

Studies on microbial bioproduction of citric acid by *Aspergillus clavatus* R-36 exposed to sodium pentadecyl sulfate

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Abstract : The influence of sodium pentadecyl sulfate on microbial bioproduction of citric acid by *Aspergillus parasiticus* R-14, *Aspergillus awamori* R-18, *Aspergillus foetidus* R-30, *Aspergillus flavus* R-32 and *Aspergillus clavatus* R-36 has been assessed. It has been observed that the fungal strain *Aspergillus clavatus* R-36 has been found most potential and effective for the citric acid fermentation process. It has been found that the micelles, i.e., sodium pentadecyl sulfate at molar concentration, i.e., 6.0×10^{-6} M has stimulatory effect on production of citric acid by *Aspergillus clavatus* R-36 and enhances the yield of citric acid to an extent of 14.399% higher in comparison to control fermentor flasks, i.e., 7.285 g/100 mL under the optimized conditions, viz. 33°C temperature, 1.8 pH, 10 days of incubation period with 30% (w/v) molasses solution.

(Keywords): Citric acid fermentation, sodium pentadecyl sulfate and *Aspergillus clavatus* R-36)

Introduction

Biotechnology is an ever expanding field of science. As the field has expanded, research into bioseparation processes has also expanded. The need for more cost effective ways to deliver products that are in high demand is greater now than ever. Bioseparation processes are a very costly part of the overall process of product development. While traditional separation process are effective, there is promise in many new techniques being developed which have the potential to lower the costs of bioseparations.

Micelles are approximately spherical in shape. Other phases, including shapes such as ellipsoids, cylinders, and bilayers are also

possible. The shape and size of a micelle is a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, pH, and ionic strength. The process of forming micellae is known as micellisation and forms part of the phase behaviour of many lipids according to their polymorphism.

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.

Therefore, micellar effect for specially citric acid and other fermentation process may be significant and useful for its improved yield¹⁻⁵. 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 workers⁶⁻¹⁵ 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 fermentation¹⁶⁻²² and in view of this the authoress has studied the influence of sodium pentadecyl sulfate on citric acid production by *Aspergillus clavatus* R-36.

Experimental

The influence of sodium pentadecyl sulfate on production of citric acid by *Aspergillus clavatus* R-36.

The composition of production medium for the production of citric acid by *Aspergillus clavatus* R-36 is prepared as follows:

Molasses : 30% (w/v), NH_4NO_3 : 0.45%
 KH_2PO_4 : 0.45%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.45%, 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" on production of citric acid by *Aspergillus clavatus* R-36. Now, the same production of citric acid by *Aspergillus clavatus* R-36 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 pentadecyl 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 pentadecyl sulfate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

$A \times 10^{-x} \text{ M}$, $1.0 \times 10^{-5} \text{ M}$ to $10.0 \times 10^{-5} \text{ M}$

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 33°C and analysed after 6, 10 and 14 days for citric acid²³ formed and molasses²⁴ left unfermented

Results and Discussion

The influence of Sodium pentadecyl sulfate

The data recorded in the table-1 shows that Sodium pentadecyl sulfate also has stimulatory effect on microbial bioproduction of citric acid by *Aspergillus clavatus* R-36.

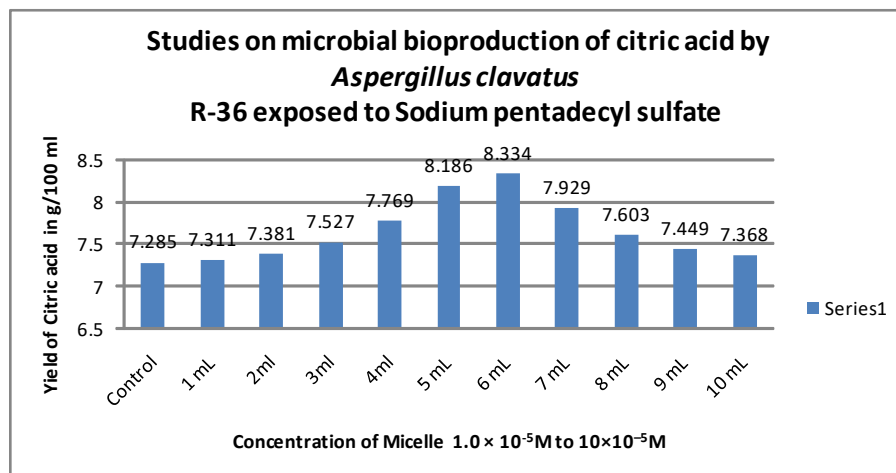
The data (table-1) reveals that the micelle sodium pentadecyl sulfate stimulates the microbial bioproduction of citric acid by

