

# Biotransformation of Molasses to Citric Acid Exposed to DL-5-(4-Hydroxyphenyl)-5-Phenyl Hydantoin

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**Abstract:** The efficacy of DL-5-(4-hydroxyphenyl)-5-phenyl hydantoin on biotransformation of molasses to citric acid by fungal strains such as *Aspergillus flavus* NCIM-540, *Aspergillus oxyzae* NCIM - 553, *Aspergillus niger* NCIM - 501, *Aspergillus foetidus* NCIM - 937 and *Aspergillus wentii* NCIM-941 has been assessed. It has been observed that the fungal strain *Aspergillus niger* NCIM - 501 has been found most significant and effective for the citric acid fermentation process. It has been found that the compound, i.e. DL-5-(4-hydroxyphenyl)-5-phenyl hydantoin has stimulatory effect on production of citric acid by *Aspergillus niger* NCIM - 501 and enhances the yield of citric acid to an extent of 12.477% higher in comparison to control fermentor flasks, i.e., 7.989g/100 ml under the optimized conditions, viz. 30C temperature, 2.0 pH, 12 days of incubation period with 26% molasses solution.

**Keywords:** *Aspergillus niger* NCIM-501, Citric acid fermentation, DL-5-(4-hydroxyphenyl)-5-phenyl hydantoin.

## I. INTRODUCTION

There are large group of some organic molecules which when introduced to the submerged fermentation medium can affect the enzyme responsible for the biosynthesis of micro and macro molecules in the microbial cells as well bioconversion of raw substrate into desired products and such organic compounds may be referred to as Physiologically Active Organic Compounds (PAOC). Though biologically active organic compounds are not essentially growth promoter for some or all microbes yet a few organic molecules are utilized by some or all microbes for their nutritional requirements. An attempt has been made to study the influences of different biologically active organic compounds on mycological biosynthesis of citric acid by using the fungal strain of *Aspergillus niger* NCIM 501. These include following:

- DL-5-(4-hydroxyphenyl)-5-phenyl hydantoin(HPPH)
- $\alpha,\alpha$ -diethyl- $\beta$ -ethyl succinimide(DEES)
- 5-Hydroxyindole-3- acetic acid(HIAA)
- $\alpha$ -Keto adipic acid(KAA)

*Experimental:* The influence of HPPH on mycological biosynthesis of citric acid by using the fungal strain of *Aspergillus niger* NCIM 501.

The composition of the production medium for Biotransformation of molasses to citric acid by using fungus *Aspergillus niger* NCIM-501 has been prepared as follows.

*Molasses:* 26 % (w/v),  $\text{NH}_4\text{NO}_3$ :0.280%, $\text{KH}_2\text{PO}_4$ :0.380%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ :0.358%,

PH: 2.0

The  $\text{P}^{\text{H}}$  of the medium was adjusted to 2.0 by adding requisite amount of KCl-HCl buffer solution, and the 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.

Now, the same production for citric acid fermentation by *Aspergillus niger* NCIM-501 was prepared for 99 fermentor flasks i.e. each fermentor flask containing 100ml of production medium.

The above fermentor flasks were then arranged in ten sets, each comprising 9 fermentor flask. Each set was again rearranged in three subsets, each comprising of 3 fermentor flasks. The remaining nine fermentor flasks out of 99 fermentor flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now M/100 solution of DL-5-(4-hydroxyphenyl)-5-phenyl hydantoin (HPPH) was prepared and 1.0,2.0,3.0,4.0,5.0,6.0,7.0 ,8.0,9.0 and 10.0 ml of this solution was added to the fermentor flasks of 1<sup>st</sup> to 10<sup>th</sup> sets respectively. The control fermentor flasks contain no physiologically active organic compounds. Now the total volume in each fermentor flask were made upto 100ml by adding requisite amount of distilled water. Thus the concentration of DL-5-(4-hydroxyphenyl)-5-phenyl hydantoin (HPPH) in 1<sup>st</sup>,2<sup>nd</sup>,3<sup>rd</sup>,4<sup>th</sup>,5<sup>th</sup>,6<sup>th</sup>,7<sup>th</sup>,8<sup>th</sup>,9<sup>th</sup> and 10<sup>th</sup> subsets were approximately as given below.

$$A \times 10^{-x} \text{ M}$$

Where A= Amount of PAOC in ml ( from 1 to 10 ml)

x = Molarity of the solution containing PAOC

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analyzed after 10,12 and 14 days for citric acid formed and molasses sugars left unfermented. The experimental procedure for the study of influence of others PAOC were exactly same as described above with the only difference that in place of M/100 solution of DL-5-(4-hydroxyphenyl)-5-phenyl hydantoin (HPPH) other PAOC under trials were added to the citric acid fermentation medium respectively.

Temperature: 30°C

Distilled Water: To make 100 ml

Sterilization: At 15 lbs steam pressure for 15 minutes at 121°C.

Organism Used: Aspergillus niger NCIM-501 was used as source of enzymes for fungal synthesis of citric acid by

submerged fermentation. It was stored in a refrigerator and reinoculated before immediate use. The strain was inoculated into 1 L baffled shake flask containing 100 ml production medium. These were grown at 30°C overnight for 48 hours and then inoculated into fermenter.

Inoculum: 0.5 ml conidial suspension of Aspergillus niger NCIM-501. Age of the inoculum: 48 hours old.

Incubation Period: 10, 12 and 14 days

Optimum incubation period: 12 days.

## II. RESULT AND DISCUSSION

The results obtained in the study of the efficacy of some PAOC on biotransformation of molasses to citric acid by fungal strains, Aspergillus niger NCIM - 501, are tabulated in the following table.

TABLE I: A COMPARATIVE ASSESSMENT OF THE INFLUENCE OF DIFFERENT PAOC ON MYCOLOGICAL BIOSYNTHESIS OF CITRIC ACID BY USING THE FUNGAL STRAIN OF ASPERGILLUS NIGER NCIM 501

PAOC USED	Optimum Conc. of Micelle $A \times 10^{-4} \text{ M}$	Maximum Yield of Citric Acid in the Control Fermentor Flasks in g/100 ml	Maximum Yield of Citric Acid Exposed to Different PAOC in g/100 ml <sup>2</sup>	% Difference in the Yield of Citric Acid Increased (+) or Decreased in 12 Days After 12 hrs
HPPH	$7.0 \times 10^{-4} \text{ M}$	7.98986	8.98677	(+)12.47718
DEES	$5.0 \times 10^{-4} \text{ M}$	8.04305	8.86238	(+)10.18680
HIAA	$1.0 \times 10^{-4} \text{ M}$	8.07315	7.80634	(+)3.30490
KAA	$2.0 \times 10^{-4} \text{ M}$	8.07917	8.14818	(+)0.85417

It was very interesting to note that HPPH was very effective amongst PAOC used which could improve significantly the yield of citric acid to an extent of 12.47710% in comparison of the control fermentor flask under same experimental parameter.

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