J. Chemtracks 27 (1&2) 35-42, 2025 Abstracted / Indexed in
A division of the American Chemical Society
Chemical Abstract U.S.A.
ISSN 0973-239X
CODEN:JCOHF7
IMPACT FACTOR : 7.866

# Studies on parametric variables for efficient lactic acid bioproduction

**Prachi Prakash**
Department of Chemistry, B.N. Mandal University, Madhepura, Bihar
Email: prach.mahi@gmail.com

*Manuscript received online 18 February 2025, accepted on 29 March 2025*

**Abstract** : The parameters for effective lactic acid bioproduction when exposed to certain microorganisms, including *Lactobacillus casei* NCIM-2063, *Lactobacillus pentosus* NCIM-2256, *Lactobacillus leichamannii* NCIM-2172, and *Lactobacillus delbrueckii* NCIM-2353, are the focus of this communication. It has been discovered that the *Lactobacillus pentosus* NCIM-2256 LABs strain is an effective lactic acid producer. Effective lactic acid bioproduction has been found to work best when a cheap raw material molasses solution (w/v) of 28% is allowed to ferment for seven days at 34$^0$C with a pH 6.0 maintained, along with a few other bioirigradients that are essential supplements needed by *Lactobacillus pentosus* NCIM-2256.

**(Key words :** Lactic acid fermentation, molasses, *Lactobacillus pentosus* NCIM-2256, pH, temperature and incubation period)

## Introduction

In many industries, such as food, medicine, and bioplastics, lactic acid fermentation is an essential procedure. Lactic acid fermentation parameters must be optimized in order to increase production yield and cost-effectiveness. To improve the fermentation process, this entails determining the most important variables and their typical ranges. Optimized parameters can improve lactic acid production rates and yields while lowering energy, raw material, and downstream processing costs. It also contributes to lower contaminants and consistent product quality.

Parametric analysis of the microbiological search for *Lactobacillus pentosus* NCIM-2256's lactic acid production is almost as crucial to a lactic acid fermentation's success as choosing the right organism to carry out the fermentation.[1–4]. The medium provides nutrients for energy production, cell substance synthesis, growth, and fermentation bioprocess product biosynthesis. Cellular development and the yield of fermentation products might be negatively impacted by a poor choice of medium components.[5-8] The types and ratios of products from among those for which a microorganism has biosynthetic capability can be partially or completely influenced by the optimization of parameters such as temperature, incubation period, hydrogen ion concentration, and concentration of a chosen raw material.[9–12]. Therefore, it is crucial to determine the lactic acid fermentation by *Lactobacillus pentosus* NCIM-2256 parametrically. Various workers have explored how to optimize culture for different lactic acid fermentations.At 32–33°C and pH 6.1, Liu and Chen[18] observed a notable production of lactic acid from11-23% sucrose[13–17]. According to Tiwari and Pandey[19], *L. bugaricus* produced the most lactic acid when a 20% molasses solution was left to ferment for six days at 47°C and a pH of 5.8 to 6.0. According to Singh *et al*.[20], *L. delbrueckii* produces the most lactic acid when a 5% sugar solution with a pH of 6.2 is fermented for six days at 46$^0$C.

Singh *et al*.[21–27] and a few others[28–49] investigated the fermentation of lactic acid by several strains of lactic acid bacteria and discovered that their optimal activity occurred when the concentration of sugar substrate was

between 10–30%, the pH was between 5.8 and 6.2, the temperature was between 30 and 50$^0$C, and the incubation period was between 5 and 8 days.

The authoress of this communication has focused her research on employing the *Lactobacillus pentosus* NCIM-2256 bacterial strain to ferment lactic acid as much as possible while optimizing the parameters.

**Experimental**

**Medium :**

The composition of the production medium for each fermentor flask containing 100 mL production medium is as below :

Molasses : 28% (w/v) , Malt Extract :1.50%, Yeast Extract :1.50%, Peptone: 1.50% $(NH_4)_2HPO_4$ :1.50%, $CaCO_3$ : 12%, pH: 6.0, Distilled water, To make up 100 ml.

