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8. Lactobacillus Bulgaricus NCIM-2359: Influence of Active Organic Molecule

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ABSTRACT

The influence of some AOM (Active Organic Molecule) on lactic acid fermentaiton by Lactobacillus bulgaricus NCIM-2359 describes effect of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea , 5-aminoaorotic acid, mandelic acid, 2-hydroxybutyric acid on lactic acid fermentation by Lactobacillus bulgaricus NCIM-2359.

KEYWORDS

Lactic Acid, Lactic acid fermentation, Lactobacillus bulgaricus, NCIM- 2359, organic molecules.

Introduction:

Organic molecules are the chemicals of life, compounds composed of more than one type of element, that are found in, and produced by, living organisms. The feature that distinguishes an organic from inorganic molecule is that organic contain carbon-hydrogen bonds, whereas inorganic molecules do not.

The four major classes of organic molecules include carbohydrates, proteins, lipids and nucleic acids. It has been found that a few physiologically and pharmacologically active organic molecules are very active and play biological properties of vital importance in the biosynthesis of some useful micro and macro organic molecules. Though biologically active organic molecules are not essentially growth promoter for some or all microbes yet a few organic molecules are utilized by some or all microbes for their nutritional requirements [1-17].

A biologically active compound is defined as one that has a direct physiological effect on a plant, animal, or another microorganism. Many known compounds with biological activity are found only in trace amounts in soil.

Research has shown that there are essential, highly active organic molecules that can, even in extremely small quantities, vastly influence the fermentative actions and interactions. A number of organic molecules and their derivatives are well known to show physiological and pharmacological property.

Information regarding their role in biological system is very much limited and still unsettled. Although a group of workers have tried to explore the effect of some organic molecules and their derivatives on microbial enzymes systems, yet there is no definite opinion regarding its influence on submerged fermentation processes.

There are large group of some organic molecules which when introduced to the submerged fermentation medium can effect the enzyme responsible for the biosynthesis of micro and macro molecules in the microbial cells as well bioconversion of raw substrate into desired products and such organic compounds may be referred to as physiologically active organic compounds.

Experimental:

The influence of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359.

The composition of the production medium for the production of lactic acid by *Lactobacillus bulgaricus* NCIM-2359 was prepared as follows:

Molasses	:	20% (w/v)
Malt Extract	:	0.60 %
Yeast Extract	:	0.60 %
Peptone	:	0.60%
$(NH_4)_2HPO_4$	:	0.60%
$CaCO_3$	:	8.0%
pH	:	6.1
Distilled water	:	To make up 100 ml.

The pH of the medium was adjusted to 6.1 by adding requisite amount of phosphate-buffer solution, and the pH was also ascertained by a pH meter. The above compostion medium represents volume of a fermentor flask, i. e., "100ml" production medium for lactic acid fermentation.

Now, the same produciton medium for lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 was prepared for 99 fermentor flasks, i. e., each fermentor flask containg '100 ml' of production medium.

The above fermentor flasks were then arranged in ten sets, each comprising 9 fermentor flask. Each set was again rearranged in three subsets, each comprising of 3 fermentor flasks. The remaining nine fermentor flasks out of 99 fermentor flasks were kept as control and these were also rearranged in three subsets each consisting of three fermentor flasks.

Now M/1000 solution/suspension of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea 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 1st to 10th sets respectively.

The control fermentor flasks containing no active organic molecule. Now the total volume in each fermentor flask were made up to 100ml by adding requisite amount of distil water. Thus, the concentration of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea in 1st, 2nd, 3rd, 4th, 5th, 6th, 7th, 8th, 9th and 10th subsets were approximately as given below :

	$A \times 10^{-x}M$	$6.0 \times 10^{-5}M$	
i. e.,	$1.0 \times 10^{-5}M$	$7.0 \times 10^{-5}M$	Where A = amount of
	$2.0 \times 10^{-5}M$	$8.0 \times 10^{-5}M$	active organic molecule in ml,
	$3.0 \times 10^{-5}M$	$9.0 \times 10^{-5}M$	i.e., 1.0 ml to 10ml.
	$4.0 \times 10^{-5}M$	$10.0 \times 10^{-5}M$	x = molarity of the
	$5.0 \times 10^{-5}M$		solution containing AOM

The fermentor flasks were then sterilized, cooled, inoculated, incubated and analysed after 3, 6 and 9 days for lactic acid formed and molasses sugars left unfermented as described in the experimental portion, i. e., chapter II of this thesis.

