

## Valorization of molasses waste to lactic acid by *Lactobacillus casei* NCIM-2732 and 4-methylurazole : A sustainable bioprocess

Sadhana Kumari<sup>1</sup>, Reena Kumari<sup>2</sup> and Nilima<sup>3</sup>

<sup>1,2</sup>Department of Chemistry, Magadh Mahila College, Patna University, Patna (Bihar)

<sup>3</sup>Assistant Professor Guest Faculty, Department of Chemistry, B.S. College Danapur, Patna Bihar  
Email:sadhana.bits@gmail.com; reenakumari3103@gmail.com ; nilimak16@gmail.com

Manuscript received online 24 January 2025, accepted on 11 February 2025

**Abstract :** The sustainable conversion of agro-industrial waste streams into value-added biochemicals is a critical component of circular bioeconomy strategies. In this study, molasses waste was utilized as an inexpensive carbon source for enhanced lactic acid production by *Lactobacillus casei* NCIM-2732. The bioprocess was further evaluated in the presence of 4-methylurazole, a heterocyclic nitrogen compound hypothesized to act as a metabolic modulator. Fermentation kinetics, substrate utilization, biomass formation, and lactic acid productivity were monitored to determine the synergistic effects of molasses valorization and 4-methylurazole supplementation. Results indicated that optimized concentrations of 4-methylurazole significantly improved lactic acid yield, shortened fermentation time, and enhanced carbon-to-product conversion efficiency compared to control fermentations. The observed stimulatory impact is likely associated with improved redox balance and enzyme activation pathways within *L. casei*. This integrated approach demonstrates a cost-effective and eco-friendly route for converting low-value molasses waste into high-value lactic acid, supporting sustainable industrial fermentation processes and reducing environmental burdens. It has been observed that the compound, i.e.; 4-methylurazole at molar concentration  $6.0 \times 10^{-5} \text{M}$  increases the formation of lactic acid to an extent of 20.491% higher in comparison to control, i.e.; 8.540 g/100mL when allowed to ferment at pH 6.0, temperature 34°C and 6 days of incubation period with 20%(w/v) molasses solution.

**(Keywords :** Valorization, molasses, lactic acid, *Lactobacillus casei* NCIM-2732 and 4-methylurazole).

### Introduction

The global transition toward sustainable

and circular bioprocessing has intensified the search for low-cost, renewable, and underutilized feedstocks for industrial fermentations. Among these, molasses, a viscous by-product of the sugar industry, represents an abundant carbohydrate-rich substrate that is often treated as waste due to its limited direct economic value and disposal challenges. Molasses contains high levels of sucrose, glucose, fructose, minerals, and nitrogenous compounds, making it a promising carbon source for microbial biotransformations. Effective valorization of this agro-industrial residue not only reduces environmental burden but also contributes to economically viable biomanufacturing systems.

This study investigates the co-application of molasses waste and 4-methylurazole for the enhanced bioproduction of lactic acid using *Lactobacillus casei* NCIM-2732. The work focuses on evaluating fermentation performance, metabolic responses, and overall process sustainability. By integrating a low-cost substrate with a novel biochemical enhancer, the research aims to develop a greener and more efficient bioprocess, contributing to waste valorization strategies and advancing sustainable industrial biotechnology.

**Lactic acid (LA)** is a high-value platform chemical with expanding applications in food preservation, pharmaceuticals, biodegradable plastics (PLA), textile fibers, and biochemical syntheses. Although *Lactobacillus casei* is a robust lactic acid bacterium capable of fermenting diverse carbohydrate substrates, its

metabolic efficiency can be limited by inhibitory by-products, suboptimal redox balance, and stress responses associated with impure waste substrates such as molasses. Therefore, enhancing microbial tolerance and productivity is crucial to improving process yields.

Recent studies have explored the role of small heterocyclic modulators in improving microbial metabolism and fermentation robustness. 4-Methylurazole, a substituted urazole derivative, has attracted attention for its redox-active and antioxidant-like properties, which may influence intracellular electron transfer, mitigate oxidative stress, and improve enzymatic functioning in bacterial systems. Its potential role as a metabolic booster in lactic acid fermentations, her, remains largely unexplored.

Though biologically active biomolecules are not essentially growth promoters for some microbes yet a few organic biomolecules are utilized by some or all microbes for their nutritional benefits. Although a group of workers<sup>1-6</sup> have tried to explore the efficacy of some bioactive and their derivatives on biotic enzymes system, yet there is no definite opinion regarding its impact on bioproduction of lactic acid.

In view of the insufficient information about involvement of bioactive compounds to any fermentation processes specially lactic acid fermentation process the authors have confined their study to valorization of molasses waste to lactic acid by *Lactobacillus casei* NCIM-2732 and 4-methylurazole :A sustainable bioprocess.

