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Research Article

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Mycological Production of Citric Acid Exposed to 3-Acetamidocoumarin

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ABSTRACT

The mycological production of citric acid by Aspergillus niger NCIM-683 has been carried out in the presence of 3-acetamidocoumarin. The effect of coumarin compound on mycological production of citric acid has been observed and it was found that coumarin compound enhances the yield of citric acid to an extent of 7.793% higher in comparison to control fermentor flasks i.e. 6.880 g/100ml in 8 days of optimum incubation period, 1.8 pH and 30 ^{0}C temperature with 20% (w/v) molasses solution.

Keywords: Molasses; Citric acid fermentation; 3-acetamidocoumarin; Aspergillus niger NCIM-683

INTRODUCTION

Coumarins comprise a very large class of compounds found throughout the plant kingdom. They are found at high levels in some essential oils, particularly cinnamon bark oil (7,000 ppm), cassia leaf oil (up to 87,300 ppm) and lavender oil. Coumarin is also found in fruits (e.g. bilberry, cloudberry), green tea and other foods such as chicory (Lake, 1999). Most coumarins occur in higher plants, with the richest sources being the Rutaceae and Umbelliferone.

It is used for its fragrance in many personal care products (perfumes, deodorants, soaps and in tobacco), in household and industrial products to masks unpleasant odour and in some countries as flavouring agent in food and beverages. It has also been used to treat several medical conditions. The fermentation product of coumarin in dicoumarol which is a potent anticoagulant. However natural coumarins are rendered inactive in human gastrointestinal tract and on oral administration of the therapeutic doses these herbs are not considered to anticoagulant. Coumarins are considered as phytoalexins since plants produce them as defence subantances when woonded are attacked by other organism [1-7].

Coumarines in the field of biotechnology has assumed great importance. Some coumarines and its derivatives are also used in medicine today and many attempts has been established the structure activity relationship of some coumarin derivatives. The correlation of chemical structure with anticoagulant activity of some coumarin derivatives have also been studied by many workers [8-36]**.**

Literature survey reveals that a little work has been done on efficacy of coumarins on citric acid fermentation therefore the authors have employed 3-acetamidocoumarin on citric acid fermentation by *Aspergillus niger* NCIM-683

EXPERIMENTAL SECTION

The influence of 3-acetamidocoumarin on mycological production of citric acid by *Aspergillus niger* NCIM-683. The composition of the production medium for mycological production of citric acid by *Aspergillus niger* NCIM-683 has been prepared as follows.

Molasses: 20% (w/v), NH_4NO_3:0.60%, KH_2PO_4:0.06%, $MgSO_4.7H_2O$:0.60%, pH: 1.8

The pH of the production medium was adjusted to 1.8 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 the fermentor flask i.e. "100 ml" production medium for mycological production of citric acid by *Aspergillus niger* NCIM-683 was prepared for 99-fermentar flask i.e. each contained 100 ml of the production medium.

The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then arranged in 3-subsets each consisting of 3-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.

After preparing the above sets of fermentor flasks M/1000 solution 3-acetamidocoumarin was prepared and from the above coumarine solution 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml was added to the fermentation flasks of above I^{st} to 10^{th} sets respectively. The control fermentor flasks contained no coumarin.

Now, the total volume in each fermenter flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of 3-acetamidocoumarin in 1^{st} , 2^{nd} , 3^{rd} , 4^{th} , 5^{th} , 6^{th} , 7^{th} , 8^{th} , 9^{th} and 10^{th} subsets were approximately as given below-

$AX10^{-5}$ M i.e.

$1.0X10^{-5}$	$6.8X10^{-5}$M
$2.0X10^{-5}$	$7.0X10^{-5}$M
$3.0X10^{-5}$	$8.0X10^{-5}$M
$4.0X10^{-5}$	$9.0X10^{-5}$M
$5.0X10^{-5}$	$10.0X10^{-5}$M

A=Amount of coumarin in ml i.e. 1.0 ml ……..10.0 ml

X= Molarity of the coumarin solution

The above fermentor flasks were then sterilized, cooled inoculated and incubated at 30 ^oC and analyzed after 6,8, and 10 days for citric acid formed.

Table 1: Mycological production of citric acid exposed to 3-acetamidocoumarin

Concentration of coumarin used $AX10^{-5}$M	Yield of citric acid * in g/100 ml			% of citric acid increases (+) in 8 days optimum incubation period
	06 Days	08 Days	10 Days	
Cotrol	5.795	6.89	6.65	
$1.0X10^{-5}$ M	5.859	6.987	6.737	(+) 1.407
$2.0X10^{-5}$ M	5.87	7	6.743	(+) 1.596
$3.0X10^{-5}$ M	5.877	7.015	6.757	(+) 2.814
$4.0X10^{-5}$ M	5.94	7.153	6.877	(+) 3.817
$5.0X10^{-5}$ M	6.009	7.288	6.949	(+) 5.776
$6.0X10^{-5}$ M**	6.169	7.427	7.155	(+) 7.793
$7.0X10^{-5}$ M	6.106	7.365	7.089	(+) 6.894
$8.0X10^{-5}$ M	6.001	7.22	6.943	(+) 4.789
$9.0X10^{-5}$ M	5.899	7.089	6.818	(+) 2.888
$10.0X10^{-5}$ M	5.893	7.076	6.814	(+) 2.699

* Mean of three observations

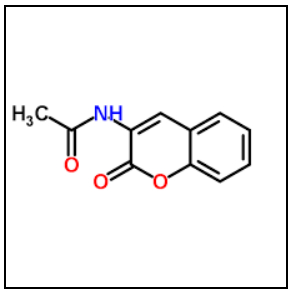
** Optimum concentration of coumarin

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

Experimental deviation (+/-) 1.5% to 3.5%

RESULT AND DISCUSSION

The data recorded in table-1 shows that the coumarin compound 3-acetamidocoumarin was found to be increasing upto its concentration from $1.0X10^{-5}$ M to $6.0X10^{-5}$ M but it is less effective. It has also been observed that the gradual addition of 3-acetamidocoumarin to the fermentation medium gradually increases the mycological production of citric acid by *Aspergillus niger* NCIM-683. The productions of citric acid on these concentrations were not very much significant and could favour mycological production of citric acid by *Aspergillus niger* NCIM-683 in the range from 1.407% to 7.793% only.



CONCLUSION

It has been observed that higher concentration of 3-acetamidocoumarin i.e. on $6.0X10^{-5}$M and onwards has retarded mycological production of citric acid by *Aspergillus niger* NCIM-683.
The maximum yield of citric acid has been recorded at $6.0X10^{-5}$M concentration of 3-acetamidocoumarin i.e. 7.427g/100 ml in 6 days of optimum incubation period which is 7.793% higher in comparison to the control fermentor flasks i.e. 6.880 g/100 ml in the same set of experimental parameters for mycological production of citric acid by *Aspergillus niger* NCIM-683.

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