Micellar Action of Sodium Cocoyl Glutamate (SCG) in Enhancing Ethanol Production from Molasses by *S. cerevisiae* NCIM-1267

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Abstract: The application of eco-friendly surfactants in fermentation processes has gained increasing attention for enhancing microbial efficiency and product yield. In this study, the micellar role of Sodium Cocoyl Glutamate (SCG), a biodegradable amino acid-based surfactant, was investigated in ethanol production from natural source molasses using Saccharomyces cerevisiae NCIM-1267. The micellar system formed by SCG was found to improve substrate availability, facilitate nutrient transport, and reduce inhibitory stress on yeast cells during fermentation. Enhanced cell membrane permeability and better interaction between hydrophobic substrates and microbial cells contributed to higher ethanol yield compared to control fermentations without SCG. The findings suggest that the incorporation of SCG in the fermentation medium can serve as a sustainable and effective strategy to optimize ethanol bioproduction from molasses, with potential applications in industrial-scale bioethanol 'production. In the present communication micellar action of SCG in enhancing ethanol production from molasses by *Saccharomyces cerevisiae* NCIM-1267 has been assessed. It has been observed that the micelle SCG acts as a good modulator and stimulator, therefore; enhances the yield of ethanol to an extent of 3.768%higher in comparison to control when 25% molasses solution is allowed to ferment at pH 5.2, temperature 30^0C and incubation period of 50 hrs along with some rich ingredients.

(Keywords : Micelles, SCG, Ethanol fermentation and *Saccharomyces cerevisiae* NCIM-1267).

Introduction

Molasses, a low-cost by-product of the sugar industry, remains a cornerstone feedstock for first-generation bioethanol due to its high fermentable sugar content, established supply chains, and compatibility with robust Saccharomyces cerevisiae strains. Recent work continues to refine molasses-based processes through strain selection, co-culture strategies, and process intensification to overcome variability and inhibitor loads intrinsic to industrial molasses streams. For example, co-fermentation approaches and targeted strain screening have shown measurable gains in ethanol titres and productivity under industrially relevant conditions.[1-10]

Alongside biological improvements, surfactant-assisted fermentation has emerged as a pragmatic lever to enhance mass transfer, mitigate product and substrate inhibition, and modulate cell envelope properties. Non-ionic surfactants (e.g., Tween series) are the most documented, improving enzymatic saccharification and simultaneous saccharification - fermentation (SSF) and, in several studies, increasing ethanol yields by reducing unproductive enzyme - lignin interactions and improving substrate accessibility[11-20]. Although much of this literature focuses on lignocellulosic feedstocks, the underlying mechanisms - lower interfacial tension, micelle-mediated solubilization of hydrophobic inhibitors, improved nutrient transport, and altered membrane permeability — are also pertinent to molasses systems.

Experimental

The influence of sodium cocoyl glutamate (SCG) on alcoholic fermentation by the

yeast *Saccharomyces cerevisiae* NCIM -1267

The composition of production medium for the alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM -1267 is prepared as follows:
Molasses : 25%, Malt extract : 0.35% , Yeast extract: 0.35%, Peptone : 0.50%, $(NH_4)_2HPO_4$: 0.35%
pH : 5.2
Distilled water was added to make up the volume upto 100 ml.
The pH of the medium was adjusted to 5.2 by adding requisite amount of lactic acid.
The above composition medium represents volume of a fermentor flask, i.e, 100 ml production medium for alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM -1267.

Now the same production medium for alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM -1267 was prepared for 99 fermentor-flasks, i.e., each containing 100 ml of production medium. These fermentor-flasks were then arranged in 10 sets each comprising 9 fermentor-flasks. Each set was rearranged in 3 subsets, each comprising 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.

Now, M/1000 solution of Sodium Cocoyl Glutamate (SCG) was prepared and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ml of this solution was added to the fermentor-flasks of first to 10th sets respectively. The control fermentor-flasks contained no micelles. The total volume in each fermentor-flask was made upto 100 ml by adding requisite amount of distilled water.
Thus, the concentration of Sodium Cocoyl Glutamate (SCG) in first, second, third, fourth, fifth, sixth, seventh, eighth, ninth and tenth subsets were approximately as given below :
$a \times 10^{-x}$ M i. e., 1.0×10^{-5} M to 10.0×10^{-5} M
Where a = amount of micelles in ml, ie; from 1.0 ml to 10ml. x = molarity of the micellar solution.

