

Efficacy of sodium hexadecyl sulfate on microbial bioproduction of citric acid

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Abstract : The efficacy of sodium hexadecyl 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 hexadecyl sulfate at molar concentration, i.e., 5.0×10^{-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 19.360% higher in comparison to control fermentor flasks, i.e., 7.350 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 hexadecyl sulfate and *Aspergillus clavatus* R-36)

Introduction

Citric acid has high economic potential owing to its numerous applications. It is mostly produced by microbial fermentation using *Aspergillus niger*. In view of surges in demand and growing markets, there is always a need for the discovery and development of better production techniques and solutions to improve production yields and the efficiency of product recovery. To support the enormous scale of production, it is necessary and important for the production process to be environmentally friendly by utilizing readily available and inexpensive agro-industrial waste products, while maintaining high production yields. This article reviews the biochemistry of citric acid formation, choices of

citric-acid producing microorganisms and raw materials, fermentation strategies, the effects of various fermentation conditions, citric acid recovery options and the numerous applications of citric acid, based on information drawn from the literature over the past 10 years.

Micelles are not a very easy thing to understand and learn. Micelles are amphiphilic colloidal structures having a particle diameter ranging from 5 to 100 nm. Micelles constitute of molecules having two regions of different affinities for water. The amphiphilic molecules forming micelles associate at certain temperatures and certain concentrations. The critical micelle concentration is the concentration at which aggregation begins and the micelles are formed. Similarly, the critical micellar temperature is the temperature at which the micellar molecules aggregate and below which no micelles are formed and exist as monomers. The number of monomer molecules forming a micelle is known as the aggregation number of the micelle. The aggregation of the amphiphilic molecules leading to the formation of micelles occurs due to the removal of the hydrophobic fragments of the micelles from the aqueous environment and the formation of hydrogen bonds in water which leads to a decrease in free energy of the system. Micelles are used as drug carriers in various pharmaceutical industries as they can carry lipophilic drugs within its core while the micellar surface binds polar molecules. Polymeric micelles are used in therapeutics as they provide an improved solubility and hence better intestinal permeability of micelles. Polymeric micelles are

Table - 1
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Concentration of micelle used $A \times 10^{-3}M$	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	3.345	7.891	-
(-) Micelle	10	7.350	4.886	-
	14	5.115	4.768	-
$1.0 \times 10^{-3}M$	6	3.415	7.821	-
(+) Micelle	10	7.515	4.722	+ 2.244
	14	5.313	4.618	-
$2.0 \times 10^{-3}M$	6	3.562	7.675	-
(+) Micelle	10	7.837	4.405	+ 6.625
	14	5.411	4.311	-
$3.0 \times 10^{-3}M$	6	3.749	7.487	-
(+) Micelle	10	8.247	3.995	+ 12.204
	14	6.116	3.858	-
$4.0 \times 10^{-3}M$	6	3.893	7.343	-
(+) Micelle	10	8.574	3.663	+ 16.653
	14	6.215	3.538	-
$5.0 \times 10^{-3}M^{**}$	6	3.987	7.249	-
(+) Micelle	10	8.773***	3.588	+ 19.360
	14	6.364	3.475	-

* 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%

formed of amphiphilic block copolymers¹. Polymeric micelles have long circulating characteristics and significant tumour accumulation which provide an important use in tumour targeting². In contrast with the surfactant micelles, polymeric micelles are usually more stable, have a lower critical micelle concentration, a slower dissociation rate and achieving high drug accumulation at the target. The narrow size range of the micelles are similar to that of viruses and lipoproteins which is a crucial and significant factor in determining their body arrangement when an enhanced permeation retention effect is involved³⁻⁷.

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.

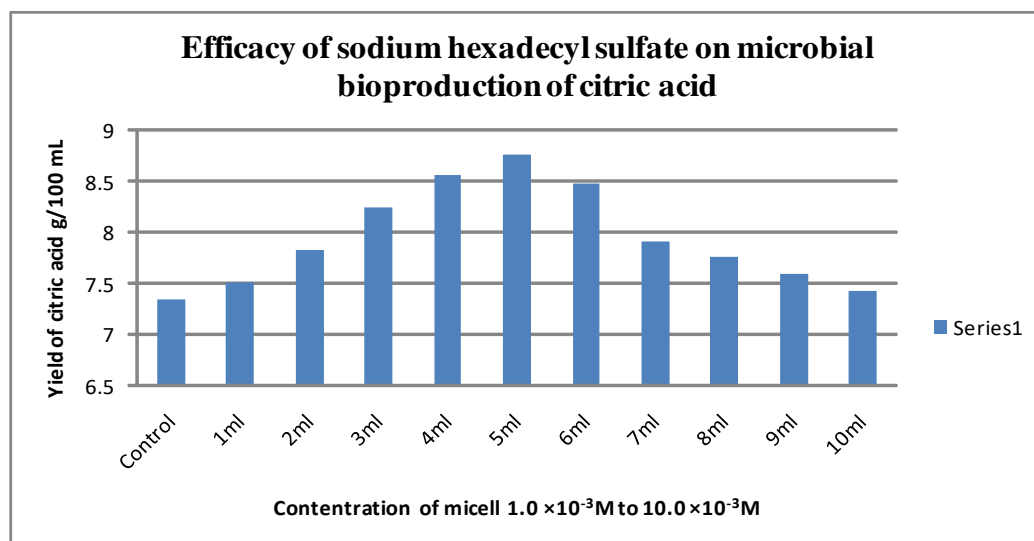
Micelles are approximately spherical in shape. Other phases, including shapes such as ellipsoids, cylinders, and bilayers are also possible. The shape and size of a micelle is a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, 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.

