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EFFECT OF CETYLTRIMETHYLAMMONIUM HYDROGEN SULPHATE ON PRODUCTION OF CITRIC ACID BY ASPERGILLUS NIGER NCIM-2101

Umesh Prasad Chaurasia
Department of Chemistry, TU, Thakur Ram Multiple Campus, Birgunj, Nepal
E-mail: uchaurasia1000@gmail.com

Abstract

The ability of cetyltrimethylammonium hydrogen sulphate on production of citric acid by some fungal strains such as Aspergillus-niger NCIM -2101 has been assessed. The micelle; i.e. cetyltrimethylammonium hydrogen sulphate under trial has stimulatory effect on bioproduction of citric acid and enhances the yield of citric acid to an extent of 7.694% higher in comparison to control fermenter flasks, i.e., 8.825 g/100 ml in 12 days of optimum incubation period, 1.8 pH and 30°C temperature with 28% (w/v) molasses solution along with other nutritional ingredients.

Key words

Molasses; citric acid fermentation; cetyltrimethylammonium hydrogen sulphate; Aspergillus niger NCIM-2101

Introduction

A micelle is an aggregate of surfactant molecules dispersed in a liquid colloid. Micelles are spheres of *lipids* that form in aqueous solutions. In humans, they form from *bile salts*. These micellar aggregates help transport the digestive products of lipids to the intestine to be absorbed. Also, they are used as detergents.

A typical micelle in aqueous solution forms an aggregate with the hydrophilic "head" regions in contact with surrounding solvent, sequestering the hydrophobic single-tail regions in the micelle centre (Röhr, 1998) . A micelle is formed when a variety of molecules including soaps and detergents are added to water. The molecule may be a fatty acid, a salt of a fatty acid (soap), phospholipids, or other similar molecules (Bayraktar and Mehmetoglu, 2000). The molecule must have a strongly polar "head" and a non-polar hydrocarbon chain "tail". The polar head of the molecule presents itself for interaction with the water molecules on the outside of the micelle. Micelles either accelerate or retard the organic reactions depending on its nature (Rabeyrin *et al.*, 2000). It is assumed that micelles are moderators of enzyme actions in

some biological systems (Papagianni, 2007) .There are several known micelles, but a very few micelles have been used in submerged fermentation processes (Srivasta and Kamal, 1979). Since micellar effect on fermentation studies especially citric acid fermentation is relatively new and almost unexplored, it needs careful and specific experimentations. In the present investigation the author has made an attempt to study the effect of cetyltrimethylammonium hydrogen sulphate on citric acid fermentation *by* Aspergillus *niger* NCIM-2101.

Method

The influence of cetyltrimethylammonium hydrogen sulphate on production of citric acid by *Aspergillus niger* NCIM-2101 is observed as : The composition of the production medium for production of citric acid by *Aspergillus niger* NCIM-2101 has been prepared as follows : Molasses: 28% (w/v), NH_4NO_3: 0.25%, KH_2PO_4:0.25%, $MgSO_4.7H_2O$:0.25%, pH:1.8, The pH value of the production medium was adjusted to 1.8 by adding requisite amount of KCl-HCl buffer solution, and this pH was also verified by a pH meter. The above composition medium represents volume of a fermenter flask, i.e., "100ml" production medium for production of citric acid by *Aspergillus niger* NCIM-2101. Now, the same production medium for production of citric acid by *Aspergillus niger* NCIM-2101 was prepared for 99-fermenter flask, i.e; each contained '100ml' of production medium.

Then a 99-fermenter flask was then arranged to 11-sets, each comprising of 9-fermenter flasks respectively. The fermenter flasks out of 99-fermenter flasks were kept as control and these were also rearranged in 3-subsets each consisting of 3-fermenter flasks.

After preparing the above sets of fermenter flasks M/1000 solution of cetyltrimethylammonium hydrogen sulphate was prepared and from the above cetyltrimethylammonium hydrogen sulphate 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 fermenter flasks contained no cetyltrimethylammonium hydrogen sulphate.

The total volume in each fermenter flasks was made 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of cetyltrimethylammonium hydrogen sulphate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 10^{-x} M, i.e., 1.0×10^{-5}M 2.0×10^{-5}M 3.0×10^{-5}M 4.0×10^{-5}M 5.0×10^{-5}M	PHASE-I
6.0×10^{-5}M 7.0×10^{-5}M 8.0×10^{-5}M 9.0×10^{-5}M 10.0×10^{-5}M	PHASE-II

A = amount of cetyltrimethylammonium hydrogen sulphate, in ml, i.e., 1.0 ml ... to 10 ml.

x = Molarity of the cetyltrimethylammonium hydrogen sulphate solution

The fermenter flasks used were then properly sterilized, cooled inoculated and

incubated at 30^{O}C and analysed after 8, 12 and 14 days for citric acid formed.