The experimental procedure for the study of influence of other active organic molecules were exctly the same as described above with the only difference that in place of M/1000 solution of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea other active organic molecule under trials were added to the lactic acid fermentation medium respectively.

Results and Discussion:

The results obtained in the study of the influence of some active organic molecules on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 are tabulated in the tables from 1 to 4.

Table - 1

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl] urea

Concentration of AOM used A x 10^{-x} M	Incubation period in hours	Yield of lactic acid* in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 3, 6, 9 days of incubation pd.
Control (–AOM)	3	5.8367560	4.1632443	—
	6	7.9115967	2.1864033	—
	9	7.4181653	2.0173518	—
1.0 x 10^{-5} M (+ AOM)	3	5.8993879	4.1006129	(+) 1.07306010
	6	8.0457850	2.1453130	(+) 1.6960963
	9	7.4948316	1.9985373	(+)1.0334940
2.0 x 10^{-5} M (+ AOM)	3	5.9139596	4.0861370	(+) 1.3227141
	6	8.1356740	2.1341618	(+) 2.8322639
	9	7.5623459	1.9761815	(+) 1.9437390
3.0 x 10^{-5} M (+ AOM)	3	3.0426261	3.9573812	(+) 3.5271321
	6	8.3675937	2.1251719	(+) 5.1636532
	9	7.7419296	1.95303907	(+) 4.3644794
4.0 x 10^{-5} M (+AOM)	3	6.1169896	3.8831573	(+) 4.8011875
	6	8.5050567	2.1031316	(+) 7.5011406
	9	7.8752418	1.9374262	(+) 6.16158418
5.0 x 10^{-5} M (+AOM)	3	6.1817193	3.8182413	(+) 5.9101888
	6	8.6078985	2.0927563	(+) 8.8010274
	9	7.9530261	1.9261819	(+) 7.2101493
Control	3	5.8367560	4.1632443	—
	6	7.9115967	2.1864033	—

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
(– AOM)	9	7.4181653	2.0173518	—
6.0×10^{-5} M**	3	6.3276381	3.6723610	(+) 8.4101870
	6	8.8380566***	2.0031182	(+) 11.7101507
(+ AOM)	9	8.1674852	1.8931375	(+) 10.1011485
7.0×10^{-5} M	3	6.2634339	3.8516542	(+) 7.3101890
	6	8.7193818	2.1137063	(+) 10.2101400
(+ AOM)	9	8.0420441	1.9141853	(+) 8.4101496

Table - 1

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl] urea

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0×10^{-5} M	3	6.1466988	3.8971656	(+) 5.3101894
	6	8.5540293	2.1573642	(+) 8.1201383
(+ AOM)	9	7.8936897	1.9474253	(+) 6.4102696
9.0×10^{-5} M	3	****	–	–
	6	****	–	–
(+ AOM)	9	****	–	–
10.0×10^{-5} M	3	****	–	–
	6	****	–	–
(+AOM)	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation $\pm$ 2.5 – 3.5%

Table - 2

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 5-aminoaorotic acid

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
Control	3	5.8142791	4.1861163	—
	6	7.8926956	2.1066513	—
(–AOM)	9	7.3997267	2.0911836	—
1.0 x 10^{-5} M	3	5.8439419	4.1564539	(+) 0.5101715
	6	7.9881983	2.0831698	(+) 1.2100137
(+ AOM)	9	7.4596754	2.0411369	(+) 0.8101474
2.0 x 10^{-5} M	3	5.8607944	4.1376972	(+) 0.8000183
	6	8.0197789	2.0795156	(+) 1.6101457
(+ AOM)	9	7.4670752	2.0394013	(+) 0.9101484
3.0 x 10^{-5} M	3	5.8962715	4.1032065	+ 1.4101903
	6	8.2005218	2.0718169	+ 3.9001402
(+ AOM)	9	7.6291293	2.0324095	+ 3.1001496
4.0 x 10^{-5} M	3	5.9481186	4.0816362	+ 2.3019014
	6	8.3504829	2.06960305	+ 5.8001388
(+AOM)	9	7.7852635	2.0301084	+ 5.2101491
	3	6.1340754	4.0683956	+ 5.5001883
	6	8.6267162***	2.0593015	+ 9.2999988

