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Influence Of Different Coumarin Derivatives On Mycological Production Of Citric Acid By *Aspergillus Niger* Mutagens

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ABSTRACT:

Citric acid are being used as industrial raw material due to its expanding applications. The growing market and demands there are always need to develop better technique and procedure to enhance the production of yield and also the efficacy of product recovery. The efficacy of different coumarin derivatives was studied on citric acid production with the help of *Aspergillus niger* NCIM-683.

Keywords: Citric acid, *Aspergillus niger* NCIM-683, Coumarin.

INTRODUCTION:

The microbial production of citric acid became important after World War I. In 1893 Wehmer discovered that fungi have ability to produce citric acid and now a day's commercially citric acid produced mostly comes from mold fermentation. An American food chemists James Curie in 1917 found out that many strains of *Aspergillus niger* are efficient citric acid producers whereas Pfizer in 1919 and Citrique Belge in 1929 used this technique to produce citric acid at industrial level.

The factors which are responsible to produce citric acid by fungal strains are molasses and its concentration which are basically carbohydrate source. The other factors are hydrogen ion concentration, temperature, incubation period as well as incorporation of trace metals.

Citric acid is being used as acidulants in pharmaceuticals and food industry due to its

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low toxicity. The low toxicity increased the worldwide demand of citric acid in other applications like cosmetics, toiletries etc. Now the Global demand of it are reached more than 1.4 million tones and annual growth are more than 4% with respect to demand/consumption ratio. Thus the way which have potential to enhance the productivity of citric acid always have possibility of attention to producers and hence all possible ways should be considered to achieve it.

The composition medium is one of the key controlling factors during production of acid. The selection of strains such as hybrid or mutants which are developed by certain producers play an important role to increase the yield percentage of citric acid up to certain level.

Submerged fermentation of molasses are generally used for commercial production of citric acid by use of filamentous fungus *Aspergillus niger*. Whereas synthetically it is produced from acetone or glycerol (Torres *et al*[1]., 1998; Fernando *et al*[2]., 2000; Adachi *et al*[3]., 2003; Haq *et al*[4]., 2004). Pandey[5], 2003 worked on solid state fermentation which is an alternate of submerged fermentation. In this process micro-organisms were cultivated in non-soluble material having low water environment which acts as both nutrient source and physical support. Solid state fermentation have many advantages over submerged fermentation such as low operating costs, high yield and low water requirement but it has many disadvantages also such as low heat, oxygen and nutrient transfer. Different species of *Aspergillus niger* such as (*A. niger*, *A. awamori*, *A. foetidus*, *Penicillium restrictum* (Mattey and Allan[7], 1990; Kubicek[8], 1998). have been evaluated by different workers for citric acid production. Other than *A. niger* many workers have also worked on yeast like *Candida lipolytica*, *C. intermedia* and *Saccharomyces cerevisiae* (Crolla and Kennedy[9], 2001; Archer *et al*.[10], 2001; Kamzolova *et al*[11]. 2003. Due to high yield, easy handling and fermentation

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with variety of easily available source raw materials (Schuster *et al*[12]. 2002) the filamentous *Aspergillus niger* remained the first choice for citric acid production. The cost reduction is always an important factors in industrial production which may be achieved by cheap agricultural waste like cotton waste, kiwi fruit peel, orange peel apple and grapes pomace and cane molasses (Kiel *et al*[13]., 1981; Hang and Woodams[14], 1986; 1987; Khare *et al*[15]., 1995;).

Experimental: In view of the importance and good physiological response and activities of the coumarins, study was carried out with *Aspergillus niger* NCIM-683 along with coumarin derivatives to observe the production of citric acid. These coumarins are 3-Acetamidocoumarin, 6-amino-3,4-benzocoumarin, 3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium salt and 7-nitro-3, 4-benzocoumarin. The composition for the production medium has been prepared as to study the mycological production of citric acid by *A niger* mutagens NCIM 683.

Molasses: 20% (w/v)

NH_4NO_3 : 0.60%

KH_2PO_4 : 0.60%

$MgSO_4.7H_2O$: 0.60%

pH: 1.8

In the composition the buffer solution of KCl-HCl has been used to adjust the pH: 1.8. The same were assured by pH meter. The composition volume have represents 100 ml production medium in fermentor flask to produce citric acid by *Aspergillus niger* NCIM-683. Similar citric acid production medium were also prepared for the 99 fermentor flask in which each having 100 ml of this medium.

