

Natural production of ethanol exposed to 5,7,8-trimethoxycoumarin

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Abstract: Coumarins are a class of phenolic compounds found in plant, microorganism, and sponges that have many biological properties and therapeutic applications. The efficacy of 5,7,8-trimethoxycoumarin on natural production of ethanol by *Saccharomyces cerevisiae* NCIM -2086 has been assessed. It has been found that the coumarin, i.e., 5,7,8-trimethoxycoumarin under trial has stimulatory effect on natural production of ethanol by *Saccharomyces cerevisiae* NCIM -2086 and enhances the yield of ethanol to an extent of 18.823% higher in comparison to control fermentor flasks, i.e., 4.25 ml/100mL in 47 hrs of incubation period, 4.9 pH and 31°C temperature with 16% (w/v) molasses solution.

(Key words : Molasses, Coumarins, Ethanol, 5,7,8-trimethoxycoumarin, *Saccharomyces cerevisiae* NCIM-2086)

Introduction

Plants synthesize many types of phenolic compounds. Depending on their carbon skeletons, these phenolic compounds can be divided into four groups¹⁻³. Coumarins contain a backbone of 1,2-benzopyrone and are classified into four groups; simple coumarins, furanocoumarins, pyranocoumarins, and prenylated coumarins. Simple coumarins include scopoletin, umbelliferone, esculetin, and others⁴. Coumarins are plant secondary metabolites that are produced as either defensive compounds against pathogens^{5,6} or iron chelators in the soil⁷. Biological activities of coumarins and their

derivatives include antibacterial, antiviral, antifungal, anti-inflammatory, anticancer, anticoagulant, and antihypertensive activities⁸. As more impacts of coumarins on human have emerged, natural coumarins are serving as backbones for the synthesis of a range of new potentially useful coumarin derivatives⁹⁻¹².

Model microorganisms have been used to synthesize plant phenolic compounds. Hydroxycinnamic acid, flavonoid, and stilbene have successfully been synthesized in *Escherichia coli*¹³⁻¹⁸.

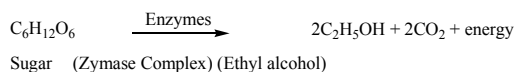
Ethanol is not only found in sugarcane, barley, corn, wheat, and potatoes but the same is also found in natural substances like petroleum and oils. There are various methods to extract natural alcohol from plant oil, one of which is distillation on natural oils, this process is often known as steam distillation.

In 2023, global ethanol consumption was over 131 million metric tons, which is back to pre-pandemic levels. In 2024, consumption is expected to surpass this, reaching almost 149 million metric tons by 2030.

A primary industrial alcohol is ethanol, or ethyl alcohol (C₂H₅OH). Alcoholic fermentation is one of the oldest, best known and most important of industrial fermentations. In this process, ethyl alcohol is produced from

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carbohydrate materials (such as sugars) by yeasts. The process is extracellular. The overall biochemical change accomplished by the yeast is represented by the following equation.



Market size

The global ethanol market was valued at USD 103.62 billion in 2023, and is expected to reach USD 182.88 billion by 2034.

Ethanol, also known as ethyl alcohol, is a colorless chemical compound with a pleasant taste and odor. Naturally, it is made by the fermentation of sugar and starch-based biological materials and cellulosic feedstock, including wood, sugarcane, barley, corn, and wheat. Synthetically, the product is synthesized from coal, natural gas, and ethylene.

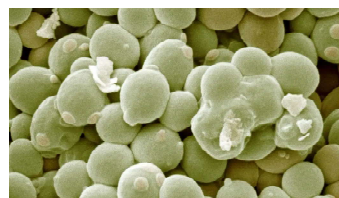
The increasing product advancement to make it more sustainable, coupled with the rising product demand from beverages, fuel and fuel additives, and solvents, is expected to boost market growth. The rising government regulations to produce eco-friendly products and high product demand from disinfectant applications are predicted to surge the market during the forecast period. Source: <https://www.fortunebusinessinsights.com/industry-reports/ethanol-market-101567>

Literature survey reveals that a little work has been done on natural production of ethanol with yeast exposed to coumarins, therefore, the authors have employed 5,7,8-trimethoxycoumarin on natural production of ethanol by *Saccharomyces cerevisiae* NCIM -2086.