**Table - 1**

**Impact of different carbohydrates on production of lactic acid by *L. pentosus* NCIM-2256**

| S. No. | Carbohydrates substrates used | Yield of lactic acid* in g/100 ml | Sugar left Unfermented g/100 ml. |
|---|---|---|---|
| 1 | Arabinose | 1.682 | - |
| 2 | Rhamnose | 0.920 | - |
| 3 | Xylose | 1.110 | - |
| 4 | Glucose | 8.670 | - |
| 5 | Fructose | 6.810 | - |
| 6 | Galactose | 6.630 | - |
| 7 | Sorbose | 0.888 | - |
| 8 | Lactose | 5.759 | - |
| 9 | Sucrose | 8.420 | - |
| 10 | Maltose | 3.331 | - |
| 11 | Starch | 0.659 | - |
| 12 | Inuline | 0.586 | - |
| 13 | Dextrine | 0.811 | - |
| 14 | Raffinose | 3.380 | - |
| 15 | Mannitol | 2.137 | - |
| 16 | **Molasses** 28% (w/v) | 9.336 | 1.578 |

**Formulation of culture :**

Formation of enriched culture medium for production of lactic acid by *Lactobacillus pentosus* NCIM-2256 the following bio-ingredients has been employed :

Glucose : 0.50%, Lactose : 0.50%

Sodium-Acetate : 200.00 mgs

Liver-Extract 500.00 mgs, Peptone* : 500.00 mgs

Salt Solution A : 0.50 ml,

Salt solution B : .50ml pH : 6.0-6.2

Sterilization : 15lbs for 25-30 minutes

Sub-culture : Once a month

Distilled water : To make up 100 ml

Requisite amount of distilled water was added to make the total volume 100 ml.

**Preparation of Salt Solution A**

It was prepared by mixing the following with water:

$KH_2PO_4$ : 25.00 g

$K_2HPO_4$ : 25.00 g

Requisite amount of distilled water was added to make the volume 250ml.

**Preparation of Salt solution B:**

It was prepared by mixing the following with water:

$MgSO_4.7H_2O$ : 10.00 g

NaCI : 500.00 mgs

$MnSO_4.5H_2O$ : 500.00 mgs

$FeSO_4.7H_2O$ : 500.00 mgs

The combination of above amount were dissolved in requisite amount of distilled water to make up the volume up to 250 ml.

The volume of the culture medium was taken in a dry and clean flask and was plugged with non-absorbent cotton wool plugs. About 12 clean and dry culture tubes were similarly plugged with non-absorbent cotton. These culture tubes and culture medium were sterilized in an autoclave at 1.71 Kg/ Cm$^2$ steam pressure for 25-30 minutes. For solid growth medium 2.0 % agar-agar was added in this solution before sterilization. After cooling, 5.0 ml of the culture medium from the conical flask was transferred to

**Table -2**
**Impact of concentration of molasses substrate, pH, temperature and incubation period on production of lactic acid by *L. pentosus* NCIM-2256**

| % of Molasses | pH | Temp. in ($^0$C) | Incubation Period in days | Corresponding yield of lactic acid* in g/100ml | | | |
|---|---|---|---|---|---|---|---|
| 3 | 5.2 | 10 | 1 | 16.115 | 3.989 | 1.310 | 0.956 |
| 5 | 5.4 | 15 | 3 | 4.302 | 4.869 | 2.349 | 2.116 |
| 10 | 5.6 | 20 | 4 | 4.803 | 5.993 | 6.265 | 5.893 |
| 15 | 5.8 | 30 | 7** | 4.940 | 8.956 | 8.113 | 9.765*** |
| 20 | 6.0** | 34*** | 9 | 5.980 | 9.677*** | 9.818*** | 8.618 |
| 25 | 6.2 | 36 | 11 | 7.886 | 7.320 | 7.317 | 7.105 |
| 28** | 6.4 | 38 | 13 | 9.580*** | 6.667 | 6.810 | 6.320 |
| 30 | 6.6 | 40 | 15 | 9.210 | 6.012 | 5.118 | 5.904 |
| 32 | 6.8 | 45 | 18 | 8.402 | 5.116 | 8.336 | 4.314 |
| 35 | 6.9 | 50 | 20 | 7.501 | 4.527 | 3.428 | 3.115 |

* Each value represents mean of three observations. ** Optimum concentration of molasses pH, Temp and IP. *** Optimum yield of lactic acid.

each culture tube. The culture tubes were then allowed to stand in slant position overnight.

