

Assessment of citric acid production in the presence of 3-[4-(bromomethyl)phenyl]-7-(diethylamino)

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Abstract : The efficacy of 3-[4-(bromomethyl) phenyl]-7-(diethylamino) on bioproduction of citric acid by some fungal strains such as *Aspergillus carbonarius* NCIM -1962, *Aspergillus saitoi*, NCIM -1364, *Aspergillus usumii* NCIM -1576, *Aspergillus wentii* NCIM -1981 and *Aspergillus niger* NCIM -2690 has been assessed. It has been found that the fungal strain *Aspergillus niger* NCIM -2690 has been found most suitable to give higher yield of citric acid. The coumarin; i.e. 3-[4-(bromomethyl) phenyl]-7-(diethylamino) under trial has stimulatory effect on bioproduction of citric acid and enhances the yield of citric acid to an extent of 12.324% higher in comparison to control fermenter flasks, i.e., 6.776 g/100 ml in 12 days of optimum incubation period, 2.0pH and 25°C temperature with 20% (w/v) molasses solution alongwith other nutritional ingredients.

(Key words : Molasses, citric acid fermentation, 3-[4-(bromomethyl) phenyl]-7-(diethylamino) and *Aspergillus niger* NCIM-2690)

Introduction

Coumarin can be prepared by a number of name reactions, with the Perkin reaction between salicylaldehyde and acetic anhydride being a popular example. The Pechmann condensation provides another route to coumarin and its derivatives, as does the Kostanecki acylation, which can also be used to produce chromones. 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.

Coumarin, an organic compound having the characteristic odour of new-mown hay, obtainable from the tonka tree (native to Guyana) or by chemical synthesis. It is used in perfumes and flavourings and for the preparation of other chemicals. Coumarins in the field of biotechnology has assumed great importance¹⁻¹⁵. Some coumarins and its derivative are also used in medicine today. The corelation of chemical structure with anticoagulant activity of some coumarin derivatives has also been studied by many workers¹⁶⁻³⁴. Literature survey reveals that a little work has been done on different fermentative products with respective microorganism³⁵⁻³⁹ exposed to coumarins, therefore, the author has employed 3-[4-(bromomethyl) phenyl]-7-(diethylamino) on production of citric acid by *Aspergillus niger* NCIM -2690.

Experimental

Assessment of citric acid production in the presence of 3-[4-(bromomethyl) phenyl]-7-(diethylamino)

The composition of the production medium for citric acid fermentation by *Aspergillus niger* NCIM-2690 has been prepared as follows :
Molasses : 20% (w/v), NH_4NO_3 : 0.25%
 KH_2PO_4 : 0.25%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.30%, pH : 2.0

The pH of the production medium was adjusted to 2.0 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" citric acid fermentation by *Aspergillus niger* NCIM-2690. Now, the same production

Table - 1
Assessment of citric acid production in the presence of
3-[4-(bromomethyl)phenyl]-7-(diethylamino)

Concentration of coumarin used $A \times 10^{-x}M$	Yields of citric acid* in g/100 ml			% of Citric acid increased after 12 days
	10 Days	12 Days	14 Days	
Control	5.169	6.775	7.665	-
$1.0 \times 10^{-5}M$	5.225	6.850	5.727	+ 1.107
$2.0 \times 10^{-5}M$	5.282	6.925	5.789	+ 2.214
$3.0 \times 10^{-5}M$	5.401	7.080	5.919	+ 4.501
$4.0 \times 10^{-5}M$	5.494	7.202	6.021	+ 6.302
$5.0 \times 10^{-5}M$	5.551	7.278	6.084	+ 7.424
$6.0 \times 10^{-5}M$	5.623	7.372	6.163	+ 8.811
$7.0 \times 10^{-5}M$	5.722	7.500	6.271	+ 10.701
$8.0 \times 10^{-5}M^{**}$	5.804	7.610***	6.361	+ 12.324
$9.0 \times 10^{-5}M$	5.598	7.338	6.135	+ 8.309
$10.0 \times 10^{-5}M$	5.401	7.080	5.919	+ 4.501

* Each value represents mean of three trials ** Optimum concentration of coumarin used

***Optimum yield of citric acid (+) values indicate % increase in the yield of citric acid after 12 days.
 Experimental deviation ($\pm$) 1.5-3%

medium for citric acid fermentation by *Aspergillus niger* NCIM-2690 was prepared for 99-fermentor flask, i. e; each contained '100ml' of production medium. The above 99-fermentor flasks were then arranged to 11-sets each comprising of 9-fermentor flasks. Each set was then rearranged 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 of 3-[4-(Bromomethyl)phenyl]-7-(diethylamino) was prepared and from the above coumarin 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 1st to 10th sets respectively. The control fermentor flasks contained no coumarin. 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 3-[4-(Bromomethyl)phenyl]-7-(diethylamino) in

1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

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

Where : A = amount of chemical coumarin, in ml,
 i.e. 1.0 ml to 10 ml.

x=Molarity of the coumarin solution and respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at $25^{\circ}C$ and analysed after 10, 12 and 14 days for citric acid⁴⁰ formed.

Results and Discussion

The data recorded in the table-1 shows that 3-[4-(bromomethyl) phenyl]-7-(diethylamino) was found to be significant for higher promotion of citric acid up to its concentration from 1.0×10^{-5} to $8.0 \times 10^{-5} M$ and above this concentration the citric acid fermentation by *Aspergillus niger* NCIM-2690 was found to be decreasing. However, citric acid fermentation by *Aspergillus niger* NCIM-2690

used were higher than that of control flasks.

The maximum yield of citric acid was observed at 8.0×10^{-5} M concentration of 3-[4-(bromomethyl)phenyl]-7-(diethylamino) i.e., 7.610g/ 100 ml in 12 days of optimum incubation

period which is 12.324% higher in comparison to control, i.e., 6.775g/100 ml in the same experimental conditions and optimum incubation period.

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