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# Production of citric acid by *Candida lipolytica* G-25 exposed to n-octadecyl p-bromobenzenesulfonate

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**Abstract**: The influence of n-octadecyl p-bromobenzenesulfonate on transformation of molasses pollutant to citric acid by *Candida lipolytica* G-25 has been assessed. It has been found that the micelle, i.e., n-octadecyl p-bromobenzenesulfonate under trial has stimulatory effect on transformation of molasses pollutant to citric acid by *Candida lipolytica* G-25 and enhances the yield of citric acid to an extent of 8.905% higher in comparison to control fermentor flasks, i.e., 7.904 ml/100mL in 13 days of incubation period, 5.2 pH and 29$^0$C temperature with 24% (w/v) molasses solution.

(**Key words :** Molasses, micelle, citric acid, n-octadecyl p-bromobenzenesulfonate and *Candida lipolytica* G-25 )

## Introduction

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. Micelle formation is favored when the cross-sectional area of the head group is greater than that of the acyl side chain(s), as in free fatty acids, more notably lysophospholipids (phospholipids lacking one fatty acid), and many detergents, such as sodium dodecyl sulfate.These single chain fatty acids will usually form micelles as opposed to Glycolipids and other phospholipids with two hydrocarbon tails. Fatty acids from Glycolipids and phospholipids with their two hydrophobic chains are too bulky to fit into the a spherical shape as micelles do. Thus, glycolipids and phospholipids are more commonly found in "lipid bilayers", which are discussed in the next section.

Micelles are known to have an anisotropic water distribution within their structure[1]. 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 bioavailability[2-6]. Within this context, the utilization of micelles as drug carriers presents some advantages when compared to other alternatives such as soluble polymers and liposomes.

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,

**Table - 1**
**Production of citric acid by *Candida lipolytica* G-25 exposed to n-octadecyl p-bromobenzenesulfonate**

| 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 | 12 | 6.451 | 4.799 | - |
| (-) Micelle | 13 | 7.804 | 3.445 | - |
| | 14 | 7.456 | 3.118 | - |
| 1.0x10-5M | 12 | 6.483 | 4.758 | - |
| (+) Micelle | 13 | 7.844 | 3.417 | +0.512 |
| | 14 | 7.493 | 3.009 | - |
| 2.0x10-5M | 12 | 6.560 | 4.716 | - |
| (+) Micelle | 13 | 7.938 | 3.361 | +1.717 |
| | 14 | 7.582 | 2.910 | - |
| 3.0x10-5M | 12 | 6.605 | 4.619 | - |
| (+) Micelle | 13 | 7.992 | 3.320 | +2.409 |
| | 14 | 7.634 | 2.880 | - |
| 4.0x10-5M | 12 | 6.689 | 4.571 | - |
| (+) Micelle | 13 | 8.093 | 3.279 | +3.703 |
| | 14 | 7.731 | 2.811 | - |
| 5.0x10-5M | 12 | 6.760 | 4.518 | - |
| (+) Micelle | 13 | 8.180 | 3.210 | +4.818 |
| | 14 | 7.813 | 2.710 | - |

* 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 13 days.
Experimental deviation (±) 1.5-3%

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 aggregates[9-23].

Thus, from the above brief review it is evident that micelles are required for genetic manupulation and exploitation specially for citric acid fermentation and in view of this the authors have studied the influence of n-octadecyl p-bromobenzenesulfonate on transformation of molasses pollutant to citric acid by *Candida lipolytica* G-25 .

**Experimental**

The influence of n-octadecyl p-bromobenzenesulfonate on production of citric acid by *Candida lipolytica* G-25. The composition of production medium for the production of citric acid by *Candida lipolytica* G-25 is prepared as follows:

Molasses : 24% (w/v), $NH_4NO_3$ : 0.25%
$KH_2PO_4$ : 0.25%, $MgSO_4.7H_2O$ : 0.25%, pH : 5.2

The pH of the production medium was adjusted to 5.2 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

Table-1 Continued .....

**Table - 1**
**Production of citric acid by *Candida lipolytica* G-25 exposed to n-octadecyl p-bromobenzenesulfonate**

| 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 | 12 | 6.451 | 4.799 | - |
| (-) Micelle | 13 | 7.804 | 3.445 | - |
| | 14 | 7.456 | 3.118 | - |
| 6.0x10-5M | 12 | 6.844 | 4.426 | - |
| (+) Micelle | 13 | 8.282 | 3.179 | +6.125 |
| | 14 | 7.910 | 2.749 | - |
| 7.0x10-5M** | 12 | 7.025 | 4.498 | - |
| (+) Micelle | 13 | 8.499*** | 3.229 | +8.905 |
| | 14 | 8.119 | 2.799 | - |
| 8.0x10-5M | 12 | 6.812 | 4.535 | - |
| (+) Micelle | 13 | 8.242 | 3.290 | +5.612 |
| | 14 | 7.873 | 2.820 | - |
| 9.0x10-5M | 12 | 6.670 | 4.593 | - |
| (+) Micelle | 13 | 8.070 | 3.325 | +3.408 |
| | 14 | 7.709 | 2.841 | - |
| 10.0x10-5M | 12 | 6.599 | 4.685 | - |
| (+) Micelle | 13 | 7.984 | 3.397 | +2.306 |
| | 14 | 7.627 | 3.105 | - |