### Experimental

#### Compositions of the production medium :

The composition of the production medium for biotransformation of molasses pollutant to lactic acid by *Lactobacillus casei* NCIM -2732 exposed to 4-methylurazole is as follows :

**Molasses** : 20% (W/V); Malt extract : 0.80%; yeast extract : 0.75% , Peptone : 0.75%, (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> : 0.8%; CaCO<sub>3</sub> : 10%, pH :6.0, (Adjusted by adding requisite amount of phosphate-buffer solution). Distilled water : To make up 100 ml.

**Assay methods** : Evaluation of lactic acid formed and molasses left unfermented was made colorimetrically<sup>7-8</sup>

**Sterilization** : The growth and production medium was sterilized in an autoclave maintained at 15 lbs steam pressure for 30 minutes.

**Strain** : *Lactobacillus casei* NCIM-2732 has been employed in the present study. The strain was procured from NCL - Pune, India

**Age of the inoculum** : 48 hours old.

**Quantum of the inoculum**:0.5 ml bacterial suspension of *Lactobacillus casei* NCIM-2732

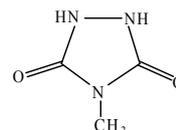
**Incubation period** : 4, 6 and 8 days

**Concentration of 4-methylurazole** :

M/1000 solution of 4-methylurazole under trial has been prepared and  $1.0 \times 10^{-5}$ M to  $10 \times 10^{-5}$ M molar concentration of 4-methylurazole has been employed.

### Results and Discussion

#### The influence of 4-methylurazole on lactic acid fermentation



**4-methylurazole Compound**

The addition of 4-methylurazole vide table -1 in the production medium for bioproduction of lactic acid by *Lactobacillus casei* NCIM-2732 has been found significant. It has been found that there is a gradual increase in the production of lactic acid with stepping up of the compound urazole till the maximum yield of lactic acid, i. e., 10.290g/100 ml was obtained at its molar concentration of  $6.0 \times 10^{-5}$ M which is 20.491% higher in comparison to control fermentor flasks in 6 days of optimum incubation period.

**Table - 1**  
**Efficacy of 4-methylurazole on lactic acid formation by *Lactobacillus casei* NCIM-2732**

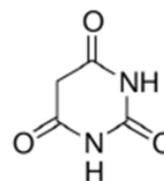
Concentration of 4-methylurazole	Incubation period in days	Yield of lactic acid* in g/100 ml	Molasses* left unfermented in g/100 ml	% of lactic acid increased in 6 days
Control	6	8.540	1.720	–
$1 \times 10^{-5}$ M	6	8.841	1.421	(+) 3.524
$2 \times 10^{-5}$ M	6	9.300	0.963	(+) 8.899
$3 \times 10^{-5}$ M	6	9.592	0.670	(+) 12.318
$4 \times 10^{-5}$ M	6	9.960	0.360	(+) 16.627
$5 \times 10^{-5}$ M	6	10.095	0.170	(+) 18.208
$6 \times 10^{-5}$ M**	6	10.290***	0.096	(+) 20.491
$7 \times 10^{-5}$ M	6	9.822	0.438	(+) 15.011
$8 \times 10^{-5}$ M	6	9.336	0.922	(+) 9.320
$9 \times 10^{-5}$ M	6	9.172	1.088	(+) 7.400
$10 \times 10^{-5}$ M	6	8.799	1.462	(+) 3.032

\*Each value represents mean of three trials. \*\* Optimum concentration of 4-methylurazole under trial. \*\*\* Optimum yield of lactic acid in 6 days Experimental deviation  $\pm 1.5 - 3.0\%$  +ve values indicate % increase in the yield of lactic acid.

The enzymes activities of the 4-methylurazole may be attributed to the presence of  $>C=O$  groups of the nucleus and  $-NH-CO-NH$ -linkage, i.e., peptide linkage, present in the molecule. It has been found that many organic molecules having the  $-NH-CO-NH$ -linkage have been found of great biological significant for the maximum growth and activity of different microbes. The compound taken under trial, i.e., urazole possesses active unsaturated  $>C=O$  groups directly attached with ring system which may serve as a more efficient source of energy and influences the growth and activity of the enzyme system associated with *Lactobacillus casei* NCIM-2732.

Margalith and Pagani<sup>9-10</sup> during their industrial investigations successfully studied and compared different derivatives of barbituric acid, i.e., barbiturates and reported that the organic molecules, i.e., barbiturates has been found to be most effective and useful for various industrial fermentations process. Barbiturates in general has been found most effective and useful in different biological processes and a lot of

questions are still unsettled and open concerning the mode of action of these barbiturate molecules on the enzymes catalysed systems involved in the pathways leading to the mode of enzyme functions. However, whatever their biological functions may be, these organic molecules should be incorporated in to the fermentation medium for the better functioning of the process and improved yield of the desired products. It is a secondary factor that influences the fermentation technique associated with enzymes of *Lactobacillus casei* NCIM-2732