The fermentor-flasks were then steam sterilized, cooled, inoculated, incubated at 30°C and analysed colorimetrically after 30, 50, and 60 hours for the ethanol[21] formed and molasses left[22] unfermetned.

Results and Discussion
The influence of sodium cocoyl glutamate (SCG) on alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM -1267.
The data given in the table - 1 show that the Sodium Cocoyl Glutamate (SCG) was found to be stimulating for alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM -1267 upto its concentration from 1.0×10^{-5} M to 10.0×10^{-5} M in two phases:

In the first phase, i.e., from 1.0×10^{-5} M to 3.0×10^{-5} M the effect of the micelle sodium Cocoyl Glutamate (SCG) is significant and the production of alcohol increases gradually till a maximum value is reached at 3.0×10^{-5} M i.e., 7.16 ml/100 ml in 50 hrs. of optimum incubation period which is 3.76811% higher in comparison to control, i.e., 6.90 ml/100 ml.

In the second phase of micellar effect, i. e., from 4.0×10^{-5} M and onwards the production of alcohol has been increased but in lesser amount. However, the alcoholic fermentation by the yeast *Saccharomyces cerevisiae* NCIM -1267 in the presence of sodium cocoyl glutamate (SCG) at all its concentration used has been greater than that of control fermentor flasks.

The micelles are long chain molecules containing twelve and more member of carbon atoms in the hydrocarbon tail with a head group. The surfactant sodium cocoyl glutamate (SCG) is surface active and lower the surface tension of water when dissolved in it. Surfactants undergo self-assembly thus sodium cocoyl glutamate (SCG) also undergo self assembly, which depends on the chemical structure and nature of the media specially production medium containing different bioingredients. Opposite forces of repulsion

Table - 1
Micellar Action of Sodium Cocoyl Glutamate (SCG) in Enhancing Ethanol
Production from Molasses by *Saccharomyces cerevisiae* NCIM-1267

Concentration of micelle used $A \times 10^{-x}$ M	Incubation Period in hours	Yield of alcohol* in ml/100 ml	Blackstrap molasses sugars* left unfermented in g/100 ml	% Difference in yield of alcohol in comparison to control after 50 hrs.
Control	30	4.55	3.00	–
(-) micelle	50	6.90	1.15	–
	60	6.70	1.10	–
1.0×10^{-5}M	30	4.59	2.98	+0.87912
(+) micelle	50	7.01	1.08	+1.59420
	60	6.82	1.06	+1.79104
2.0×10^{-5}M	30	4.61	2.96	+1.31868
(+) micelle	50	7.12	1.06	+3.18840
	60	6.91	1.04	+3.13432
3.0×10^{-5}M**	30	4.65	2.84	+2.19780
(+) micelle	50	7.16***	1.05	+3.76811
	60	6.92	1.04	+3.28358
4.0×10^{-5}M	30	4.64	2.95	+1.97802
(+) micelle	50	7.14	1.06	+3.47826
	60	6.90	1.05	+2.98507
5.0×10^{-5}M	30	4.63	2.96	+1.075824
(+) micelle	50	7.10	1.07	+2.89855
	60	6.89	1.06	+2.83582
6.0×10^{-5}M	30	4.62	2.97	+1.53846
(+) micelle	50	7.09	1.08	+2.75362
	60	6.87	1.07	+2.53731
7.0×10^{-5}M	30	4.60	2.99	+1.09890
(+) micelle	50	7.07	1.10	+2.46376
	60	6.85	1.08	+2.23880
8.0×10^{-5}M	30	4.59	3.00	+0.87912
(+) micelle	50	7.03	1.11	+1.88405
	60	6.83	1.10	+1.94029
9.0×10^{-5}M	30	4.58	3.01	+0.65934
(+) micelle	50	7.02	1.12	+1.73913
	60	6.82	1.11	+1.79104
10×10^{-5}M	30	4.57	3.03	+0.43956
(+) micelle	50	6.95	1.13	+0.72463
	60	6.74	1.12	+0.59701

* Each value represents mean of three trials. ** Optimum concentration of the micelle used.
*** Optimum yield of alcohol in 50 hours. (+)Values indicate % increase in the yield of alcohol in comparison to control. Experimental deviation ($\pm$) 1.5–3%.

between polar head groups (charged) and attraction between the hydrocarbon chains of the surfactants especially sodium cocoyl glutamate (SCG) are responsible for self-assembly and may also influence the enzyme activity of the reaction. Due to a balance between these hydrophilic and hydrophobic interactions, a specific structure stabilises.