Results and discussion

Table - 1

Production of citric acid by *Aspergillus niger* NCIM-2101

exposed to cetyltrimethylammonium hydrogen sulphate

Control	8	6.415	5.589	-
(-) Micelle	12	8.825	3.178	-
	14	7.598	3.067	-
$1.0x10^{-5}$M	8	6.466	5.534	-
(+) Micelle	12	8.898	3.116	+ 0.827
	14	7.789	3.032	-
$2.0x10^{-5}$M	8	6.524	5.476	-
(+) Micelle	12	8.992	3.008	+ 1.892
	14	7.888	2.995	-
$3.0x10^{-5}$M	8	6.652	5.350	-
(+) Micelle	12	9.169	2.835	+ 3.898
	14	8.066	2.746	-
$4.0x10^{-5}$M	8	6.748	5.248	-
(+) Micelle	12	9.301	2.695	+ 5.393
	14	8.203	2.598	-
$5.0x10^{-5}$M**	8	6.896	5.109	-
(+) Micelle	12	9.504***	2.493	+ 7.694
	14	8.401	2.396	-

* Each value represents mean of three trials
** Optimum concentration of micelle used
*** Optimum yield of citric acid
(+) values indicate % increase in the yield of citric acid after 12 days.
Experimental deviation (±) 1.5-3%

Table – 2

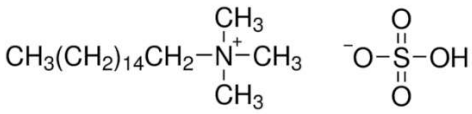
Production of citric acid by *Aspergillus niger* NCIM-2101

exposed to cetyltrimethylammonium hydrogen sulphate

Control	8	6.415	5.589	-
(-) Micelle	12	8.825	3.178	-
	14	7.598	3.067	-
$6.0x10^{-5}$M	8	6.806	5.198	-
(+) Micelle	12	9.380	2.629	+ 6.288
	14	8.279	2.540	-
$7.0x10^{-5}$M	8	6.684	5.319	-
(+) Micelle	12	9.213	2.789	+ 4.396
	14	8.115	2.695	-
$8.0x10^{-5}$M	8	6.605	5.398	-
(+) Micelle	12	9.098	2.912	+ 3.093
	14	8.037	2.824	-
$9.0x10^{-5}$M	8	6.543	5.459	-
(+) Micelle	12	9.019	2.985	+ 2.198
	14	8.003	2.896	-
$10.0x10^{-5}$M	8	6.479	5.529	-
(+) Micelle	12	8.930	3.115	+ 1.189
	14	7.810	3.031	-

* Each value represents mean of three trials
** Optimum concentration of micelle used
*** Optimum yield of citric acid
(+) values indicate % increase in the yield of citric acid after 12 days.
Experimental deviation (±) 1.5-3%

The influence of cetyltrimethylammonium hydrogen sulphate



cetyltrimethylammonium hydrogen sulphate

As per the recorded observation and above mentioned data in table-1 and table- 2 shows that cetyltrimethylammonium hydrogen sulphate also has inspiratory effect on citric acid production by Aspergillus *niger* NCIM-2101.

The data (table-1 & table-2) reveals that the micelle cetyltrimethylammonium hydrogen sulphate stimulates the citric acid fermentation process and enhances the yield of citric acid upto its cetyltrimethylammonium hydrogen sulphate concentrations from 1.0 x 10^{-5} to 5.0 x 10^{-5}M. The effect of cetyltrimethylammonium hydrogen sulphate on the productivity (yield) of citric acid was gradually in increasing order and attains its best role at 5.0 x 10^{-5}M where maximum yield of citric acid, i.e., 8.825g/100 ml is fetched in 12 days of optimum incubation period which is 7.694% higher in comparison to control fermenter flask, i.e., 8.825 g/100 ml.

In the second phase of micellar effect the molar concentration, i. e., from 6.0 x 10^{-5}M to 10 x 10^{-5}M the production of citric acid has been bit enhanced but the order of citric acid productivity is reverse in respect to increasing molar concentrations of cetyltrimethylammonium hydrogen sulphate. However, the citric acid production by *Aspergillus niger* NCIM-2101 under the influence of each concentration of cetyltrimethylammonium hydrogen sulphate used has been stimulating and the yield of citric acid has been found greater than that obtained in the control fermenter flasks. In both the phase the order of productivity and % of citric acid formed is as below:

Phase- I (Table 1)

Concentration of cetyltrimethylammonium hydrogen sulphate from 1.0x10^{-5}M to 5.0 x 10^{-5}M.

Productivity of citric acid:

0.827%, 1.892%, 3.898%, 5.393%, 7.694%

Phase – II (Table 2)

Concentration of cetyltrimethylammonium hydrogen sulphate from

6.0 x10^{-5} M to 10.0 x 10^{-5}M.

Productivity of citric acid:

6.288%, 4.396%, 3.093%, 2.198%

The openness of fungal strain *Aspergillus niger* NCIM-2101 to cetyltrimethylammonium hydrogen sulphate may produce a variety of effects.. Although the special mode of action is not very clear, there is a consensus that the lethal effect is associated with physical damage of the membrane structure of the cell surface, which initiates further deterioration.

Conclusion

Hence, it is concluded that the cetyltrimethylammonium hydrogen sulphate maintained at lower concentrations is stimulatory and at higher concentrations is detrimental for citric acid production by *Aspergillus niger* NCIM-2101.

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