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Micellar impact of Sodium octadecyl sulfate on citric acid (H_3Cit) biosynthesis by *Aspergillus niger* T-918

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Abstract : The present study investigates the micellar impact of sodium octadecyl sulfate (SOS), a long-chain anionic surfactant, on the biosynthesis of citric acid (H_3Cit) by *Aspergillus niger* T-918. The research focuses on understanding how micellar interactions between SOS and the fungal cell membrane influence metabolic activity, substrate utilization, and overall citric acid yield. Batch fermentation experiments were conducted under controlled conditions, varying SOS concentrations to evaluate its effect on fungal growth, glucose consumption, and organic acid accumulation. Results revealed that low micellar concentrations of SOS enhanced citric acid production by improving membrane permeability and substrate transport efficiency. However, higher concentrations exhibited inhibitory effects on fungal growth and metabolic flux, likely due to surfactant-induced membrane destabilization. The study highlights the dual role of SOS micelles as both stimulatory and inhibitory agents in fungi processes, depending on their concentration and interaction dynamics. These findings suggest that controlled micellar modulation using SOS can be a promising strategy for optimizing citric acid fermentation by *A. niger* strains. In the present communication efficacy of Sodium octadecyl sulfate on citric acid biosynthesis by *Aspergillus niger* T-918 has been studied. It has been found that the micelles Sodium octadecyl sulfate has stimulatory impact on citric acid fermentation and enhances the yield of citric acid to an extent of 15.253 %higher in comparison to control under optimized conditions.

(Keywords : Sodium octadecyl sulfate, micellar effect, *Aspergillus niger* T-918, citric acid biosynthesis, surfactant-microbe interaction, fermentation enhancement).

Introduction

Citric acid (H_3Cit) is one of the most important organic acids extensively used in food, pharmaceutical, and chemical industries due to its multifaceted roles as an acidulent, preservative, antioxidant, and chelating agent. Industrially, citric acid is primarily produced by *Aspergillus niger* through submerged fermentation processes utilizing carbohydrate-based substrates. Despite decades of optimization, improvements in citric acid yield and productivity remain a major focus of biotechnological research, particularly through the manipulation of physicochemical and biochemical parameters that influence fungal metabolism.[1-11]

Micellar systems, formed by surfactants such as sodium octadecyl sulfate (SOS), represent a unique physicochemical environment capable of modifying the permeability and activity of microbial cell membranes. Surfactants can alter the transport of substrates and metabolic intermediates, influence enzyme localization, and affect overall cell physiology. The interaction between micellar aggregates and microbial cells has been reported to modulate the metabolic flux towards specific biochemical pathways, thereby offering new possibilities for enhancing product biosynthesis.

Sodium octadecyl sulfate, a long-chain anionic surfactant, can form stable micelles in aqueous media above its critical micelle concentration (CMC). These micelles may influence the solubilization of hydrophobic intermediates, modify nutrient accessibility, and alter cell surface properties of *A. niger*. Understanding such micellar effects could

provide a novel approach to optimizing citric acid production through controlled modulation of the fungal metabolic network.[12-22]

The present investigation focuses on elucidating the micellar impact of sodium octadecyl sulfate on citric acid biosynthesis by *Aspergillus niger* T-918. The study aims to explore how varying concentrations of SOS affect fungal growth, metabolic activity, and citric acid yield, thereby providing insights into the mechanistic role of micellar systems in organic acid fermentation.

Experimental

The influence of sodium octadecyl sulfate on bioproduction of H_3Cit by *Aspergillus niger* T-918.

The composition of production medium for the bioproduction of H_3Cit 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

The pH of the production medium was adjusted to 2.0 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 bioproduction of H_3Cit by *Aspergillus niger* T-918. Now, the same production medium of citric acid (H_3Cit) by *Aspergillus niger* T-918 was prepared for 99-fermentor flask, i. e; each contained '100ml' ofproduction 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 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 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., $1.0x10^{-5}$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

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 27^0C and analysed after 11, 13 and 15 days for citric acid[23] formed and molasses[24] left unfermented.

Results and Discussion

* The use of surfactants like sodium octadecyl sulfate could affect the cell membrane permeability of Aspergillus niger, potentially enhancing mass transfer and substrate uptake during fermentation.
* Micellar structures formed by such surfactants may increase the solubility of hydrophobic substrates or facilitate the removal of inhibitory byproducts, supporting sustained citric acid biosynthesis.
* The surfactant's presence could also influence the morphology of fungal pellets, indirectly affecting productivity and yield.
* Nitrogen limitation, buffer and substrate selection, and other environmental controls remain critical in maximizing citric acid yield, regardless of surfactant supplementation.

