

Assessment of citric acid production in the presence of potassium dodecylbenzenesulphonate

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Abstract: The impact of potassium dodecylbenzenesulphonate on bioproduction of citric acid by some fungal strains such as *Aspergillus carbonarius* NCIM -1962, *Aspergillus saitoi*, NCIM -1364, *Aspergillus usumii* NCIM -1576, *Aspergillus wentii* NCIM -1981 and *Aspergillus niger* NCIM -269 has been assessed. It has been found that the fungal strain *Aspergillus niger* NCIM -2690 has been found most suitable to give higher yield of citric acid. The micelle; i.e. potassium dodecylbenzene- sulphonate under trial has stimulatory effect on bioproduction of citric acid and enhances the yield of citric acid to an extent of 7.617% higher in comparison to control fermenter flasks, i.e., 6.196g/100 ml in 12 days of optimum incubation period, 2.0 pH and 25°C temperature with 20% (w/v) molasses solution alongwith other nutritional ingredients.

(Key words : Molasses, micelle, citric acid, *Aspergillus niger* NCIM-2690, potassium dodecylbenzenesulphonate)

Introduction

Micelles are spherical amphiphilic structures that have a hydrophobic core and a hydrophilic shell. The hydrophilic shell makes the micelle water soluble that allows for intravenous delivery while the hydrophobic core carries a payload of drug for therapy. The nanoscale dimensions (diameter less than 50 nm) and the hydrophilic shell of polymeric micelles serve as protection from elimination by the reticulo-endothelial system, thereby increasing their circulation time and ability to deliver the drug to the target. Micelles are lipid molecules that arrange themselves in a spherical form in aqueous solutions. 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). The polar head groups of micelles form the outside surface of the molecule, facing the aqueous environment, and their hydrophobic chains are clustered in the interior, where water is excluded since they are nonpolar. Micelles are known to have an anisotropic water distribution within their structure¹. In other words, the water concentration decreases from the surface towards the core of the micelle, with a completely hydrophobic (water-excluded) core. Consequently, the spatial position of a solubilized drug in a micelle will depend on its polarity: nonpolar molecules will be solubilized in the micellar core, and substances with intermediate polarity will be distributed along the surfactant molecules in certain intermediate positions. On the other hand, numerous drug delivery and drug targeting systems have been studied in an attempt to minimize drug degradation and loss, to prevent harmful side effects, and to increase drug bioavailability²⁻⁶.

In general, surfactants play an important role in contemporary pharmaceutical biotechnology, since they are largely utilized in various drug dosage forms to control wetting, stability, bioavailability, among other properties^{7,8}. It is important to notice that lyophobic colloids, such as polymers, require certain energy to be applied for their formation, are quite unstable from the thermodynamic point of view, and frequently form large aggregates⁹⁻²³.

Thus, from the above brief review it is

Table - 1
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Concentration of micelle used $A \times 10^{-X}M$	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left unfermented in g/100ml	% of citric acid increased after 12 days
Control	10	4.954	4.586	-
(-) Micelle	12	6.196	3.351	-
	14	5.489	3.110	-
$1.0 \times 10^{-5}M$	10	5.018	4.549	-
(+) Micelle	12	6.277	3.310	+ 1.307
	14	5.560	3.080	-
$2.0 \times 10^{-5}M$	10	5.087	4.519	-
(+) Micelle	12	6.365	3.285	+ 2.727
	14	5.637	2.981	-
$3.0 \times 10^{-5}M$	10	5.122	4.490	-
(+) Micelle	12	6.408	3.211	+ 3.421
	14	5.675	2.915	-
$4.0 \times 10^{-5}M$	10	5.221	4.460	-
(+) Micelle	12	6.532	3.197	+ 5.422
	14	5.785	2.886	-
$5.0 \times 10^{-5}M^{**}$	10	5.330	4.431	-
(+) Micelle	12	6.668***	3.156	+ 7.617
	14	5.906	2.817	-

Contd.

evident that micelles are required for genetic manipulation and exploitation specially for citric acid fermentation and in view of this the authoress has studied the influence of potassium dodecylbenzenesulphonate on citric acid production.