* 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 13 days.
Experimental deviation (±) 1.5-3%

fermentor flask, i.e., "100ml" on production of citric acid by *Candida lipolytica* G-25. Now, the same production of citric acid by *Candida lipolytica* G-25 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 n-octadecyl p-bromobenzenesulfonate 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.

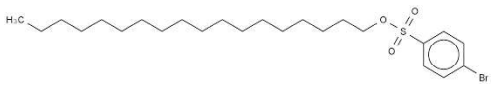
Now, the total volume in each fermentor flasks was made upto 100 ml by adding requisite amount of distilled water. Thus, the molar concentration of n-octadecyl p-bromobenzene-sulfonate in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

A x $10^{-x}$ M to 10.0x$10^{-5}$M respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 29$^0$C and analysed after 12, 13 and 14 days for citric acid[25] formed and molasses[26] left unfermented.

**Results and Discussion**

**The Influence of n-octadecyl p-bromobenzene-sulfonate.**



**n-octadecyl p-bromobenzenesulfonate**

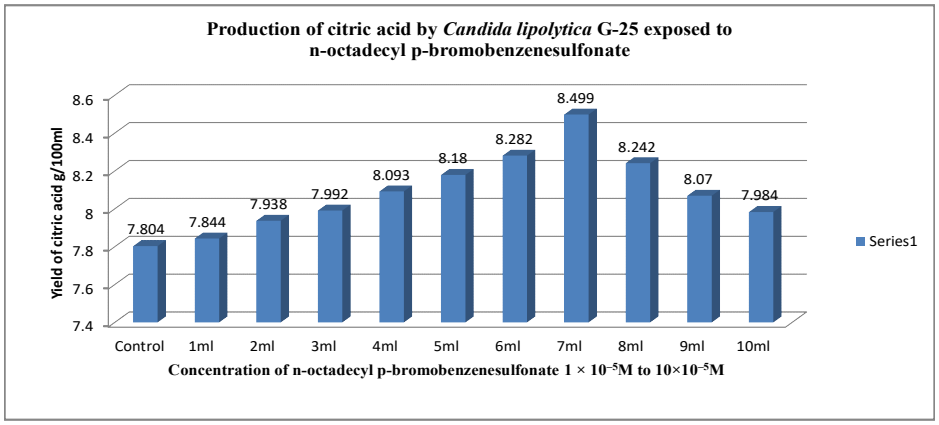
The data recorded in the table-1 shows that n-octadecyl p-bromobenzenesulfonate has stimulatory and benefical effect on production of citric acid by *Candida lipolytica* G-25.

The maximum yield of citric acid, i,e; 8.499g/100 ml in the presence of n-octadecyl p-bromobenzenesulfonate was observed at 7.0 x $10^{-5}$M molar concentration in 13 days of optimum incubation period which is 8.905% higher in comparison to control fermentor flasks, ie; 7.804 g/100 ml in the same times course and other same experimental parameters. The higher molar concentrations of n-octadecyl p-bromobenzene-sulfonate were not much effective and favourable for the production of citric acid by *Candida lipolytica* G-25. So the gradual addition of the micell n-octadecyl p-bromobenzenesulfonate after certain concentrations were not effective and beneficial for the production of citric acid by *Candida lipolytica* G-25.

It has been observed that molar concentration of the micell, i.e., n-octadecyl p-bromobenzenesulfonate from 1.0 x $10^{-5}$M to 7.0 x $10^{-5}$M enhances the yield of citric acid to a certain order being 0.512%, 1.717%, 2.409%, 3.703%, 4.818%, 6.125% and 8.905% higher in comparison to control flasks but at 8.0 x $10^{-5}$M to 10.0 x $10^{-5}$M the yield of citric acid shifted to be 5.612%, 3.408%, and 2.306% higher in comparison to previous concentrations of n-octadecyl p-bromobenzenesulfonate taken into experimental trials.

From the present investigation it is obvious that the chemical micelles used under trial, i.e., useful micelle is for production of citric acid by *Candida lipolytica* G-25 and, therefore, can be employed for the improved production of citric acid by *Candida lipolytica* G-25

It is interesting to note that the micelles used in the present investigation in the micellar molar concentrations under trial, i. e., from 1.0 x $10^{-5}$M to 10 x $10^{-5}$M has given better yield of citric acid in each case in comparison to respective controls.



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