

## Efficacy of some fluorogenic AMC compounds on bioproduction of citric acid

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**Abstract :** Fluorogenic AMC compound, i.e., 7-amino-4-methylcoumarin can be used to monitor enzyme production during fermentations, allowing for optimization of fermentation condition. In fermentation technology, various compounds can serve as substrates, inhibitors or modifiers of enzyme activity. The efficacy of 7-amino-4-methylcoumarin (AMC) on bioproduction of citric acid by GRAS fungal strains such as *Aspergillus awamori* NCIM-1869, *A. foetidus* NCIM-1859, *A. nidulans* NCIM-1651, *A. oryzae* NCIM-1756 and *Aspergillus niger* NCIM-1856 has been studied. It has been found that *A. niger* NCIM-1856 is best producer of citric acid. The compound AMC has beneficial impact on production of citric acid by *A. niger* NCIM-1856 and upgraded the yield of citric acid to an extent of 8.421% higher in comparison to control under the optimized parameters.

**(Keywords :** Citric acid, GRAS, *A. niger* NCIM-1856, AMC).

### Introduction

Coumarins are a class of compounds found in plants, fungi, and bacteria, with potential applications. In fermentation technology coumarins and citric acid fermentation have a relationship that's been explored in research, particularly regarding the impact of coumarins on citric acid bioproduction. Studies have shown that certain coumarins can effect citric acid production by *Aspergillus niger*, a fungus commonly used in citric acid fermentation.

The exact mechanism of how coumarins affect citric acid production is not fully understood, but its likely related to the impact of compounds on microbial growth and metabolism. Researchers are exploring ways to optimize fermentation conditions, including the use of coumarins to improve citric acid yields and reduce production costs. Elucidating the biochemical pathways involved in citric acid production and how coumarins interact with these pathways could leads to new strategies for improving fermentation efficiency.

Coumarins in the field of biotechnology has assumed great importance<sup>1-5</sup>. Some coumarins and its derivatives are also used in different fields to-day and many attempts have been taken to establish the structure-activity relationship of some coumarins derivative. The correlation of chemical structure with anticoagulant activity of some coumarins derivatives have also been studied by some workers<sup>6-15</sup>. Literature survey reveals that is little research work has been done on efficacy of some coumarins on citric acid fermentation<sup>16-24,25</sup>. Therefore, the authoress has employed 7-amino-4-methylcoumarin on citric acid fermentation by *A. niger* NCIM-1856

### Experimental

The impact of 7-amino-4-methylcoumarin on production of citric acid by *Aspergillus niger* NCIM-1856. The composition for production of citric acid by *Aspergillus niger* NCIM-1856 has been prepared as follows :

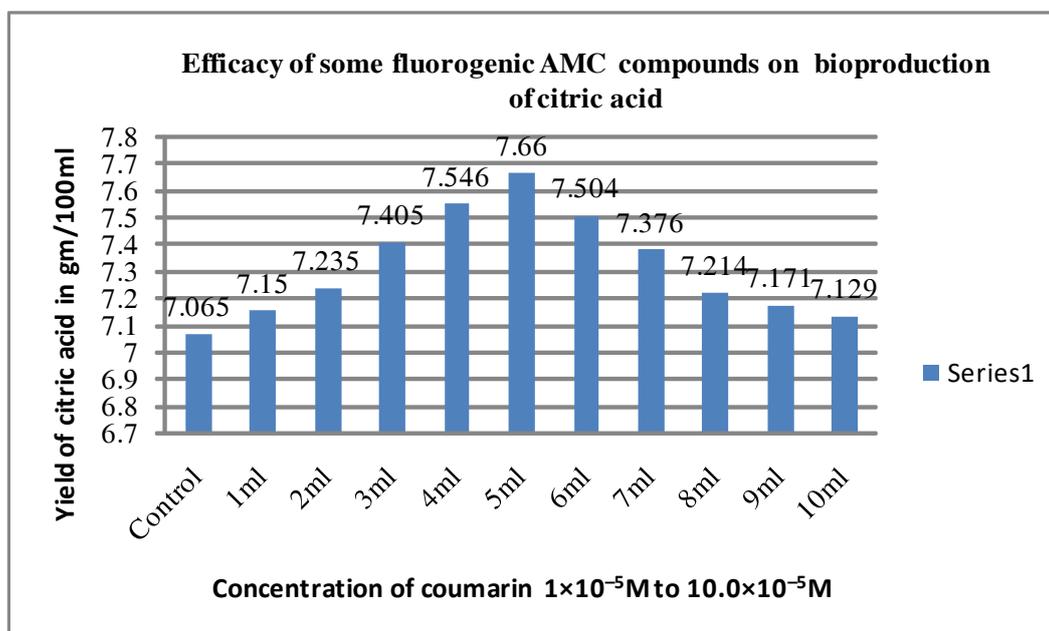
**Table - 1**  
**Efficacy of some fluorogenic AMC compounds on bioproduction of citric acid**