Experimental

The influence of potassium dodecylbenzenesulphonate on citric acid fermentation by *Aspergillus niger* NCIM-2690 The composition of citric acid fermentation by *Aspergillus niger* NCIM-2690 is prepared as follows:

Molasses : 20% (w/v), NH_4NO_3 : 0.25%
 KH_2PO_4 : 0.25%, $MgSO_4 \cdot 7H_2O$: 0.30%
 pH : 2.0

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 citric acid fermentation by *Aspergillus niger* NCIM-2690. Now, the same production medium for citric acid fermentation by *Aspergillus niger* NCIM-2690 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 potassium dodecylbenzenesulphonate 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

Contd.

Table - 1**Assessment of citric acid production in the presence of potassium dodecylbenzenesulphonate**

Concentration of micelle used $A \times 10^{-x}M$	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left unfermented in g/100ml	% of citric acid increased after 12 days
Control	10	4.954	4.586	-
(-) Micelle	12	6.196	3.351	-
	14	5.489	3.110	-
$6.0 \times 10^{-5}M$	10	5.285	4.461	-
(+) Micelle	12	6.612	3.179	+6.714
	14	5.856	2.840	-
$7.0 \times 10^{-5}M$	10	5.196	4.481	-
(+) Micelle	12	6.500	3.193	+4.906
	14	5.757	2.869	-
$8.0 \times 10^{-5}M$	10	5.082	4.510	-
(+) Micelle	12	6.358	3.227	+2.614
	14	5.631	2.915	-
$9.0 \times 10^{-5}M$	10	5.018	4.530	-
(+) Micelle	12	6.278	3.267	+1.323
	14	5.560	3.051	-
$10.0 \times 10^{-5}M$	10	4.988	4.564	-
(+) Micelle	12	6.240	3.368	+0.710
	14	5.527	3.173	-

* 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 ($\pm$) 1.5-3%

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 potassium dodecylbenzene sulphonate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

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

Where : A = amount of micelle in ml, i.e., 1.0 ml
to 10 ml.

x = Molarity of the miceller solution and
respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 25°C and analysed after 10, 12 and 14 days for citric acid²⁵ formed and molasses left unfermented²⁶.

Results and Discussion

The data recorded in the table 1 shows that potassium dodecylbenzenesulphonate has stimulatory effect on the citric acid fermentation by *Aspergillus niger* NCIM-2690.

The maximum yield of citric acid, i.e; 6.668g/100 ml in the presence of potassium dodecylbenzenesulphonate was observed at $5.0 \times 10^{-5}M$ molar concentration in 12 days of optimum incubation period which is 7.617% higher in comparison to control fermentor flasks, i.e; 6.196 g/100 ml in the same times course and other same experimental parameters. The higher molar concentrations of potassium dodecyl- benzene-sulphonate were not much favourable for the citric acid fermentation by *Aspergillus niger* NCIM-2690.

So the gradual addition of the micelle potassium dodecylbenzenesulphonate after certain concentrations were not beneficial for the citric acid fermentation process. It has been observed that molar concentration of the micelle i.e., potassium dodecylbenzenesulphonate from $1.0 \times 10^{-5}\text{M}$ to $5.0 \times 10^{-5}\text{M}$ enhances the yield of citric acid to a certain order being 1.307%, 2.727%, 3.421%, 5.422%, and 7.617% higher in comparison to control flasks but at $6.0 \times 10^{-5}\text{M}$ to $10.0 \times 10^{-5}\text{M}$ the yield of citric acid shifted to be in lower range, i.e., 6.714%, 4.906%, 2.614%, 1.323%, and 0.710% higher in comparison to previous concentrations

of potassium dodecylbenzenesulphonate taken into experimental trials.

It has been observed further that after optimum concentration, i.e., $5.0 \times 10^{-5}\text{M}$, the addition of the same micelle to the production medium causes fall in the yield of citric acid gradually and reaches to 0.710%; However, at all the experimental concentrations of potassium dodecylbenzenesulphonate used the citric acid fermentation by *Aspergillus niger* NCIM-2690 has been found higher in comparison to control fermentor flasks.

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