

Bioproduction of H₃Cit by *Aspergillus niger* T-918 exposed to sodium n-octadecyl sulfate

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Abstract : The efficacy of sodium n-octadecyl sulfate on bioproduction of citric acid by *Aspergillus foetidus* T-501, *Aspergillus fischeri* T-611, *Aspergillus giganteus* T-715, *Aspergillus oryzae* T-816 and *Aspergillus niger* T-918 has been assessed. It has been observed that the fungal strain *Aspergillus niger* T-918 has been found most significant and effective for the citric acid fermentation process. It has been found that the compound, i.e., sodium n-octadecyl sulfate has stimulatory effect on bioproduction of citric acid by *Aspergillus niger* T-918 and enhances the yield of citric acid to an extent of 15.253 % higher in comparison to control fermenter flasks, i.e., 9.375g/100 ml under the optimized conditions.

(Keywords : Citric acid fermentation, Sodium n-octadecyl sulfate and *Aspergillus niger* T-918)

Introduction

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. The molecule must have a strongly polar "head" and a non-polar hydrocarbon chain "tail". When this type of molecule is added to water, the non-polar tails of the molecules clump into the center of a ball like structure, called a micelle, because they are hydrophobic or "water hating". The polar head of the molecule presents itself for interaction with the water molecules on the outside of the micelle. Micelles are nanosized colloidal dispersions prepared from amphiphilic molecules, with a hydrophobic tail and a hydrophilic head.

The hydrophobic core acts as a reservoir for hydrophobic drugs and the hydrophilic shell stabilizes the hydrophobic core.¹⁻¹⁰ The investigation concerns chemical reactions occurring inside the particular type of biologically important aggregate, the micelles¹¹⁻¹².

Micelles either accelerates or retards the organic reactions depending on its nature¹³⁻²¹. It is assumed that micelles are moderators of enzyme actions in some biological systems. There are several known micelles, but a very few micelles have been used in submerged fermentation processes. Since micellar effect on fermentation studies especially citric acid fermentation is Efficacy of Sodium n-octadecyl sulfate on H₃Cit by *Aspergillus niger* T-918 relatively new and almost unexplored it needs careful and specific experimentations. In the present investigation the authors have made an attempt to study the effect of Sodium n-octadecyl sulfate on bioproduction of H₃Cit by *Aspergillus niger* T-918.

Experimental

The influence of sodium n-octadecyl sulfate on bioproduction of H₃Cit by *Aspergillus niger* T-918. The composition of production medium for the bioproduction of H₃Cit by *Aspergillus niger* T-918 is prepared as follows: Molasses - 30% w/v, Ammonium nitrate-0.55% Potassium dihydrogen phosphate-0.55% Magnesium sulphate-0.55%, Distilled water to make up 100 ml, pH -2.0

Table - 1
Bioproduction of H₃Cit by *Aspergillus niger* T-918 exposed to sodium n-octadecyl sulfate

Concentration of micelle used A x 10 ^{-x} M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% of citric acid increased after 13 days
Control	11	7.875	5.624	-
(-) Micelle	13	9.375	4.524	-
	15	6.376	4.380	-
1.0x10 ⁻⁵ M	11	8.016	5.483	-
1/10-3 × 10 ⁻² M	13	9.548	4.351	+1.845
(+) Micelle	15	6.490	4.279	-
2.0x10 ⁻⁵ M	11	8.213	5.286	-
2/10 ⁻³ × 10 ⁻² M	13	9.784	4.116	+4.362
(+) Micelle	15	6.650	4.047	-
3.0x10 ⁻⁵ M	11	8.449	5.053	-
3/10 ⁻³ × 10 ⁻² M	13	10.065	3.835	+7.360
(+) Micelle	15	6.841	3.614	-
4.0x10 ⁻⁵ M **	11	9.072	4.428	-
4/10 ⁻³ × 10 ⁻² M	13	10.805***	3.094	+15.253
(+) Micelle	15	7.344	3.035	-
5.0x10 ⁻⁵ M	11	8.938	4.561	-
5/10 ⁻³ × 10 ⁻² M	13	10.642	3.257	+13.514
(+) Micelle	15	7.235	3.166	-
6.0x10 ⁻⁵ M	11	8.780	4.719	-
6/10 ⁻³ × 10 ⁻² M	13	10.455	3.445	+11.520
(+) Micelle	15	7.109	3.365	-
7.0x10 ⁻⁵ M	11	8.544	4.955	-
7/10 ⁻³ × 10 ⁻² M	13	10.180	3.720	+8.586
(+) Micelle	15	6.917	3.480	-
8.0x10 ⁻⁵ M	11	8.308	5.192	-
8/10 ⁻³ × 10 ⁻² M	13	9.898	4.001	+5.578
(+) Micelle	15	6.726	3.539	-
9.0x10 ⁻⁵ M	11	8.048	5.452	-
9/10 ⁻³ × 10 ⁻² M	13	9.586	4.315	+2.250
(+) Micelle	15	6.516	4.815	-
10.0x10 ⁻⁵ M	11	7.903	5.596	-
10/10 ⁻³ × 10 ⁻² M	13	9.409	4.495	+0.362
(+) Micelle	15	6.398	4.116	-