Experimental

Microorganism used :

Saccharomyces cerevisiae.



Saccharomyces cerevisiae

Molasses :



Molasses

The composition of production medium for the ethanolic fermentation by *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: 4.9

The pH of the medium was adjusted to 4.9 by adding requisite amount of lactic acid.

The same production medium for ethanolic fermentation 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.

Now, M/1000 solution of 5,7,8-trimethoxycoumarin 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

Table - 1
Natural production of ethanol exposed to 5,7,8-trimethoxycoumarin

Concentration of coumarin used A X 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 47 hrs
Control	25	2.00	5.369	—
	47	4.25	3.118	—
	69	3.15	2.995	—
1.0 × 10 ⁻⁵ M	25	2.08	5.289	—
	47	4.40	2.968	+ 3.529
	69	3.20	2.910	—
2.0 × 10 ⁻⁵ M	25	2.15	5.220	—
	47	4.50	2.869	+ 5.882
	69	3.25	2.815	—
3.0 × 10 ⁻⁵ M	25	2.20	5.166	—
	47	4.55	2.820	+ 7.058
	69	3.30	2.784	—
4.0 × 10 ⁻⁵ M	25	2.25	5.125	—
	47	4.60	2.766	+ 8.235
	69	3.35	2.705	—
5.0 × 10 ⁻⁵ M	25	2.30	5.066	—
	47	4.80	2.566	+ 12.941
	69	3.40	2.511	—
6.0 × 10 ⁻⁵ M**	25	2.40	4.965	—
	47	5.05***	2.316	+ 18.823
	69	3.45	2.284	—
7.0 × 10 ⁻⁵ M	25	2.35	5.018	—
	47	5.00	2.371	+ 17.647
	69	3.50	2.316	—
8.0 × 10 ⁻⁵ M	25	2.34	5.030	—
	47	4.95	2.417	+ 16.470
	69	3.54	2.359	—
9.0 × 10 ⁻⁵ M	25	2.28	5.089	—
	47	4.90	2.473	+ 15.294
	69	3.58	2.384	—
10.0 × 10 ⁻⁵ M	25	2.18	5.188	—
	47	4.80	2.567	+ 12.941
	69	3.62	2.417	—

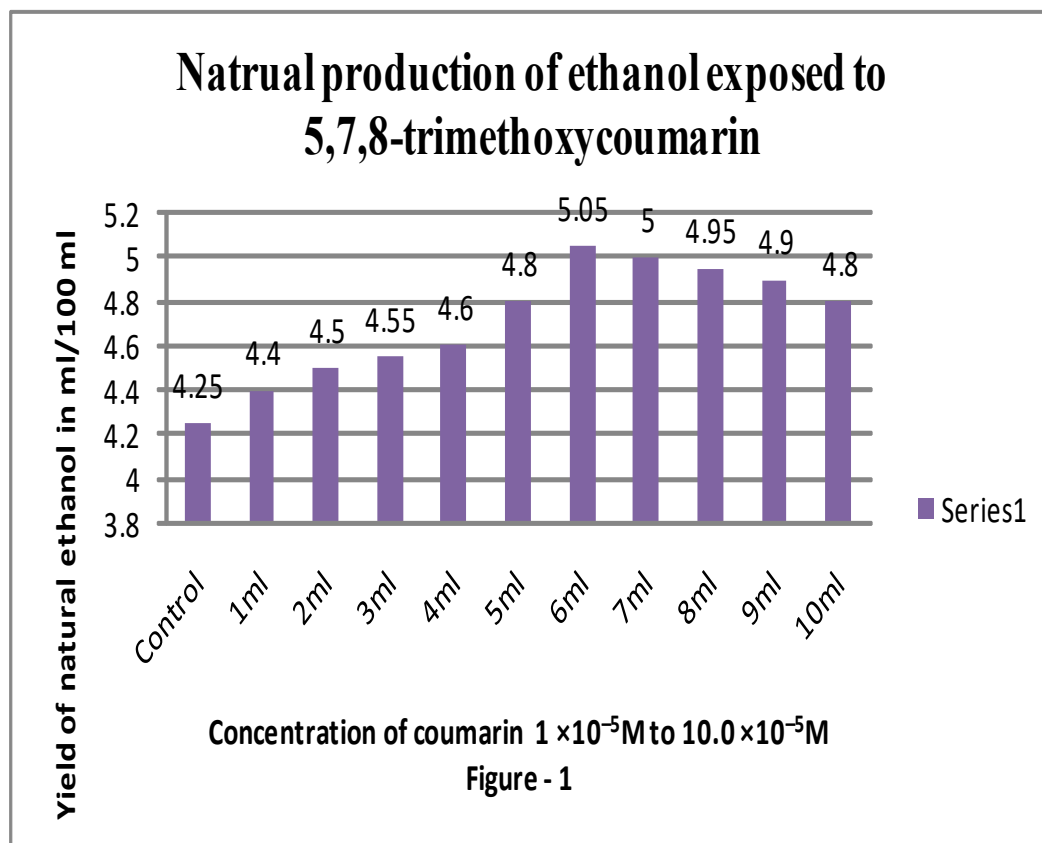
* Each value represents mean of three trials.