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
5.0×10^{-5} M (+AOM)	9	8.0509137	2.0261137	+ 8.8001493
Control (– AOM)	3	5.8142791	4.1861163	—
	6	7.8926956	2.1066513	—
	9	7.3997267	2.0911836	—
6.0×10^{-5} M** (+ AOM)	3	6.0590713	4.0715632	(+) 4.2101900
	6	8.4767661	2.0634873	(+) 7.4001396
	9	7.8668595	2.0319425	(+) 6.3128385
7.0×10^{-5} M (+ AOM)	3	6.0113460	4.0886956	(+) 3.3893608
	6	8.3112384	2.0759413	(+) 5.3029132
	9	7.7549245	2.0509362	(+) 4.8001489

Table - 2

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 5-aminoaorotic acid

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0×10^{-5} M (+ AOM)	3	****	–	–
	6	****	–	–
	9	****	–	–
9.0×10^{-5} M (+ AOM)	3	****	–	–
	6	****	–	–
	9	****	–	–
	3z	****	–	–
	6	****	–	–

Concentration of AOM used A x 10^{-x} M	Incubation period in hours	Yield of lactic acid* in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 3, 6, 9 days of incubation pd.
10.0 x 10^{-5} M (+AOM)	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation $\pm$ 2.5 – 3.5%

Table - 3

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to mandelic acid

Concentration of AOM used A x 10^{-x} M	Incubation period in hours	Yield of lactic acid* in g/100 ml	Molasses substrate* left unfermented in g/100 ml	% of lactic acid increase in 3, 6, 9 days of incubation pd.
Control (–AOM)	3	5.8545703	4.1463012	—
	6	7.9347015	2.0658130	—
	9	7.4368268	2.0057311	—
1.0 x 10^{-5} M (+ AOM)	3	5.8786842	4.1221869	+ 0.4118816
	6	7.9921379	2.0627129	+0.7238633
	9	7.4758560	2.0046638	+ 0.5248098
2.0 x 10^{-5} M (+ AOM)	3	5.8898986	4.1036368	+ 0.6034311
	6	8.0389525	2.0605697	+1.3138616
	9	7.5048593	2.0039879	+ 0.9148054

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
3.0 x 10^{-5} M (+ AOM)	3	5.9025069	4.0813694	+ 0.8187893
	6	8.0855709	2.0583167	+ 1.9013872
	9	7.5427875	2.0031610	+1.4248106
4.0 x 10^{-5} M (+AOM)	3	5.9078579	4.0798137	+ 0.9101880
	6	8.1497429***	2.0517160	+ 2.7101384
	9	7.5941205	2.0029320	+2.1150647
5.0 x 10^{-5} M (+AOM)	3	5.8855290	4.0829157	+ 0.5287954
	6	8.1269378	2.0598762	+ 2.4227288
	9	7.5643538	2.0032613	+ 1.7148039
Control (– AOM)	3	5.8545703	4.1463012	—
	6	7.9347015	2.0658130	—
	9	7.4368268	2.0057311	—
6.0 x 10^{-5} M** (+ AOM)	3	5.8732925	4.0886931	+ 0.3197877
	6	8.0317231	2.0627010	+1.2227504
	9	7.4825476	2.0036813	+0.6147890
7.0 x 10^{-5} M (+ AOM)	3	5.8615250	4.0923000	+ 0.1187909
	6	7.9986892	2.0696588	+ 0.8064285
	9	7.4749112	2.0039982	+0.5121055

Table - 3

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to mandelic acid

Concentationof AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0×10^{-5} M	3	****	–	–
	6	****	–	–
(+ AOM)	9	****	–	–
9.0×10^{-5} M	3	****	–	–
	6	****	–	–
(+ AOM)	9	****	–	–
10.0×10^{-5} M	3	****	–	–
	6	****	–	–
(+AOM)	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation $\pm$ 2.5 – 3.5%