* Each value represents mean of three observations ** Optimum concentration of micelle used

*** Optimum yield of citric acid (+) values indicate % increase in the yield of citric acid after 13 days. Experimental deviation (±) 1.5-3%

The pH of the production medium was adjusted to 2.0 by adding requisite amount of KCl-HCl 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 bioproduction of H₃Cit by *Aspergillus niger* T-918.

Now, the same production medium of citric acid (H₃Cit) by *Aspergillus niger* T-918 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.

After preparing the above sets of fermentor flasks M/1000 solution of sodium n-octadecyl sulfate 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 sodium n-octadecyl sulfate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below : $A \times 10^{-x}$ M i.e., 1.0×10^{-5} M to 10.0×10^{-5} M

Where :

A = amount of micelle, in ml, i.e. 1.0 ml .. to 10 ml.,
x = Molarity of the micelle solution

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 27°C and analysed after 11, 13 and 15 days for citric acid²³ formed and molasses²⁴ left unfermented.

Results and Discussion

The Influence of sodium n-octadecyl sulfate

The data recorded in the table 1 shows that sodium n-octadecyl sulfate has stimulatory and beneficial effect on bioproduction of H₃Cit by *Aspergillus niger* T-918.

The maximum yield of citric acid (H₃Cit), i.e; 10.805g/100 ml in the presence of sodium n-octadecyl sulfate was observed at 4.0×10^{-5} M molar concentration in 13 days of optimum incubation period which is 15.253% higher in comparison to control fermentor flasks, i.e; 9.375 g/100 ml in the same times course and other same experimental parameters.

The higher molar concentrations of sodium n-octadecyl sulfate were not much effective and favourable for the bioproduction of H₃Cit by *Aspergillus niger* T-918. So the gradual addition of the micell, i.e. sodium N-octadecyl sulfate after certain concentrations were not effective and beneficial for the bioproduction of H₃Cit by *Aspergillus niger* T-918.

It has been observed that molar concentration of the micell, i.e., sodium n-octadecyl sulfate from 1.0×10^{-5} M to 4.0×10^{-5} M enhances the yield of citric acid to a certain order being 1.845%, 4.362%, 7.360%, and 15.253% higher in comparison to control flasks but at 5.0×10^{-5} M to 10.0×10^{-5} M the yield of citric acid shifted to be 13.514%, 11.520%, 8.586%, 5.578%, 2.250% and 0.362% higher in comparison to previous concentrations of sodium n-octadecyl sulfate taken into experimental trials.

From the present investigation it is obvious that the chemical micelle used under trial, i.e., sodium n-octadecyl sulfate is useful micelle for bioproduction of H₃Cit by *Aspergillus niger* T-918 and, therefore, can be employed for the improved bioproduction of H₃Cit by *Aspergillus niger* T-918

It is interesting to note that the micelle

used in the present investigation and its molar concentrations under trial, i. e., from $1.0 \times 10^{-5}\text{M}$ to $10 \times 10^{-5}\text{M}$ has given better yield of citric acid

(H_3Cit) in each case in comparison to respective controls.

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