Concentration of coumarin used $A \times 10^{-5} M$	Incubation Period in days	Yield of citric acid* in g/100 ml	Sucrose* left Unfermented in g/100 ml	% difference of citric acid increased after 8 days
Control	8	7.065	3.194	–
$1.0 \times 10^{-5} M$	8	7.150	3.190	+1.203
$2.0 \times 10^{-5} M$	8	7.235	3.185	+2.406
$3.0 \times 10^{-5} M$	8	7.405	3.182	+4.812
$4.0 \times 10^{-5} M$	8	7.546	3.178	+6.808
$5.0 \times 10^{-5} M^{**}$	8	7.660***	3.173	+8.421
$6.0 \times 10^{-5} M$	8	7.504	3.177	+6.213
$7.0 \times 10^{-5} M$	8	7.376	3.179	+4.401
$8.0 \times 10^{-5} M$	8	7.214	3.181	+2.108
$9.0 \times 10^{-5} M$	8	7.171	3.188	+1.500
$10.0 \times 10^{-5} M$	8	7.129	3.189	+0.905

\* Mean of three observations \*\*Optimum concentration of coumarin

\*\*\* Optimum yield of citric acid (+) ve values indicate % increase in the yield of citric acid

Experimental deviation ( $\pm$ ) 1.5% to 3.5%



Sucrose: 16% w/v,  $\text{NH}_4\text{NO}_3$  : 0.45%  
 $\text{KH}_2\text{PO}_4$  : 0.45%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  : 0.50 %  
 pH: 2.1

The pH of the production medium was adjusted to 2.1 by adding requisite amount of KCl-HCl buffer solution and this pH was also ascertained by a pH meter.

The above composition medium represents volume of a fermentor flask, i. e., "100ml" production medium for citric acid fermentation by *Aspergillus niger* NCIM-1856. Now, the same production medium for citric acid fermentation by *Aspergillus niger* NCIM-1856 was prepared for 99-fermentor flask, i. e.; each contained 100ml of production medium. Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water.

Thus, the molar concentration of 7-amino-4-methylcoumarin in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th 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

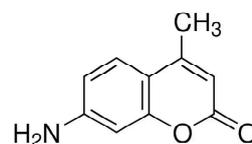
A = amount of 7-amino-4-methylcoumarin in ml, i.e., 1.0 ml.. to 10 ml.

x = Molarity of the 7-amino-4-methylcoumarin solution.

The above fermentor flasks were then

sterilized, cooled inoculated, incubated at 29°C and analysed colorimetrically after 6, 8 and 10 days for citric acid<sup>26</sup> formed and sucrose<sup>27</sup> left unfermented during the course of present investigation.

## Results and Discussion



7-amino-4-methylcoumarin

The data recorded in the table-1 shows that 7-amino-4-methylcoumarin was found to be significant for higher promotion of citric acid up to its concentration from  $1.0 \times 10^{-5}$  to  $5.0 \times 10^{-5} \text{ M}$  and above this concentration the the production of citric acid for citric acid production by *Aspergillus niger* NCIM-1856 was found to be decreasing. However, the production of citric acid by the production of citric acid by *Aspergillus niger* NCIM-1856 used were higher than that of control flasks.

The maximum yield of citric acid was observed at  $5.0 \times 10^{-5} \text{ M}$  concentration of 7-amino-4-methylcoumarin, i.e., 7.660 g/100 ml in 10 days of optimum incubation period which is 8.421% higher in comparison to control, i.e., 7.065g/100 ml in the same experimental conditions and optimum incubation period.

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