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Production of citric acid by *Aspergillus niger* NCIM -1986 exposed to N,N-dimethyldodecylamine

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Abstract : The efficacy of micelles, i.e., N,N-dimethyldodecylamine on production of citric acid by fungal strains such as *Aspergillus aculeatus* NCIM -1636, *Aspergillus awamori* NCIM -1645, *Aspergillus foetidus* NCIM-1765, *Aspergillus wentii* NCIM - 1789 and strain *Aspergillus niger* NCIM-1986 has been assessed. It has been observed that the fungal strain *Aspergillus niger* NCIM-1986 has been found most potential and effective for the citric acid fermentation process. It has been found that the micelles, i.e., N,N-dimethyldodecylamine at molar concentration, i.e., 4.0×10^{-5}M has stimulatory effect on production of citric acid by *Aspergillus niger* NCIM-1986 and enhances the yield of citric acid to an extent of 6.847% higher in comparison to control fermentor flasks, i.e., 8.835g/100 ml under the optimized conditions, viz. 30^0C temperature, 1.8 pH, 11 days of incubation period with 32% (w/v) molasses solution.

(Keywords: Citric acid fermentation, N,N-dimethyldodecylamine, micelle and *Aspergillus niger* NCIM-1986)

Introduction

In aqueous solution, molecules having both polar or charged groups and non polar regions (amphiphilic molecules) form aggregates called micelles. In a micelle, polar or ionic heads form an outer shell in contact with water, while non polar tails are sequestered in the interior. Hence, the core of a micelle, being formed of long non polar tails, resembles an oil or gasoline drop. The length of the non polar tail, the nature and size of the polar or ionic head, the acidity of the solution, the temperature, and the presence of added salts are the most important factors determining the kind of the obtained aggregate. If those parameters are changed, it is possible to change shape and size of the micelles. The number of amphiphilic molecules forming the aggregate is called aggregation number; it is a way to describe the size of the micelle.

Micelles are widely used in industrial and biological fields for their ability to dissolve and move non polar substances through an aqueous medium, or to carry drugs which are, often, scarcely soluble in water. The carrying ability of micelles can be altered if parameters determining their size and shape are changed.

An aggregate of molecules in a colloidal solution, such as those formed by detergents In aqueous solution, molecules having both polar or charged groups and non polar regions form aggregates called micelles. In a micelle, polar or ionic heads form an outer shell in contact with water, while non polar tails are sequestered in the interior. Hence, the core of a micelle, being formed of long non polar tails, resembles an oil or gasoline drop. The length of the non polar tail, the nature and size of the polar or ionic head, the acidity of the solution, the temperature, and the presence of added salts are the most important factors determining the kind of the obtained aggregate. Therefore, micellar effect for specially citric acid and other fermentation process may be significant and useful for its improved yield[1-5]. Micellar effect on fermentation studies especially citric acid fermentation is relatively new and unexploited. The organic reactions may depend on its nature as it happens probably due to electrostatic and hydrophobic interactions among substrates, reactants and micelles. Moreover, a review of the literature clearly indicates that micelles has been used as a specific model for enzyme-catalyzed reactions of fermentation processes. In recent years a group of workers[6-15] have reported some micelles very useful for fermentation process specially for ethanolic, lactic and citric acid fermentations.

Thus, from the above brief review it is evident that micelles are required for exploitation specially for citric acid fermentation[16-19] and in view of this the authors have studied the influence of N,N-dimethyldodecylamine on citric acid production by *Aspergillus niger* NCIM-1986.

Experimental

The influence of N,N-dimethyldodecylamine on production of citric acid by *Aspergillus niger* NCIM -1986.

The composition of production medium for the production of citric acid by *Aspergillus niger* NCIM -1986 is prepared as follows:

Molasses : 32% (w/v), NH_4NO_3 : 0.75%
KH_2PO_4 : 0.75%, $MgSO_4.7H_2O$: 0.75%, pH : 1.8