Table - 1
Studies on microbial bioproduction of citric acid by *Aspergillus clavatus*
R-36 exposed to Sodium pentadecyl sulfate

Concentration of micelle used $A \times 10^{-x}M$	Incubation period in days	Yield of citric acid* in g/100mL	Molasses* left Unfermented in g/100 mL	% of citric acid increase after 10 days
Control	6	3.335	7.794	-
(-) Micelle	10	7.285	5.044	-
	14	4.986	5.035	-
$1.0 \times 10^{-3}M$	6	3.341	7.789	-
(+) Micelle	10	7.311	5.018	+ 0.356
	14	5.115	5.009	-
$2.0 \times 10^{-3}M$	6	3.375	7.759	-
(+) Micelle	10	7.381	4.947	+ 1.317
	14	5.202	4.873	-
$3.0 \times 10^{-3}M$	6	3.441	7.688	-
(+) Micelle	10	7.527	4.802	+ 3.321
	14	5.310	4.701	-
$4.0 \times 10^{-3}M$	6	3.551	7.578	-
(+) Micelle	10	7.769	4.569	+ 6.643
	14	5.415	4.492	-
$5.0 \times 10^{-3}M$	6	3.741	7.388	-
(+) Micelle	10	8.186	4.144	+ 12.369
	14	6.112	4.043	-
$6.0 \times 10^{-3}M^{**}$	6	3.808	7.322	-
(+) Micelle	10	8.334***	3.995	+ 14.399
	14	6.188	3.859	-
$7.0 \times 10^{-3}M$	6	3.625	7.504	-
(+) Micelle	10	7.929	4.405	+ 8.840
	14	5.315	4.315	-
$8.0 \times 10^{-3}M$	6	3.475	7.653	-
(+) Micelle	10	7.603	4.726	+ 4.365
	14	5.211	4.666	-
$9.0 \times 10^{-3}M$	6	3.405	7.725	-
(+) Micelle	10	7.449	4.836	+ 2.251
	14	5.198	4.768	-
$10.0 \times 10^{-3}M$	6	3.375	7.750	-
(+) Micelle	10	7.368	4.963	+ 1.139
	14	5.116	4.839	-

* 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 10 days. Experimental deviation ($\pm$) 1.5-3%



Aspergillus clavatus R-36 and enhances the yield of citric acid upto its sodium pentadecyl sulfate concentrations from 1.0×10^{-3} to $6.0 \times 10^{-3} \text{M}$. The effect of sodium pentadecyl sulfate on the productivity (yield) of citric acid was gradually in increasing order and attains its best role at $6.0 \times 10^{-3} \text{M}$ where maximum yield of citric acid, i.e., 8.334g/100 mL is fetched in 10 days of optimum incubation period which is 14.399% higher in comparison to control fermentor flask, i.e., 7.285% g/100 mL.

In the second phase of micellar effect the molar concentration, i. e., from $7.0 \times 10^{-3} \text{M}$ to $10 \times 10^{-3} \text{M}$ the bioproduction of citric acid has been found bit enhanced but the order of citric acid productivity is reverse in respect to increasing molar concentrations of Sodium pentadecyl sulfate. However, the microbial bioproduction of citric acid by *Aspergillus clavatus* R-36 under the influence of each concentration of Sodium pentadecyl sulfate 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 Sodium pentadecyl sulfate from $1.0 \times 10^{-3} \text{M}$ to $6.0 \times 10^{-3} \text{M}$.

Productivity of citric acid :

3.356%, 1.317%, 3.323%, 6.643%, 12.367% and 14.399%

Phase - II

Concentration of Sodium pentadecyl sulfate from $7.0 \times 10^{-3} \text{M}$ to $10.0 \times 10^{-3} \text{M}$.

Productivity of citric acid :

8.840%, 4.365%, 2.251%, and 1.139%

Exposure of fungal strain *Aspergillus clavatus* R-36 to Sodium pentadecyl sulfate may produce a variety of effects. Depending upon the concentration of Sodium pentadecyl sulfate to which fungal strain *Aspergillus clavatus* R-36 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 Sodium pentadecyl sulfate at lower concentrations is stimulatory and at higher concentrations is deterioratory for the microbial bioproduction of citric acid by *Aspergillus clavatus* R-36

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