** Optimum concentration of the chemical coumarin used.

*** Optimum yield of ethanol in 47 hours.

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

Experimental deviation (±) 1.5–3%.



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 5,7,8-trimethoxycoumarin in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :

$$A \times 10^{-x} \text{M}, \\ 1.0 \times 10^{-5} \text{M to } 10.0 \times 10^{-5} \text{M}$$

Where,

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

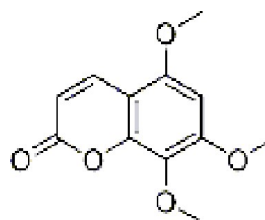
and x = molarity of the solution respectively.

The fermentor-flasks were then steam

sterilized, cooled, inoculated, incubated at 31°C and analysed colorimetrically after 25, 47, and 69 hours for ethanol¹⁹ formed and molasses sugars²⁰ left unfermented.

Results and Discussion

The influence of 5,7,8-Trimethoxycoumarin on natural production of ethanol by *Saccharomyces cerevisiae* NCIM -2086



5,7,8-Trimethoxycoumarin

The data recorded in the table-1 shows that 5,7,8-trimethoxycoumarin has stimulatory effect on ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086.

The data (table-1) reveals that the coumarin, i.e., 5,7,8-trimethoxycoumarin stimulates the ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086 and enhances the yield of ethanol upto its (5,7,8-trimethoxycoumarin) concentrations from 1.0×10^{-5} M to 6.0×10^{-5} M in two phases :

In the first phase, ie; from 1.0×10^{-5} M to 6.0×10^{-5} M the effect of 5,7,8-trimethoxycoumarin on the productivity (the yield) of ethanol was gradually in increasing order and achieves its best function at 6.0×10^{-5} M where maximum yield of ethanol, i.e; 5.05 ml/100 ml is obtained in 47 hours of optimum incubation period which is 18.823% higher in comparison to control fermentor flasks (4.25 ml/100 ml).

In the second phase of coumarins effect the molar concentrations, ie; from 7.0×10^{-5} M to

10.0×10^{-5} M the production of ethanol has been enhanced but the order of ethanol productivity is found reversed in respect to increasing molar concentrations of 5,7,8-trimethoxycoumarin. However, the ethanol formation by the yeast *Saccharomyces cerevisiae* NCIM-2086 under the influence of each concentration of 5,7,8-trimethoxycoumarin used has been stimulating and the yield of ethanol has been found greater than that obtained in the control fermentor flasks. Concentration of 5,7,8-trimethoxycoumarin from 1.0×10^{-5} M to 6.0×10^{-5} M .

Productivity of ethanol: 3.529%, 5.882%, 7.058%, 8.235%, 12.941%, and 18.823%
Concentration of 5,7,8-trimethoxycoumarin from 7.0×10^{-5} M to 10×10^{-5} M .
Productivity of ethanol : 17.647%, 16.470%, 15.294% and 12.941%.

Thus, it is concluded that 5,7,8-trimethoxycoumarin at lower concentrations is found stimulatory and at higher concentrations it is deterioratory for ethanolic fermentation by *Saccharomyces cerevisiae* NCIM -2086.

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