**Sterilization :** The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 30 min.

**Strain :** *Lactobacillus pentosus* NCIM-2256 was used in the present study.

**Assay methods:** Evaluation of lactic acid formed was made colorimetrically [50].

**Age of the inoculum:** 50 hours old.

**Quantum of the inoculum:** 0.05 ml bacterial suspension of *Lactobacillus pentosus* NCIM-2256

**Molasses concentration :** 3%, 5%, 10%, 15%,20%, 25%, 28%, 30%, 32% and 35%

**pH :** 5.2,5.4,5.6,5.8,6.0,6.2,6.4,6.6,6.8 and 6.9

**Temperature** (in$^0$C) : 10,15,20,30,34,36,38,40,45 and 50$^0$C

**Incubation period:** 1,3,4,7,9,11,13,15,18 and 20 days

**Study of the impact of different carbohydrates :**

16-sets of conical flasks (each set of three flasks) were prepared with the only difference that in place of molasses different carbohydrates in question were taken in flasks of the 1st to 15th set to provide 10.00 g of carbon substrate source to each of the 15 sets of the flasks. Then the total volume in the conical flasks were made up to 100 ml by adding requisite amount of distilled water.

The flasks were then plugged with non-absorbent cotton wool plugs and steam sterilized, cooled and then inoculated with 0.05 ml of *Lactobacillus pentosus* NCIM-2256 and incubated at 34$^0$C. The contents of the flasks were analysed colorimetrically for lactic acid produced after 7 days of incubation period.

**Study of the impact of substrate concentrations:**

10-sets, each of 3-conical flasks were prepared. The concentrations of sugar (molasses) in flasks of 1st to 10th sets was 3%, 5%, 10%, 15%,20%, 25%, 28%, 30%, 32% and 35% respectively. The above flasks were then steam sterilized, cooled and incubated at 34$^0$C for 7 days. The contents of the flasks were analysed colorimetrically for lactic acid produced and molasses left unfermented.

**Study of the impact of hydrogen ion concentration :**

10 - sets, each consisting of 3-flasks were prepared and requisite amount of phosphate-buffer solution were added to first 5 sets, to adjust the pH at 5.2, 5.4, 5.6, 5.8, 6.0, In the remaining 5 sets, the pH values 6.2, 6.4,6.6,6.8 and 6.9 were maintained respectively. the flasks were then steam sterilized, cooled and inoculated with 0.05 ml of *Lactobacillus pentosus* NCIM-2256 and incubated at .34$^0$C for 7 days. The contents of the flasks were analysed colorimetrically for lactic acid produced.

**Study of the temperature :**

10 sets, each consisting of 3 flasks, were prepared . These flasks were steam sterilized, cooled and inoculated with 0.05 ml of *Lactobacillus pentosus* NCIM-2256 . 1st to 10th sets of conical flasks were incubated at 10,15,20,30,34,36,38,40,45 and 50$^0$C respectively for 7 days. The contents of the conical flasks were analysed colorimentrically for lactic acid formed .

**Study of the impact of Incubation period of fermentation medium :**

10 sets,each of 3 conical flasks were prepared. The flasks were sterilized, cooled, and inoculated with 0.05 ml of *Lactobacillus pentosus* NCIM-2256 and were incubated at 34ºC in an incubator. The contents of the flasks were analysed colorimentrically after 1, 3, 4, 7, 9, 11, 13, 15, 18 and 20 days of incubation period for lactic acid produced.

**Results and Discussion**

The data recorded in the table-1 shows the study of effect of different carbohydrate material on production of lactic acid by *Lactobacillus pentosus* NCIM-2256 . A series of fermentation experiments were performed in 100 ml Erlenmeyer conical flask to examine the effect of different carbon sources viz. Arabinose, Rhamnose, Xylose, Glucose, Fructose, Galactose, Sorbose, Lactose, Sucrose, Maltose, Starch, Inuline, Dextrine, Raffinose and Mannitol were studied. Each substrate was taken in flask at a concentration (10g/100ml) in the fermentation medium. This was autoclaved at 121$^0$C for 15 min. Each flask was inoculated with the prepared inoculum. These flasks were incubated for maximum 6 days of incubation time under shake flask conditions at 36$^0$C. Some bacteria have the ability to ferment carbohydrates, particularly sugars. Among them, each bacteria can ferment only some of the sugars, while it cannot ferment the others. Thus, the sugars, which a bacteria can ferment and the sugars, which it cannot is the characteristic of the bacteria. The carbohydrate fermentation test is performed to test, separately, the ability of bacteria to ferment the sugars like glucose, sucrose, lactose, maltose and xylose as well as their alcoholic derivatives like aesculin, salicin, adonitol, dulcitol and sorbitol.