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These are then arranged in 11 sets in which each have 9 fermentor and 9 fermentor are again rearranged in three subsets consisting of three flasks. Out of these 99 flasks 9 fermentor flasks were kept as control which are again arranged in three subsets each consisting of three flasks. The M/1000 solution of 6-amino-3,4-benzocoumarin were added in the fermentor flasks of 1st to 10th sets in the 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10 ml respectively. The control fermentor flask does not have coumarin. Required amount of distilled water were added to make total volume of each fermentor flask 100ml. Thus from 1st to10th subsets the molar concentration of 6-amino-3, 4-benzocoumarin were approximately (A x 10^{-x} M Where A = amount of coumarin in ml i.e. 1.0 ml to 10 ml and x =Molarity of the coumarin solution) 1.0x10^{-5}M to 10.0x10^{-5}M respectively. The sterilized fermentor flasks were then inoculated and incubated at 30^{0}C and analyzed after six, eight and ten days to observe the citric acid formed and molasses left unfermented. The experimental procedure for the study of the influence of rest coumarins to be incorporated in the production medium were exactly the same as described above, only with the difference that in place of M/1000 solution of 6-amino-3, 4-benzocoumarin other coumarins under trials were also incorporated into the production medium similarly along with *Aspergillus niger* NCIM-683 and production of citric acid were observed.

Results and Discussion: The comparative study of different coumarin derivatives shows that the maximum yield percentage of citric acid production was found at 8 .0 x 10^{-5} M concentration. The maximum yield of citric acid at this concentration was found 7.451 gram at per 100ml. The experiment was carried out at 8 days of incubation period. It is 8.299% higher than control ie 6.880g/100ml. In the same experimental condition and optimum incubation period ie 8 days in the presence of Aspergillus niger NCIM 683 the yield percentage increases from concentration 1.0 x 10^{-5} to 5.0 x 10^{-5} M but it is less effective. The

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yield percentage increases in the range 1.744% to 5.801%. It is found maximum at 5.0 x 10^{-5} M concentration the yield is 7.277g/100ml. in comparison with control flask ie 6.880g/100mlwhich is 5.801% higher. The 3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium saltderivatives in the presence of *Aspergillus niger* NCIM-683 shows beneficial and encouraging result from concentration 1.0 x 10^{-5} to 8.0 x 10^{-5} M and maximum production was observed at 8.0 x 10^{-5} M concentration. It is 7.349g/100 ml whereas control have 6.881g/100 ml in the same condition and incubation period. Thus in this case the yield percentage is 6.801% higher in comparison to control.

Comparative data for different coumarins are summarized in the table:

Coumarin used	**Coumarin (Concentration AX10^{-5}M) ****	**Citric acid (yield in g/100 ml) ***			**% difference of citric acid increased after 8 days incubation period**
		06 Days	**08 Days *****	**10 Days**	
	Control	5.830	6.880	6.574	……..
6-amino-3, 4-benzocoumarin	8.0X10^{-5} M	6.232	7.451	7.068	(+) 8.299
7-nitro-3, 4-benzocoumarin	5.0X10^{-5} M	6.048	7.277	6.940	(+) 5.801
3-Acetamidocoumarin	6.0X10^{-5} M	6.169	7.427	7.155	(+) 7.793

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3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium salt	$8.0X10^{-5}$ M	6.109	7.349	7.063	(+) 6.801

*** Observations (Mean of three), ** Coumarin concentration, *** Citric acid yield, (+) ve values indicate % increase in the yield of citric acid, Possible Experimental deviation (+/-) 1.5% to 3.5%**

CONCLUSIONS:

Thus experimental data shows that different coumarins have different action by *Aspergillus niger* species on citric acid production. The best result was found with 6-amino 3,4-benzocoumarin which enhances the yield more than 8% (ie 8.299% or 7.451g/100ml) at 8 days of incubation period. On the other hand coumarins like 3-(α-acetonylbenzyl)-4-hydroxycoumarin sodium salt and 7-nitro-3, 4-benzocoumarin enhances the yield approximately 6.0% to 7.793% in comparison to control at same incubation period.

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