

Micellar effect on citric acid fermentation by novel strain of *Aspergillus niger* NCIM-705

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Abstract : The efficacy of a novel class of compounds bearing a micelle entity, sodium dodecylbenzenesulfonate on bioproduction of citric acid by *Aspergillus niger* NCIM-705, *Aspergillus carbonarius* NCIM 790, *Aspergillus saitoi* NCIM -796, *Aspergillus usumii* NCIM - 774 and *Aspergillus wentii* NCIM -726 has been targeted. It has been observed that the fungal strain *Aspergillus niger* NCIM-705 has been found most potential and effective for the citric acid fermentation process. It has been found that the micelles, i.e., sodium dodecylbenzenesulfonate at molar concentration, i.e., $8.0 \times 10^{-3}M$ has stimulatory effect on production of citric acid by *Aspergillus niger* NCIM-705 and enhances the yield of citric acid to an extent of 11.525% higher in comparison to control fermentor flasks, i.e., 6.490 g/100 ml under the optimized conditions, viz. 27°C temperature, 2.1 pH, 11 days of incubation period with 22% (w/v) molasses solution.

(Keywords: Citric acid fermentation process, sodium dodecylbenzenesulfonate and *Aspergillus niger* NCIM-705)

Introduction

A tiny particle made of substances that are soluble in water and that come together to form a ball like shape. These particles can carry other substances inside them. Today, science has found various practical applications for micelles. The properties of surfactant substances in aqueous media, been studied by scientists and there are currently several practical applications of the phenomenon of micelles formation.

The process of forming micelles is known as micellisation and forms part of the phase behaviour of many lipids according to their polymorphism¹⁻³. A number of recent review articles have been written on the rheology and applications of wormlike micelle solutions⁴⁻¹⁶.

Thus, from the above brief review it is evident that micelles are required for exploitation specially for citric acid fermentation¹⁷⁻²¹ and in view of this the authoresses has studied the influence of sodium dodecylbenzenesulfonate on citric acid production by *Aspergillus niger* NCIM-705

Experimental

The influence of sodium dodecylbenzenesulfonate on citric acid production by novel *Aspergillus niger* NCIM-705.

The composition of the production medium for citric acid production by novel *Aspergillus niger* NCIM-705 isolate has been prepared as follows :

Molasses : 22% (w/v) NH_4NO_3 : 0.45%
 KH_2PO_4 : 0.45%, $MgSO_4 \cdot 7H_2O$: 0.45%
pH : 2.1

The pH of the production medium was adjusted to 2.1 by adding requisite amount of KCl-HCl buffer solution, and this pH was also ascertained by a pH meter.

Table - 1
Micellar effect on citric acid fermentation by novel strain of *Aspergillus niger* NCIM-705

Concentration of micelles 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	% difference of citric acid increased after 11 days
Control	9	4.980	4.382	-
(-) Micelle	11	6.490	2.879	-
	13	5.960	2.610	-
1.0x10 ⁻⁵ M	9	5.034	4.365	-
(+) Micelle	11	6.562	2.803	+ 1.109
	13	6.025	2.579	-
2.0x10 ⁻⁵ M	9	5.094	4.310	-
(+) Micelle	11	6.640	2.780	+ 2.311
	13	6.025	2.545	-
3.0x10 ⁻⁵ M	9	5.209	4.279	-
(+) Micelle	11	6.789	2.721	+ 4.607
	13	6.234	2.513	-
4.0x10 ⁻⁵ M	9	5.263	4.256	-
(+) Micelle	11	6.860	2.701	+ 5.701
	13	5.299	2.471	-
5.0x10 ⁻⁵ M	9	5.323	4.221	-
(+) Micelle	11	6.938	2.681	+ 6.902
	13	6.371	2.419	-

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

The above composition medium represents volume of a fermentor flask, i.e., "100ml" citric acid production by novel *Aspergillus niger* NCIM-705 isolate

Now, the same production medium for citric acid production by novel *Aspergillus niger* NCIM-705 isolate 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 dodecylbenzenesulfonate was prepared and from the above micelle 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 micelles.

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

Table - 1
Micellar effect on citric acid fermentation by novel strain of *Aspergillus niger* NCIM-705

Concentration of micelles used $A \times 10^{-x}$ M	Incubation Period in days	Yield of citric acid* in g/100 ml	Molasses* left Unfermented in g/100 ml	% difference of citric acid increased after 11 days
Control	9	4.980	4.382	-
(-) Micelle	11	6.490	2.879	-
	13	5.960	2.610	-
6.0×10^{-5} M	9	5.343	4.201	-
(+) Micelle	11	6.965	2.665	+ 7.318
	13	6.395	2.369	-
7.0×10^{-5} M	9	5.438	4.189	-
(+) Micelle	11	7.088	2.629	+ 9.214
	13	6.508	2.353	-
8.0×10^{-5} M**	9	5.552	4.171	-
(+) Micelle	11	7.238***	2.603	+ 11.525
	13	6.645	2.318	-
9.0×10^{-5} M	9	5.333	4.191	-
(+) Micelle	11	6.952	2.633	+ 7.118
	13	6.383	2.336	-
10.0×10^{-5} M	9	5.189	4.253	-
(+) Micelle	11	6.783	2.698	+ 4.206
	13	6.210	2.371	-

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

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

Where

A = amount of chemical micelle in ml

x=Molarity of the micelle solution respectively.