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Concentration of micelle used $A \times 10^{-3}M$	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	3.345	7.891	-
(-) Micelle	10	7.350	4.886	-
	14	5.115	4.768	-
$6.0 \times 10^{-3}M$	6	3.860	7.376	-
(+) Micelle	10	8.491	3.747	+ 15.523
	14	6.220	3.675	-
$7.0 \times 10^{-3}M$	6	3.599	7.637	-
(+) Micelle	10	7.922	4.315	+ 7.782
	14	5.395	4.259	-
$8.0 \times 10^{-3}M$	6	3.525	7.712	-
(+) Micelle	10	7.760	4.478	+ 5.578
	14	5.269	4.386	-
$9.0 \times 10^{-3}M$	6	3.452	7.785	-
(+) Micelle	10	7.597	4.645	+ 3.360
	14	5.235	4.554	-
$10.0 \times 10^{-3}M$	6	3.381	7.855	-
(+) Micelle	10	7.434	4.804	+ 1.142
	14	5.159	4.710	-

* 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%



Therefore, micellar effect for specially citric acid and other fermentation process may be significant and useful for its improved yield⁸⁻¹². 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 workers¹³⁻²² 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 fermentation²³⁻²⁹ and in view of this the authoress has studied the influence of sodium hexadecyl sulfate on citric acid production by *Aspergillus clavatus* R-36.

Experimental

The influence of sodium hexadecyl 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 hexadecyl 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 hexadecyl 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 acid³⁰ formed and molasses³¹ left unfermented.

Results and Discussion

The efficacy of sodium hexadecyl sulfate

The data given in the table -1 shows that the micelle sodium hexadecyl sulfate has also been found stimulatory for the microbial bioproduction of citric acid by *Aspergillus clavatus* R-36. From the data given in the table-1 it is obvious that sodium hexadecyl sulfate influences the citric acid fermentation

process in different phases. The main characteristics of the Sodium hexadecyl sulfate is as follows :

(i) Sodium hexadecyl sulfate is stimulatory at its all molar concentrations used during the course of fermentation for microbial bioproduction of citric acid by *Aspergillus clavatus* R-36 i. e., from $1.0 \times 10^{-3}\text{M}$ to $10.0 \times 10^{-3}\text{M}$.

(ii) The molar concentration $1.0 \times 10^{-3}\text{M}$, $2.0 \times 10^{-3}\text{M}$, $3.0 \times 10^{-3}\text{M}$, $4.0 \times 10^{-3}\text{M}$ and $5.0 \times 10^{-3}\text{M}$ of Sodium hexadecyl sulfate influence the yield of citric acid in a approximately regular increasing order after each state, i. e., 2.244%, 6.625%, 12.204%, 16.653% and 19.360%

(iii) The molar concentration $6.0 \times 10^{-3}\text{M}$ to $10.0 \times 10^{-3}\text{M}$ of Sodium hexadecyl sulfate also influences the productivity of citric acid in a regular decreasing manner. The % increase in

the yield of citric acid at respective molar concentration of Sodium hexadecyl sulfate has been found to be as follows : 15.523%, 7.782%, 5.578%, 3.360%, and 1.142%

(iv) The maximum yield of citric acid, i.e; 8.773g/100 mL in the presence of Sodium hexadecyl sulfate was observed at $5.0 \times 10^{-3}\text{M}$ molar concentration in 10 days of optimum incubation period which is 19.360% higher in comparison to control fermentor flasks, ie; 7.350% g/100 mL in the same times course and other same experimental parameters.

(v). The higher molar concentrations of sodium hexadecyl sulfate were not much favourable for the microbial bioproduction of citric acid by *Aspergillus clavatus* R-36 So the gradual addition of the micelle Sodium hexadecyl sulfate after molar concentrations $6.0 \times 10^{-3}\text{M}$ and onwards were not beneficial for the citric acid fermentation process.

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