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## Study on citric acid production by *Aspergillus candidus* NCIM-883 exposed to 3- amino-4-hydroxycoumarin

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**Abstract-** The influence of 3-amino-4-hydroxycoumarin on production of sucrose to citric acid by *Aspergillus candidus* NCIM-883 has been assessed. It has been found that 3- amino-4-hydroxy coumarin has stimulatory effect on citric acid fermentation and enhances the production of citric acid to an extent of 11.52095% higher in comparison to control fermentor flasks i.e. 5.43158 g/100 ml in 10 days of optimum incubation period.

**Keywords :** Citric acid fermentation, 3-amino-4-hydroxy coumarin & *Aspergillus candidus* NCIM-883

### INTRODUCTION

Coumarin was first isolated by Vogel<sup>1</sup> in 1820 from the fruit of *Dipteryx odorata* Wild (Tonk bean) of Munich, he mistook it for benzoic acid<sup>2</sup>. Coumarin is 5,6 - benzo-2-pyrone and an important class of oxygen heterocycles, Also Nicholas Jean Baptiste Gaston Guibourt of France in 1820<sup>2</sup>, independently isolated coumarin; he realized that it was not benzoic acid.<sup>3</sup> In 1835, the French pharmacist Guillemette proved that Vogel and Guilbourt had isolated the same substance.<sup>4</sup> Coumarin was first synthesized in 1868 by English chemist William Henry Perkin<sup>5</sup>. More than 1350 coumarins have been isolated till 1997.

Coumarin is a volatile compound that is formed mainly from a non-volatile glucose derivative upon senescence or injury. Coumarins are important class of

organic compounds having multiple applications in perfume, cosmetic and pharmaceutical industrial production & furthermore, they are used in the alignment layers of liquid crystal<sup>6</sup> optical brighteners and dispersed fluorescent & laser dyes<sup>7</sup>.

With expectation that coumarins might be useful in the citric acid fermentation & production protocol, the final elucidation of coumarins structure and its reactivity by introducing different groups at 3 and 4 positions into the basic skeleton of coumarins has been taken into consideration for the production of citric acid. In view of the importance and good physiological activities of the coumarins, the author has employed coumarins on production of citric acid by *Aspergillus candidus* NCIM-883.

The present study was undertaken for the production of citric by *Aspergillus candidus* NCIM-883 exposed to 3- amino-4-hydroxycoumarin.

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**MATERIALS AND METHODS**

The influence of 3- amino-4-hydroxycoumarin on production of citric acid by *Aspergillus candidus* NCIM-883: The composition of production medium for the production medium for the production of citric acid by *Aspergillus candidus* NCIM-883 is prepared as follows:

Sucrose:15%;NH<sub>4</sub>NO<sub>3</sub>: 0.18%: KH<sub>2</sub>PO<sub>4</sub>: 0.25% ; MgSO<sub>4</sub>. 7H<sub>2</sub>O: 0.35%; pH :2.2

The pH of the production medium was adjusted to 2.2 by adding requisite amount of KCl-HCl buffer solution & this pH was also ascertained by a pH meter.

**Table-1- Study on citric acid production by *Aspergillus candidus* NCIM-883 exposed to 3- amino-4-hydroxycoumarin**

| Concentration of coumarin | Incubation period in day | Yield of citric acid* in g/100 ml | Sucrose left unfermented in g/100 ml | % of citric acid increased in 7 days |
|---------------------------|--------------------------|-----------------------------------|--------------------------------------|--------------------------------------|
| Control                   | 10                       | 5.43158                           | 3.20250                              | ---                                  |
| 1.0x10 <sup>-4</sup> M    | 10                       | 5.51446                           | 3.11962                              | (+) 1.52589                          |
| 2.0x10 <sup>-4</sup> M    | 10                       | 5.58706                           | 3.04703                              | (+) 2.86251                          |
| 3.0x10 <sup>-4</sup> M    | 10                       | 5.72015                           | 2.91395                              | (+) 5.31281                          |
| 4.0x10 <sup>-4</sup> M    | 10                       | 5.80772                           | 2.76445                              | (+) 6.92505                          |
| 5.0x10 <sup>-4</sup> M    | 10                       | 5.83970                           | 2.79438                              | (+) 7.51383                          |
| 6.0x10 <sup>-4</sup> M    | 10                       | 5.91069                           | 2.72336                              | (+) 8.82082                          |
| 7.0x10 <sup>-4</sup> M**  | 10                       | 6.05735 ***                       | 2.57674                              | (+) 11.52095                         |
| 8.0x10 <sup>-4</sup> M    | 10                       | 6.01954                           | 2.61456                              | (+) 10.82484                         |
| 9.0x10 <sup>-4</sup> M    | 10                       | 6.00906                           | 2.62509                              | (+) 10.63189                         |
| 10.0x10 <sup>-4</sup> M   | 10                       | 5.93736                           | 2.69673                              | (+) 9.31183                          |

\* Each value represents means of three trials  
 \*\* Optimum concentration of coumarin  
 \*\*\* Optimum yield of citric acid (+) ve values indicate % increase the yield of citric acid Experimental deviation (+) 1.5 % to 3.5%.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 28°C and analysed after 7,10 and 15 days for citric acid formed and sucrose sugar left unfermented.

**RESULTS & DISCUSSION**

The data recorded in table-1 shows that like 4-hydroxycoumarin the compound 3-amino-4-hydroxy coumarin was also found to be increasing upto its concentration from 1.0x10<sup>-4</sup> M to 7.0x10<sup>-4</sup> M. It has been observed thus gradual addition of 3-amino-4 hydroxycoumarin to the fermentation medium gradually increases the production of citric acid. The production of citric acid on these production of citric acid. The production of citric acid on these concentrations were very much significant and could favour the production to be range of 1.52589 % to 11.52095 % only.

**CONCLUSION**

It has been observed that higher concentration of 3- amino-4-hydroxycoumarin, i.e. on 7.0x10<sup>-4</sup>M and onwards has retarded the yield of citric acid by *Aspergillus candidus* NCIM-883.

The maximum yield of citric acid has been recorded at 7.0 x 10<sup>-4</sup> M concentration of 3-amino-4-hydroxy

coumarin, i.e, 6.05735 g/100 ml in 10days of optimum incubation period which is 11.52095% higher in comparison to the control ferment flasks i.e. 5.43158 g/ 100 ml in the same set of experimental parameters for bioconversion of sucrose to citric acid by *Aspergillus candidus* NCIM-883.

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