The data recorded in the table-1 shows the study of impact of different carbohydrate material on production of lactic acid . From the results it is clear that sugar substrate becomes very smooth and easy as the molecular size and structure configuration of the substrate molecules becomes simple. The monosaccharides, especially glucose and fructose sugars both have been found much fermentable amongst monosaccharides due to the presence of active carbonyl group being common in glucose and fructose (aldehydic and ketonic groups) are easily phosphorelated due to energy conversation of living cells which is fundamental properties of

microbes. Galactose is close to glucose and differs in one hydrogen and one hydroxyl group at position C–4. The degree of fermentability of galactose is very much near to glucose and fructose. The fermentation value of arabinose, rhamnose, and Sorbose sugars were almost in traces. Living cells produce useful currency of energy-ATP, which is regarded as the cells's energy currency. Microbes have the property of maintaining a stock of ATP, which is possible due to consumption of sugars like glucose and fructose.

The primary ingredient in sugarcane juice is sucrose, which is made up of one glucose molecule joined to one fructose molecule. The glucose and fructose units that enter the energy metabolization machinery to produce energy are broken apart as the initial stage of microbial activity. The sugar will gradually break down into ever-tinier molecules if microorganisms are allowed to flourish in an aerobic medium, eventually releasing simple invert sugars. Among the disaccharides examined in this study, sucrose has been found to be the most effective and appropriate substrate for maximizing its conversion to lactic acid.

When lactic acid was being produced, lactose did not provide a substantial output. It has also been discovered that mannitol is far less fermentable to make lactic acid. When it comes to polysaccharides, *Lactobacillus pentosus* NCIM-2256 found that starch, inulin, and dextrin were completely inappropriate and undesired for producing lactic acid.

Depending on the substrate, the fermentation-related bacteria use a variety of metabolic pathways. Therefore, it can be said that among the substrate monosaccharides, glucose and among the disaccharides, sucrose, have high fermentability values and are best suited for *Lactobacillus casei* NCIM-2256's generation of lactic acid.

Additionally, the molasses can be used to produce lactic acid. Molasses, commonly referred to as black-strap molasses, is a by-product of processing sugarcane and is composed of 22% invert sugars and 52% total sugars, which are calculated as sucrose (30 percent sucrose).This molasses is thought to have roughly 50% fermentable sugars when employed as a component of a fermentation medium. Molasses has been chosen as a carbohydrate source for the current study, which involves *Lactobacillus pentosus* NCIM-2256 producing lactic acid, due to its high sugar content and affordability.

The information in Table 2 illustrates the impact of varying sugary raw material concentrations, such as molasses substrate. When*Lactobacillus pentosus* NCIM-2256 is permitted to produce lactic acid using a 28% molasses substrate solution, the best results have been seen. Higher molasses solutions have been shown to disrupt bacterial enzyme function and hence slow down *Lactobacillus pentosus* NCIM-2256's generation of lactic acid, whereas lower molasses concentrations have been shown to produce no discernible yield of lactic acid.

The data recorded in table-2 shows the influence of different pH on fermetative production of lactic acid by *Lactobacillus pentosus* NCIM-2256.

It was investigated how pH affected *Lactobacillus pentosus* NCIM-2256's ability to produce lactic acid. For the best lactic acid generation, the pH range between 5.2 and 6.0 was examined. The buffer solution was used to change the pH. It has been discovered that as pH rises toward the acidic side of neutrality, more lactic acid is produced. The strong acidic medium solution inhibits *Lactobacillus pentosus* NCIM-2256's ability to produce lactic acid. Lactic acid production has been reported to increase as pH values rise from 5.2 to 6.0, or 3.989 g/100 ml to 9.677 g/100 ml. Additionally, *Lactobacillus*

*pentosus* NCIM-2256 produces less lactic acid when pH levels rise from 6.0 and beyond. It was thus concluded that production of lactic acid does not proceed smoothly in strong acidic as well as neutral pH medium. The optimum pH of the fermentation was thus found *to* be 6.0 which was most suitable for production of lactic acid by *Lactobacillus pentosus* NCIM-2256. and thus all the experiment conducted by the authoress for production of lactic acid by *Lactobacillus pentosus* NCIM-2256 has been maintained at optimum pH value of 6.0.