The above fermentor flasks were then sterilized, cooled inoculated, incubated at 32°C and analysed after 9, 11 and 13 days for citric acid²² formed and molasses²³ left unfermented.

Results and Discussion

The data recorded in the table 1 shows that sodium dodecylbenzenesulfonate has stimulatory effect on the citric acid production by novel *Aspergillus niger* NCIM-705 isolate.

The maximum yield of citric acid, i.e;

7.238g/100 ml in the presence of sodium dodecylbenzenesulfonate was observed at 8.0×10^{-5} M molar concentration in 11 days of optimum incubation period which is 11.525% higher in comparison to control fermentor flasks, i.e; 6.490 g/100 ml in the same times course and other same experimental parameters.

The higher molar concentrations of sodium dodecylbenzenesulfonate were not much favourable for the citric acid production by novel *Aspergillus niger* NCIM-705 isolate. So the gradual addition of the micelle sodium dodecylbenzenesulfonate after certain concentrations were not beneficial for the citric acid fermentation process.

It has been observed that molar concentration of the micelle i.e., sodium dodecylbenzenesulfonate from $1.0 \times 10^{-5}\text{M}$ to $8.0 \times 10^{-5}\text{M}$ enhances the yield of citric acid to a certain order being 1.109%, 2.311%, 4.607%, 5.701%, 6.902%, 7.318%, 9.214% and 11.525% higher in comparison to control flasks but at $9.0 \times 10^{-5}\text{M}$ to $10.0 \times 10^{-5}\text{M}$ the yield of citric acid shifted to be in lower range, i.e., 7.118% and 4.206% higher in comparison to previous concentrations of sodium dodecyl benzene-sulfonate taken into experimental trials.

It has been observed further that after optimum concentration, i.e., $8.0 \times 10^{-5}\text{M}$, the addition of the same micelle to the production medium causes fall in the yield of citric acid gradually and reaches to 4.206%; However, at all the experimental concentrations of sodium dodecylbenzenesulfonate used for the citric acid production by novel *Aspergillus niger* NCIM-705 isolate has been found higher in comparison to control fermentor flasks.

References

1. J.W. McBain, *Trans. Faraday Soc.* **9**, 99 (1993)
2. MacNaughton, Alan D.; Wilkinson, Andrew R., eds. Compendium of Chemical Terminology : IUPAC Recommendations (2nd ed.). Oxford: Blackwell Science. (1997)
3. I.W. Hamley "Introduction to Soft Matter" (John Wiley, 2007)
4. P. Schurtenberger, R. Scartazzini, L J Magid, , Leser, M E and P I Luisi, *J. Phys. Chem.*, **94**(9) 3695 (1990)
5. Tung, S-H, Huang, Y-E and Raghavan, S R, *J. Am. Chem. Soc.*, **128**, 5751 (2006).
6. F. Lequeux, and S J Candau, T. McLeish, Editor. Kluwer Academic Publishers: Netherlands. p. 181- 190 (1997).
7. R G Laughlin, Academic Press (New York) (1994).
8. J. Yang, *Current Opinion in Colloid and Interface Sci.*, **7**, 276 (2002).
9. J N Israelachvili, Academic Press (London), 296 (1985).
10. R G Larson, , The Structure and Rheology of Complex Fluids. Oxford University Press (New York) 364 (1999)
11. V J Anderson, J R A Pearson, and E S Boek, , *The British Society of Rheology*. p. 217-253. (2006).
12. C A Dreiss, , *Soft Matter*, **3**, 956 (2007).
13. M ECates and Fielding, S M, *Adv. Phys.*, **55** (7-8) 799 (2006) .
14. J L Zakin, B Lu, and N W Bewerdorff, *Rev. Chem. Eng.*, **14**(45), 253 (1998).
15. H Rehage and H. Hoffmann, *Mol. Phys.*, **74**(5) 933 (1991)
16. J N Israelachvili, Intermolecular and surface forces. 2nd ed. Academic Press (London ; San Diego). xxi, 450 (1992)
17. J. P. Kumar and S. P. Singh *J. Chemtracks* **18**(1) 57 (2016)
18. Manoj Kumar *J. Chemtracks*, **22**(1&2), 73 (2020)
19. Manoj Kumar *J. Chemtracks*, **22**(1&2), 293 (2020)
20. Manoj Kumar *J. Chemtracks*, **22**(1&2), 343 (2020)
21. Manoj Kumar, S. M. Abdullah and Renu Bala *J. Chemtracks*, **24**(1&2), 67 (2022)
22. J. R. Marrier and M. Boulet *J. Dairy Science*, **41**, 1683 (1983).
23. M. Dubois, K.A. Gilles J. K. Hamilton and F. Smith, *Anal Chem.* **28**, 350 (1956).