Table - 1
Bioproduction of H_3Cit by *Aspergillus niger* T-918 exposed to Sodium 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	8.466	5.001	-
(-) Micelle	13	9.998	3.868	-
	15	6.989	3.665	-
1.0×10^{-5}M	11	8.652	4.814	-
$1/10^{-3}\times10^{-2}$M	13	10.227	3.639	+2.290
(+) Micelle	15	7.142	3.492	-
2.0×10^{-5}M	11	8.931	4.525	-
$2/10^{-3}\times10^{-2}$M	13	10.550	3.319	+5.521
(+) Micelle	15	7.373	3.265	-
3.0×10^{-5}M	11	9.610	4.306	-
$3/10^{-3}\times10^{-2}$M	13	10.823	3.043	+8.251
(+) Micelle	15	7.548	3.001	-
4.0×10^{-5}M	11	9.481	3.985	-
$4/10^{-3}\times10^{-2}$M	13	11.234	2.632	+12.362
(+) Micelle	15	7.827	2.430	-
5.0×10^{-5}M**	11	9.735	3.732	-
$5/10^{-3}\times10^{-2}$M	13	11.523***	2.343	+15.253
(+) Micelle	15	8.037	2.214	-
6.0×10^{-5}M	11	9.312	4.154	-
$6/10^{-3}\times10^{-2}$M	13	11.030	2.836	+10.322
(+) Micelle	15	7.687	3.665	-
7.0×10^{-5}M	11	8.973	4.154	-
$7/10^{-3}\times10^{-2}$M	13	10.662	2.836	+6.641
(+) Micelle	15	7.450	2.663	-
8.0×10^{-5}M	11	8.736	4.493	-
$8/10^{-3}\times10^{-2}$M	13	10.334	3.204	+3.360
(+) Micelle	15	7.219	2.985	-
9.0×10^{-5}M	11	8.643	4.823	-
$9/10^{-3}\times10^{-2}$M	13	10.223	3.643	+2.250
(+) Micelle	15	7.135	3.434	-
10.0×10^{-5}M	11	8.550	4.916	-
$10/10^{-3}\times10^{-2}$M	13	10.110	3.759	+1.120
(+) Micelle	15	7.065	3.663	-

* 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%

* Further research integrating surfactants with classical process optimization may produce clearer insights into synergistic effects on fungal metabolism, yield improvement, and process economics for industrial citric acid manufacture.

The data recorded in the table 1 shows that the micelle, i.e., sodium octadecyl sulfate has stimulatory effect on bioproduction of H_3Cit by *Aspergillus niger* T-918.

The maximum yield of citric acid, i,e; 11.523g/100 ml in the presence of sodium octadecyl sulfate was observed at 5.0 $x10^{-5}$M molar concentration in 13 days of optimum incubation period which is 15.253% higher in comparison to control F.F. fermentor flasks, i.e; 9.998 g/100 ml in the same times course and other same experimental parameters.

The higher molar concentrations of sodium octadecyl sulfate were not much favourable for the bioproduction of H_3Cit by *Aspergillus niger* T-918. So the gradual addition of the micelle sodium octadecyl sulfate after certain concentrations were not beneficial for the citric acid fermentation bioprocess.

It has been observed that molar concentration of the micelle i.e., sodium octadecyl sulfate from 1.0 x 10^{-5}M to 5.0 x 10^{-5}M enhances the yield of citric acid to a certain order being 2.290%, 5.521%, 8.251%, 12.362%, and 15.253% higher in comparison to control flasks but at 6.0 x 10^{-5}M to 10.0 x10^{-5}M the yield of citric acid shifted to be in the range, i.e., 10.322%, 6.641%, 3.360%, 2.250% and 1.120% higher in comparison to previous concentrations of sodium octadecyl sulfate taken into experimental trials.

It has been observed further that after optimum concentration, i. e., 5.0 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 1.120%; However, at all the experimental concentrations of sodium octadecyl sulfate used the bioproduction of citric acid (H_3Cit) by *Aspergillus niger* T-918 has been found higher in comparison to control F.F. fermentor flasks.

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