The effect of temperature on lactic acid production by *Lactobacillus pentosus* NCIM-2256 has been investigated at the temperature range of $10^0$C to $50^0$C. The results are summarised in table - 2 which shows the influence of different temperature on production of lactic acid by *Lactobacillus pentosus* NCIM-2256. It has been found that production of lactic acid by *Lactobacillus pentosus* NCIM-2256 increases with increase of temperature from 10°C to 34°C. At lower temperatures, i.e., at $10^0$C, $15^0$C, and $20^0$C the yield of lactic acid was found to be much lower. While the yield of lactic acid gradually falls with the rise of temperature i.e.,$34^0$C and onwards. The $34^0$C temperature has been found most significant, suitable, and effective for maximum production of lactic acid by *Lactobacillus pentosus* NCIM-2256 from molasses and therefore, this temperature, i.e., 34°C was selected and maintained throughout the experiments.

The data recorded in the table-2 reveals the influence of different incubation period on production of lactic acid by *Lactobacillus pentosus* NCIM-2256. In lactic acid fermentation, an appropriate incubation period is important parameter. The results summarised in table -2 shows appreciable lactic acid production was increasing upto 7 days. It has been found that biotransformation of molasses to lactic acid increases with the increase in incubation period from 1 days to 7 days and then normally falls. It was also found that usually consumption of molasses corresponded with the maximum yield of lactic acid, i.e., 9.765g/100 ml has been obtained. No further increase in the yield of lactic acid has been observed with the further increase in the incubation period.

It may, therefore, be concluded that production of lactic acid by *Lactobacillus pentosus* NCIM-2256 proceeds best when a molasses solution of 28% (W/V) is allowed to ferment for 7 days of incubation period at 34°C temperature by maintaining the pH values of fermenting medium at 6.0 along with other bioingredients supplements required by the production of lactic acid by *Lactobacillus pentosus* NCIM-2256 .

## References


1. H. C Sherman, Chemistry of food and Nutrition ed.8, New-York. The Macmillan Company (1952).
2. H. Pollack, and Therapeutic Nutrition, Pub. No. 234 Halperns,S.L.National Academy of Sciences National Research Council. (1952)
3. E. W. McHenry,Basic Nutrition, J. B. Philadelphia Lippincott Compnay. (1957)
4. B. T Burton Heinz Hand Book of Nutrition New-York, Mc Grow-Hill Book Company. (1959).
5. E. Geiger, Etracaloric function of dietery components in relation to protein utilisation Federation Proc. **10**, 670 (1951).
6. J. B Allison, *Biological evaluation* of *proteins, physiol. Rev.* **35**,664 (1955).
7. I. W Dawes and Enzyme Nomenclature Academic Sutherland, I. W.New-York. (1979).
8. I. W. Dawes, and Microbial physiology Blockwell Sutherland, I. W. Scientific publication & Oxford. (1976).