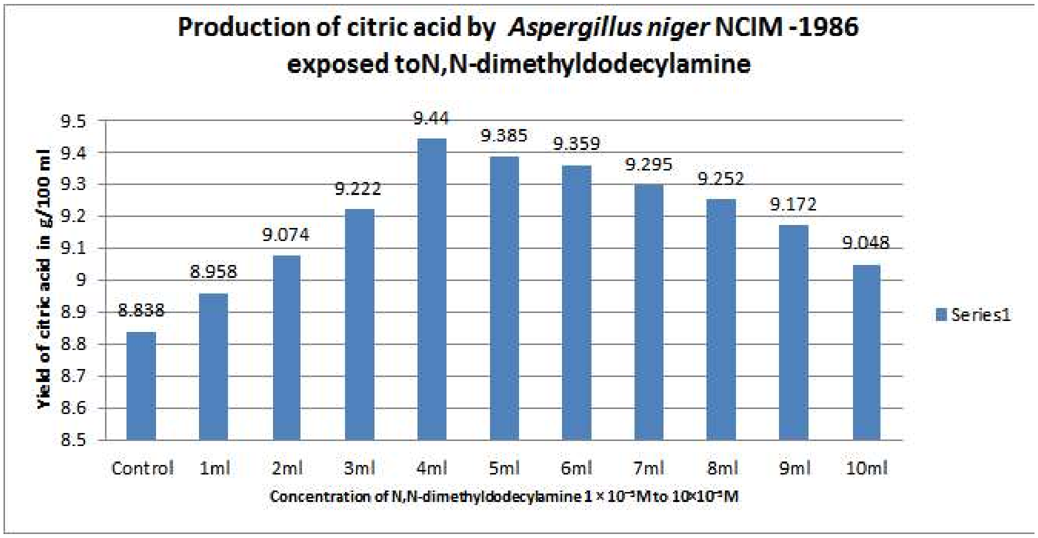
The pH of the production medium was adjusted to1.8 by adding requisite amount of KCI-HCI buffer solution, and this pH was also ascertained by a pH meter. The above composition medium represents volume of a fermentor flask, i.e., "100ml" on production of citric acid by *Aspergillus niger* NCIM -1986. Now, the same production of citric acid by *Aspergillus niger* NCIM -1986 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.

Table - 1
Production of citric acid by *Aspergillus niger* NCIM -1986 exposed toN,N-dimethyldodecylamine

Concentration of micelles used used a x 10^{-5}M	Yield of citric acid* in g/100 ml			% of citric acid increased(+) in 11 days of optimum incubation period.
	10 days	11 days	12 days	
Control	6.814	8.835	7.560	–
1.0 x 10^{-5}M	6.910	8.958	7.658	(+) 1.392
2.0 x 10^{-5}M	6.997	9.074	7.7764	(+) 2.705
3.0 x 10^{-5}M	7.113	9.225	7.892	(+) 4.414
4.0 x 10^{-5}M**	7.277	9.440***	8.074	(+) 6.847
5.0 x 10^{-5}M	7.236	9.385	8.028	(+) 6.225
6.0 x 10^{-5}M	7.216	9.359	8.006	(+) 5.930
7.0 x 10^{-5}M	7.168	9.295	7.953	(+) 5.206
8.0 x 10^{-5}M	7.134	9.252	7.915	(+) 4.719
9.0 x 10^{-5}M	7.072	7.172	7.847	(+) 3.814
10 x 10^{-5}M	6.973	9.048	7.741	(+) 2.410

* Mean of three observations; ** Optimum concentration of micelle
*** Optimum yield of citric acid; (+) ve values indicate % increase in the yield of citric acid
Experimental deviation ($\pm$) 1.5 to 3.5%



After preparing the above sets of fermentor flasks M/1000 solution of N,N-dimethyldodecylamine was prepared and from the above micell 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 micelle.

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

A x 10^{-x} M , 1.0x10^{-5}M to 10.0x10^{-5}M
Where :
A = amount of micelle, in ml, i.e. 1.0 ml .... to 10 ml.
x =Molarity of the micelle solution and respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 30^{0}C and analysed after 10, 11 and 12 days for citric acid[20] formed.

Results and Discussion

The data recorded in the table 1 shows that N,N-dimethyldodecylamine has stimulatory effect on production of citric acid by *Aspergillus niger* NCIM -1986.

The maximum yield of citric acid, i,e; 9.440g/100 ml in the presence of N,N-dimethyldodecylamine was observed at 4.0 x10^{-5}M molar concentration in 11 days of optimum incubation period which is 6.847% higher in comparison to control fermentor flasks, i.e; 8.835 g/100 ml in the same times course and other same experimental parameters.

The higher molar concentrations of N,N-dimethyldodecylamine were not much favourable for the production of citric acid by *Aspergillus niger* NCIM -1986. So the gradual addition of the micelle N,N-dimethyldodecylamine after certain concentrations were not beneficial for the citric acid fermentation process.

It has been observed that molar concentration of the micelle i.e., N,N-dimethyldodecylamine from 1.0 x 10^{-5}M to 4.0 x 10^{-5}M enhances the yield of citric acid to a certain order being 1.392%, 2.705%, 4.414%, and 6.847% higher in comparison to control flasks but at 5.0 x 10^{-5}M to 10.0 x10^{-5}M the yield of citric acid shifted to be in the range, i.e., 6.225%, 5.930%, 5.206%, 4.719%, 3.814% and 2.410%, higher in comparison to previous concentrations of N,N-dimethyldodecylamine taken into experimental trials.

It has been observed further that after optimum concentration, i. e., 4.0 x 10^{-5}M, the addition of the same micelle to the production medium causes fall in the yield of citric acid gradually and reaches to 2.410%; However, at all the experimental concentrations of N,N-dimethyldodecylamine used the production of citric acid by *Aspergillus niger* NCIM -1986 has been found higher in comparison to control fermentor flasks.

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