**Barbituric acid**

Further, a group of researchers<sup>11-17</sup> have reported stimulatory effect of barbituric acid and its derivatives possessing barbiturate nucleus and also some active organic molecules on

microbes and microbial process<sup>18-24</sup>. Since the organic molecule, i.e., 4-methylurazole also possess part structure combination of barbiturate nucleus, it may influence critically the outcome of lactic acid by the bacterial strain of *Lactobacillus casei* NCIM-2732. Singh *et al* and others<sup>25-26</sup> also found 5,5'-diphenylhydantoin and 5-phenyl hydantoin stimulatory for lactic acid and

citric acid fermentation respectively. Leena<sup>27</sup> has reported phenobarbital as a most effective organic compound for the higher production of lactic acid. Recently Kumari Reena<sup>28-29</sup> has reported the compound phenobarbital sodium salt and orotic acid monohydrate most effective stimulatory for the significant higher yield of lactic acid.

## References

1. R. Jacob, D. De Oliveira, T. Peglow, J. Nascimento, R. Bartz, *J. Braz. Chem. Soc.* **30**, 2144 (2019)
2. A.J. Pearce, R.P. Harkins, B.R. Reiner, A.C. Wotal, R.J. Dunscomb, I.A. Tonks, *J. Am. Chem. Soc.* **142**, 4390 (2020)
3. G. Mali, B.A. Shaikh, S. Garg, A. Kumar, S. Bhattacharyya, R.D. Erande, A.V. Chate, *ACS Omega*, **6**, 30734 (2021).
4. K. Kula, A. Kęcka-Zych, A. Lapczuk-Krygier, Z. Wzorek, A. K. Nowak, R. Jasiriski, *Molecules* **26**, 1364 (2021)
5. M.S. Ledovskaya, V.V. Voronin, M.V. Polynski, A.N. Lebedev, V.P. Ananikov, *Eur. J. Org. Chem.* **2020**, 4571 (2020)
6. J.N. Zhu, W. K. Wang, Z. H. Jin, Q. K. Wang, S. Y. Zhao, *Org. Lett.* **21**, 5046 (2019)
7. S. B. Barker and W. H. Summerson *J. Biol. Chem.* **138**, 535, (1941).
8. M. Dubois, K. A. Gills, J. K. Hamilton, P.A. Rebers and F. Smith; *Anal Chem.* **28**, 350, (1956).
9. P. Margalith, *Adv. Appl. Microbiol.* **6**, 85 (1964).
10. P. Margalith and H. Pagani, *J. Appl. Microbiol.* **9**, 325 (1961b).
11. S.P. Singh, and K.P. Tiwari : *Nat. Acad. Sci lett.* **1**, 146 (1978).
12. K.P. Tiwari and A. Pandey : *Zbl. Bakt. II Abt.* **136**, 70 (1981).
13. S.P. Singh, Md. Shmim and K.P. Kamal, : Fourth convention of the Indian Society of Agricultural biochemist B.H.U. PV 3 pp. 53 (1988)
14. S.P. Singh, B. Kumar, U. Kumar, A.K. Sinha, and A. Suraiya, : *Asian J. Pure & Appl. Chem.* **1**, 33 (1995).
15. F. R. Faizi, K. Ahmad, A. Suraiya and S. P. Singh : *J. Chemtracks* **8**, 69 (2006).
16. Md. Irfan, F. R. Faizi, R. Kumar, S. N. Prasad, and S. P. Singh : *J. Chemtracks* **9**, 165 (2007)
17. H. Kumar, S. M. Abdullah, and H. M. Singh : *J. Chemtracks* **9**, 173 (2007).
18. F. R. Faizi, K. Ahmad, A. Suraiya and S. P. Singh *J. Chemtracks* **8**, 69 (2006).
19. Md. Irfan, F. R. Faizi, R. Kumar, S. N. Prasad, and S. P. Singh *J. Chemtracks* **9**, 165 (2007)
20. M. Sharma, F. R. Faizi, R. Kumar, A. Suraiya, R. Kumari and S. P. Singh *J. Chemtracks* **10**, 161 (2008).
21. M. Sharma, F. R. Faizi, T. Kumari, A. K. Sinha, V. Kumar and S. P. Singh *J. Chemtracks* **10**, 171 (2008).
22. S. Singh and A. Singh *J. Chemtracks* **11**, 57 (2009).
23. S. Singh and A. Singh *J. Chemtracks* **11**, 209 (2009).
24. G. K. Mishra, Snigdha, N. Rathor, S. Kumar and S. P. Singh *J. Chemtracks* **11(2)**, 393 (2009).
25. S. Singh and A. Singh : *J. Chemtracks* **11**, 57 (2009)
26. A. P. Singh, F. R. Faizi, K. Ahmad, S. Kumar, G. Kumari and S. P. Singh *J. Chemtracks*, **11**, 605 (2009)
27. Leena *J. Chemtracks* **16(1)**, 313 (2014)
28. Reena Kumari *J. Chemtracks* **19(2)**, 171 (2017)
29. Reena Kumari *J. Chemtracks* **19(2)**, 313 (2017)