9. D. E. Koshland, *Science,* **142**, 1533 (1963).
10. R. Y. Stanier, Adelberg, :The Microbial world 4th Ed. pretice-Hall E. A. and Ingraham J.Englewood Cliffs N. J. (1976)
11. J. Mandelstam Biochemistry of Bacterial Growth and Mc Quillen Blackwell Scientific publications Oxford. (1973).
12. A. L. Lehniger, Principles of Biochemistry worth publishers New-York. (1982).
13. E. Johansen, *J.Appl. Bact.,***35**,415 (1972).
14. H. Diar,*J.Gen. Microbiol.,* **73**, 233 (1972).
15. K.Vladimir, D. Josef and J.Strejoeck, *Chem. Abstr.*, **65,** 4616b (1966).
16. K.P. Tiwari and N. Mishra, *Proc. Natl. Acad. Sci. India*, **48**, 170 (1978)
17. S. P. Singh, N.Rathor, G.Samdani, R.P. Sinha and A. K. Sinha, *Mendel,* **3,** i-iii (1986)
18. P. W. Liu and S.T. Chen. *Chem. Abstr*., **50**, 2117 (1956)
19. K. P.Tiwari and A.Pandey, *Proc. Natl. Acad. Sci. India*, **49**, 37 (1979).
20. S. P. Singh, N. Rathor,A.K Sinha and L.K. Sinha, *Mendel*,**4**,341 (1988)
21. S. P. Singh ,Md. Shamin, K.P Kamal and K.B Lal, *Indian J. agric Chem*.,**30**,73 (1997)
22. S. P. Singh,S. Kumar, U.N. varma,and B. Singh, *Orient. J. Chem.***14**, 359 (1998)
23. S. P. Singh, S. Kumar, B. Singh and B. K Singh, *Asian J. Chem.,* **10,** 377 (1998).
24. S.P. Singh, S. Kumar, and B. P. Pandey, *Asian J. Chem.* **10**,645 (1998)
25. S. P. Singh,S.Kumar and D.C Mandal, *Vijnana Parishad Anusandhan Patrika*, **43**, 73 (2000)
26. S.P. Singh,V.Kumar and S.S Chandra, *Indian J. agric. Chem.,* **34,** 80 (2001)
27. S.P. Singh, V. Kumar, *J. Chemtracks*, **3** , 68 (2001)
28. R. Kumar, A. Singh, V. Kumar, P. K Chaurasia and S.P Singh *J. Chemtracks*, **4** ,15 (2002)
29. K. Ahmad, S. Kumari, N. Rathor and S.P. Singh *J. Chemtracks,* **6**, 63 (2004).
30. R. Kumar, C. Kumari, A. K. Nag, Km. S. Sinha, Jyotimala and S. P. Singh, *J. Chemtracks* **10**(1&2), 375 (2008).
31. S. Kumar, A. Kumar, S.K Srivastava, R. Prasad, J. Kumar and S. P. Singh *J. Chemtracks,* **12** (2), 297-302 (2010)
32. Shalini Singh, Anurag Singh, M. K. Srivastava, O. P. Srivastava and S. P. Singh *Vijnana Parishad Anusandhan Patrika*, **53**, 11 (2010)
33. Ranjan Kumar, B.B. Sharma,Snigdha, S. P. Singh, A. N. Choudhary and Khursheed Ahmad *Vijnana Parishad Anusandhan Patrika*, **54**, 13(2011)
34. Dilip Kumar, Snigdha, Jyotimala, Shalini Singh and S.P. Singh *Vijnana Parishad Anusandhan Patrika*, **55**, 1 (2012)
35. V. Mandal and S. Singh Chandra *J.Chemtracks*, **14** (1), 291 (2012)
36. G.K Mishra, B. Singh, S.K. Verma, R. Kumar, A.Sinha, R. Singh and S. P. Singh *J. Chemtracks*, **14 (2**), 561 (2012)
37. A. K. Singh, A. K. Yadav and Naresh Kumar *J. Chemtracks*, **14** (2), 581 (2012)
38. R. Kumar, S. Singh , A. Singh, K. Ahmad, M. Choudhari and S. P. Singh *J.Chemtracks*, **14** (2), 615 (2012)
39. Afshan Suraiya and Kuldeep Singh *J. Chemtracks*, **14 (**2) ,633 (2012)
40. B.B. Pandey and M.K. Mishra *J. Chemtracks*, **15 (**1) ,211 (2013)
41. R. Kumari, R. K. Lal, S. Agrawal, S. Kumar, and S. P. Singh *J. Chemtracks*, **16 (**2) , 393 (2014)
42. Archna Kumari, and Renu Bala *J. Chemtracks*, **16 (**2) , 453 (2014)
43. Afshan Suraiya . *Chemtracks*, **16 (**2) , 537 (2014)
44. R. Mishra, L.Kumari, M. Prasad, M. Kumari, P.K. Sinha and S. P. Singh *J. Chemtracks*, **17 (**2) , 247 (2015)
45. P. Singh, J. P. Kumar, S. Kumari, D. K. Singh, V. Kumar, U. S. Singh and S. P. Singh *J. Chemtracks*, **17 (**2) , 333 (2015)
46. R. S. Sharma, B. K. Choudhary, and B.B. Sharma *J. Chemtracks*, **18 (**1) , 219 (2016)
47. P Manjhi, L.Kumari, P.K. Sinha and S.P. Singh *J. Chemtracks*, **19 (**1) , 45 (2017)
48. Ashok Kumar Singh and S. P. Singh *J. Chemtracks*, **21 (**1&2) , 97 (2019)
49. Kumai Priyanka Sinha *J. Chemtracks*, **22 (**1&2) , 217 (2020)
50. S. B. Barker and W. H. Summerson *J.Biol. Chem.* **138**, 535 (1941).

An International Peer Reviewed Research Journal of Chemistry
Abstracted / Indexed in : Chemical Abstract U.S.A.
JC- 1610/2K25 (ISSN:0973-239X), INDIA
*Journal Chemtracks Vol.* 27 (1&2),35-42, January to December 2025