$2.0 \times 10^{-5} M,$	$7.0 \times 10^{-5} M,$
$3.0 \times 10^{-5} M,$	$8.0 \times 10^{-5} M,$
$4.0 \times 10^{-5} M,$	$9.0 \times 10^{-5} M,$
$5.0 \times 10^{-5} M,$	$10.0 \times 10^{-5} M$

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 25, 30, 45, 55, 60, 65 and 70 hours for ethanol²² formed and molasses sugars²³ left unfermented.

Results and Discussion

The influence of 7-hydroxycoumarin-3-carboxylic acid on production of ethanol by yeast *S.cerevisiae* NT-138

The data given in the table-1 shows that the coumarin, i.e., 7-hydroxycoumarin-3-carboxylic acid has been found stimulatory for the ethanol production by yeast *S.cerevisiae* NT-138. From the data given in the table - 1, it is obvious that 7-hydroxycoumarin-3-carboxylic acid influences the ethanolic fermentation process in different phases. The main characteristics of the effect of 7-hydroxycoumarin-3-carboxylic acid on ethanol production by yeast *S.cerevisiae* NT-138 is as under.

- 7-hydroxycoumarin-3-carboxylic acid is stimulatory at its all molar concentrations used

Table -1
Biotic production of bioethanol exposed to 7-hydroxycoumarin-3-carboxylic acid

Concentration of coumarins used A X 10 ^{-x} M	Incubation Period in hours	Yield of bioethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of bioethanol after 55 hours in comparison to control.
Control	25	2.03	7.657	-
	30	3.16	6.527	-
	45	4.29	5.397	-
	55	6.55	4.426	-
	60	5.98	4.362	-
	65	5.65	4.013	-
	70	4.52	3.897	-
2.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.05	4.639	-
	55	7.46	3.517	+ 11.343
	60	6.50	3.470	-
	65	****	-	-
	70	****	-	-
2.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.16	4.528	-
	55	7.59	3.386	+13.283
	60	6.62	3.269	-
	65	****	-	-
	70	****	-	-
3.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.25	3.438	-
	55	7.80	3.178	+16.417
	60	6.78	3.088	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the coumarins used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (+) 1.5–3%. **** Insignificant value

Contd....

Table -1**Biotic production of bioethanol exposed to 7-hydroxycoumarin-3-carboxylic acid**

Concentration of coumarins used A X 10 ^{-x} M	Incubation Period in hours	Yield of bioethanol* in ml/100 ml	Molasses sugars* left unfermented in g/100 ml	% Difference in yield of bioethanol after 55 hours in comparison to control.
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	55	6.55	4.426	-
	60	5.98	4.362	-
	65	5.65	4.013	-
	70	4.52	3.897	-
3.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.45	3.238	-
	55	7.98	2.997	+ 19.104
	60	6.95	2.870	-
	65	****	-	-
	70	****	-	-
4.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.60	3.087	-
	55	8.12	2.858	+21.194
	60	7.09	2.784	-
	65	****	-	-
	70	****	-	-
4.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.76	2.928	-
	55	8.28	2.697	+23.582
	60	7.25	2.579	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the coumarins used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (+) 1.5–3%. **** Insignificant value

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	30	3.16	6.527	-
	45	4.29	5.397	-
	55	6.55	4.426	-
	60	5.98	4.362	-
	65	5.65	4.013	-
	70	4.52	3.897	-
5.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.85	2.837	-
	55	8.38	2.597	+ 25.074
	60	7.35	2.477	-
	65	****	-	-
	70	****	-	-
5.5 × 10 ⁻⁵ M**	25	****	-	-
	30	****	-	-
	45	7.05	2.638	-
	55	8.55***	2.427	+27.611
	60	7.49	2.373	-
	65	****	-	-
	70	****	-	-
6.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.82	2.867	-
	55	8.32	2.658	+24.179
	60	7.26	2.584	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the coumarins used. *** Optimum yield of bioethanol in 55 hours. (+) Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (+) 1.5–3%. **** Insignificant value

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	45	4.29	5.397	-
	55	6.55	4.426	-
	60	5.98	4.362	-
	65	5.65	4.013	-
	70	4.52	3.897	-
6.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.40	3.288	-
	55	7.90	3.075	+ 17.910
	60	7.02	3.059	-
	65	****	-	-
	70	****	-	-
7.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	6.02	3.668	-
	55	7.50	3.477	+11.940
	60	6.63	3.370	-
	65	****	-	-
	70	****	-	-
7.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.85	3.839	-
	55	7.33	3.647	+9.402
	60	6.45	3.574	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the coumarins used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (+) 1.5–3%. **** Insignificant value

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	60	5.98	4.362	-
	65	5.65	4.013	-
	70	4.52	3.897	-
8.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.56	4.128	-
	55	7.05	3.928	+ 5.223
	60	6.17	3.882	-
	65	****	-	-
	70	****	-	-
8.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.35	4.338	-
	55	6.85	4.128	+2.238
	60	5.98	4.080	-
	65	****	-	-
	70	****	-	-
9.0 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.28	4.408	-
	55	6.78	4.197	+1.194
	60	5.90	4.079	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the coumarins used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (+) 1.5–3%. **** Insignificant value

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	60	5.98	4.362	-
	65	5.65	4.013	-
	70	4.52	3.897	-
9.5 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.25	4.438	-
	55	6.75	4.227	+ 0.746
	60	5.83	4.172	-
	65	****	-	-
	70	****	-	-
10 × 10 ⁻⁵ M	25	****	-	-
	30	****	-	-
	45	5.23	4.458	-
	55	6.72	4.257	+0.298
	60	5.79	4.173	-
	65	****	-	-
	70	****	-	-
*****	25	****	-	-
	30	****	-	-
	45	****	-	-
	55	****	-	-
	60	****	-	-
	65	****	-	-
	70	****	-	-

* Each value represents mean of three trials. ** Optimum concentration of the coumarins used. *** Optimum yield of bioethanol in 55 hours. (+)Values indicate % increase in the yield of ethanol in comparison to control. Experimental deviation (+) 1.5–3%. **** Insignificant value

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during the course of the production of ethanol by yeast *S.cerevisiae* NT-138, i.e; from 1.0×10^{-5} M to 10.0×10^{-5} M.

- (ii) The molar concentration of the coumarin, i.e; 7-hydroxycoumarin-3-carboxylic acid from 0.5×10^{-5} M to 5.5×10^{-5} M influences the yield of ethanol significantly and enhances the production of ethanol regularly.
- (iii) The other molar concentrations of 7-hydroxycoumarin-3-carboxylic acid, i.e; from

6.0×10^{-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., 24.179%, 17.910%, 11.940%, 9.402%, 5.223%, 2.238%, 1.194%, 0.746% and 0.298% respectively. However, the maximum yield of ethanol has been found at 5.5×10^{-5} M concentration of 7-hydroxycoumarin-3-carboxylic acid, i.e., 8.55 ml/100 ml which is 27.611% higher in comparison to control 6.55 ml/100ml fermentor flasks.

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