However, micellar mechanism of fermentative enzyme action is not clearly known and no work has been done regarding the mechanism of influence of micelles on fermentation technology. The use of micellar systems for enzymes-catalysed reactions specially in fermentation technology may vigorously stimulate the enzyme action and thereby enhances the yield of different fermentative production. The structure of micellar system is grossly similar to that of protein and their chemical composition is infinitely less complex. However, micelles may be taken for trial for different microbial synthesis as it may be proved to be an important biological tool in the field of fermentation technology specially in alcoholic fermentation.

References

1. A. Abalos, A. Pinazo, M. Infante, M. Casals, F. Garcia and A. Manresa *Langmuir* **17**, 1367 (2001).

2. G. F. Abd-al-hussan, F. Abd, A.L. Hussein, and M. Jassim, *J. Kerbala Univ.* **14**, 186 (2016)

3. M. Abouseoud, R. Maachi, and A. Amrane, *Commun. Curr. Res. Educ. Top Appl. Microbiol.* **1**, 340 (2007).

4. A. Adamu, U.J.J. Ijah, M.L. Riskuwa, H.Y. Ismail, and U.B. Ibrahim, *Int. J. Sci. Res. Knowl.* **3**, 13 (2015).

5. S.A.M. Ali, R.Z. Sayyed, M.S. Reddy, H.E. Enshasy, and B. Hameeda, (USA: CRC Press-Taylor & Francis group), 59 (2022a).

6. S. Alsager, M. Marafi, I.M. Banat, and W. Ismail, *J. Appl. Microbiol.* **125**, 1358 (2018)

7. I.D. Astuti, I.A. Purwasena, R.K. Putri, M. Amaniyah, and Y. Sugai *J. Pet. Explor. Prod. Technol.* **9**, 2279 (2019)

8. M.K. Bafghi, and M.H. Fazaelipoor, *J. Surfactants Deterg.* **15**, 679 (2012).

9. S.S. Balan, C.G. Kumar, and S. Jayalakshmi, *Process Biochem.* **51**, 2198 (2016)

10. S. Alsager, M. Marafi, I.M. Banat, and W. Ismail, *J. Appl. Microbiol.* **125**, 1358 (2018)

11. F. Anjum, G. Gautam, G. Edgard, and S. Negi, *Bioresour. Technol.* **213**, 262 (2016)

12. H.W.C. Araújo, R.F.S. Andrade, D.M. Rodríguez, D.R. Ribeaux, C.A. Alves and G.M.C. Takaki, G. M. C. *Microb. Cell Fact.* **18**:2. ((2019).)

13. R. Bayoumi, B. Haroun, E. Ghazal, E., and Y. Maher, *J. Basic Appl. Sci.* **4**, 3484 (2010).

14. W. Bednarski, M. Adamczak, J. Tomasik, and M. Plaszczyk, Bioresour. Technol. 95, 15 (2004)

15. H. Bee, H., Khan, M. Y., and Sayyed, R. Z. Antonious (Singapore: Springer-Nature), 205 (2019).

16. S.S.Balan, C.G. Kumar and S. Jayalakshmi, *Microbiol. Res.* **194**, 1 (2017).

17. A. Bartal, A. Vigneshwari, B. Boka, M. Voros, I. Takacs, L. Kredics, et al. *Molecules* 23:2675 (2018).

18. R. Bayoumi, B. Haroun, E. Ghazal, and Y. Maher, *J. Basic Appl. Sci.* **4**, 3484 (2010)

19. F.A. Bezza, and E.M.N. Chirwa, E. M. N. *Biochem. Eng. J.* **101**, 168 (2015).

20. G. Bhardwaj, S.S. Cameotra, H.K. and Chopra . *Pet. Environ. Biotechnol.* **4** :160 (2013)

21. L. P. McCloskey and L.L. Replogle *Am. J.Enol Vitie* **25**, 194 (1974)

22. M. Dubois, K. A.Gilles, J. K. Hamilton and F.Smith, *Anal. Chem.* **28**, 350 (1956)