

Liquid substrate fermentative production of H₃Cit by *Aspergillus clavatus* NCIM 1679 exposed to calcium dodecyl benzene sulfonate

Pragati Kiran

Department of Chemistry, Magadh University, Bodh-Gaya (Bihar) India

Email : pragatikiran27@gmail.com

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Abstract: The efficacy of calcium dodecyl benzene sulfonate on liquid substrate fermentative production of citric acid (H₃Cit) has been assessed. It has been found that calcium dodecyl benzene sulfonate has stimulatory effect on liquid substrate fermentative production of citric acid (H₃Cit) by *Aspergillus clavatus* NCIM-1679 and enhances the yield of citric acid (H₃Cit) to an extent of 8.593% higher in comparison to control fermentor flasks, i.e., 9.356g/100 ml under the optimized conditions.

(Key words : Citric acid fermentation, LSF, (H₃Cit) calcium dodecyl benzene sulfonate and *Aspergillus clavatus* NCIM-1679)

Introduction

A micelle is stable formation of amphipathic molecules, or molecules that have a polar head and a nonpolar tail. Polarity is the factor that determines whether a molecule interacts with or runs away from water. A micelle, then, is a spherical structure in which the nonpolar tails of amphipathic molecules hide on the inside and are shielded from water by the polar heads that line the outside. Micelles have important roles in fat and vitamin absorption in the intestine. Micelles are lipid molecules that arrange themselves in a spherical form in aqueous solutions¹⁻⁸. The formation of a micelle is a response to the amphipathic nature of fatty acids, meaning that they contain both hydrophilic regions (polar head groups) as well as hydrophobic regions (the long hydrophobic chain). A number of recent review articles have been written on the rheology and applications of wormlike micelle solutions⁹⁻¹³.

However, as the salt concentration of micelles is increased and the head group charges are sufficiently screened, the wormlike micelles can form three-point or four-point junctions.¹⁴⁻¹⁸

Thus, from the above brief review it is evident that micelles are required for exploitation specially for citric acid fermentation¹⁹⁻²⁰ and in view of this the authors have studied the influence of calcium dodecyl benzene sulfonate on citric acid production by *Aspergillus clavatus* NCIM-1679.

Experimental

The influence of calcium dodecyl benzene sulfonate on citric acid production by *Aspergillus clavatus* NCIM-1679.

The composition of the production medium for citric acid production by *Aspergillus clavatus* NCIM-1679 has been prepared as follows: Molasses : 32% (w/v), NH₄NO₃ : 0.36%, KH₂PO₄ : 0.36%, MgSO₄·7H₂O : 0.50%, pH : 1.8

The pH of the production medium was adjusted to 1.8 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" for citric acid production by *Aspergillus clavatus* NCIM-1679

Now, the same production medium for citric acid production by *Aspergillus clavatus* NCIM-1679 was prepared for 99-fermentor flask, i. e; each contained '100ml' of production medium.

Table - 1
LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679
exposed to calcium dodecyl benzene sulfonate

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 12 days
Control	12	9.356	4.650	-
1.0x10 ⁻⁵ M	12	9.449	4.550	+ 0.994
2.0x10 ⁻⁵ M	12	9.477	4.529	+ 1.293
3.0x10 ⁻⁵ M	12	9.543	4.460	+ 1.998
4.0x10 ⁻⁵ M	12	9.627	4.383	+ 2.896
5.0x10 ⁻⁵ M	12	9.823	4.179	+ 4.991
6.0x10 ⁻⁵ M	12	9.936	4.064	+ 6.199
7.0x10 ⁻⁵ M**	12	10.160***	3.844	+ 8.593
8.0x10 ⁻⁵ M	12	9.748	4.259	+ 4.189
9.0x10 ⁻⁵ M	12	9.580	4.431	+ 2.394
10.0x10 ⁻⁵ M	12	9.477	4.528	+ 1.293

* 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 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 calcium dodecyl benzene sulfonate was prepared and from the above micellar 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 calcium dodecyl benzene sulfonate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x 10^{-x} M

1.0x10⁻⁵M to 10.0x10⁻⁵M respectively.

Where : A = amount of micelle.

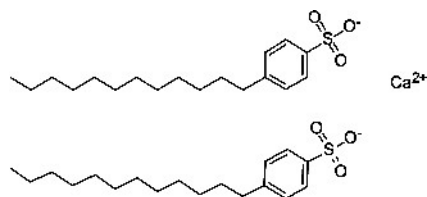
in ml, i.e. 1.0 ml to 10 ml.

x = Molarity of the micelle solution

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 32°C and analysed after 9, 12 and 15 days for citric acid²¹ formed and molasses²² left unfermented.

Results and Discussion

The influence of calcium dodecyl benzene sulfonate



(Calcium dodecyl benzene sulfonate)

The data recorded in the table-1 shows that calcium dodecyl benzene sulfonate has stimulatory effect on LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679.

The data (vide table-1) reveals that the micelle calcium dodecyl benzene sulfonate stimulates the citric acid fermentation process and enhances the LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679 upto its calcium dodecyl benzene sulfonate concentrations from 1.0×10^{-5} to 7.0×10^{-5} M. The effect of calcium dodecyl benzene sulfonate on LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679 was gradually in increasing order and attains its best role at 7.0×10^{-5} M where maximum yield of citric acid, i.e., 10.160g/100 ml is fetched in 12 days of optimum incubation period which is 8.593% higher in comparison to control fermentor flask, i.e., 9.356 g/100 ml.

In the second phase of micellar effect the molar concentration, i. e., from 8.0×10^{-5} M to 10×10^{-5} M the LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679 has been less enhanced but the order of citric acid productivity is reverse in respect to increasing molar concentrations of calcium dodecyl benzene sulfonate. However, the LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679 under the influence of each concentration of calcium dodecyl benzene sulfonate used has been stimulating and the yield of citric acid has been found greater than that obtained in the control fermentor flasks. In both the phase the order of productivity and % of citric acid formed is as below :

Phase-I

Concentration of calcium dodecyl benzene sulfonate from 1.0×10^{-5} M to 7.0×10^{-5} M.

Productivity of H₃Cit acid : 0.994%, 1.293%, 1.998%, 2.896%, 4.991%, 6.199% and 8.593%

Phase - II

Concentration of calcium dodecyl benzene sulfonate from 8.0×10^{-5} M to 10.0×10^{-5} M.

Productivity of H₃Cit acid :

4.189%, 2.394%, and 1.293% .

Exposure of fungal strain *Aspergillus clavatus* NCIM 1679 to calcium dodecyl benzene sulfonate may produce a variety of impact. Depending upon the concentration of calcium dodecyl benzene sulfonate to which fungal strain *Aspergillus clavatus* NCIM 1679 were exposed may influence disruption of cells, precipitation of cell protein, inactivation of enzymes and leakage of amino acids from the cells. 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.

Thus, it is concluded that calcium dodecyl benzene sulfonate at lower concentrations is stimulatory and at higher concentrations is deterioratory for the LSF production of H₃Cit by *Aspergillus clavatus* NCIM 1679.

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