Table - 4

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 2-hydroxybutyric acid

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
	3	5.7946637	4.1953363	—

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
Control (–AOM)	6	7.8869820	2.1053769	—
	9	7.3860495	2.0148635	—
1.0 x 10^{-5} M (+ AOM)	3	5.8235269	4.1664833	+ 0.4980996
	6	7.9501178	2.0864224	+ 0.8005064
	9	7.4312145	2.0125139	+ 0.6114906
2.0 x 10^{-5} M (+ AOM)	3	5.8311225	4.1593015	+ 0.6291788
	6	7.9748176	2.0837150	+1.1136782
	9	7.4459868	2.0104131	+ 0.8114933
3.0 x 10^{-5} M (+ AOM)	3	5.8478256	4.1381632	+ 0.9174285
	6	7.9826562	2.0816182	+ 1.2130647
	9	7.4617486	2.0093131	+ 1.0248929
4.0 x 10^{-5} M (+AOM)	3	5.8496320	4.1071618	+ 0.9486020
	6	8.0062844***	2.0785357	+ 1.5126495
	9	7.4698735	2.0082416	+ 1.1348962
5.0 x 10^{-5} M (+AOM)	3	5.6903590	4.1181396	- 1.8000130
	6	7.6721435	2.0799836	- 2.7239633
	9	7.2301926	2.0089716	- 2.1101523
Control (– AOM)	3	5.7946637	4.1953363	—
	6	7.8869820	2.1053769	—
	9	7.3860495	2.0148635	—

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
6.0 x 10^{-5} M** (+ AOM)	3	5.6149287	4.1359782	- 3.1017330
	6	7.4453100	2.0825163	- 5.6000127
	9	7.0673482	2.0093116	- 4.3149088
7.0 x 10^{-5} M (+ AOM)	3	5.5380193	4.1505693	- 4.4289783
	6	7.2864703	2.0956012	- 7.6139605
	9	6.9122423	2.0098363	- 6.4148933

Table - 4

Lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 exposed to 2-hydroxybutyric acid

Concentration of AOM used A x 10^{-x} M	**Incubation period in hours**	**Yield of lactic acid* in g/100 ml**	**Molasses substrate* left unfermented in g/100 ml**	**% of lactic acid increase in 3, 6, 9 days of incubation pd.**
8.0 x 10^{-5} M (+ AOM)	3	****	–	–
	6	****	–	–
	9	****	–	–
9.0 x 10^{-5} M (+ AOM)	3	****	–	–
	6	****	–	–
	9	****	–	–
10.0 x 10^{-5} M (+AOM)	3	****	–	–
	6	****	–	–
	9	****	–	–

* Each value represents mean of three trials.

** Optimum concentration of AOM.

*** Optimum yield of lactic acid

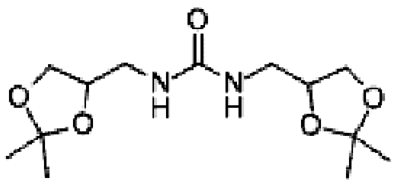
**** Insignificant value

(+) Values indicate % increases in the yield of lactic acid

Experimental deviation ± 2.5 – 3.5%

Discussion:

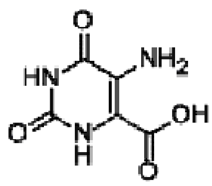
The influence of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea



1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea Compound - I

The addition of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea vide table -1 in the production medium for lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 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 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea till the maximum yield of lactic acid, i. e., 8.8380566 g/100 ml was obtained at its molar concentration of 6.0×10^{-5}M which is 11.7101507% higher in comparison to control fermentor flasks in 6 days of optimum incubation period.

The influence of 5-aminoorotic acid:

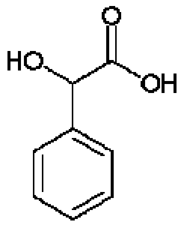


5-aminoorotic acid *[Compound II,]*

The data recorded in the table-2 shows that the addition of 5-aminoorotic acid into the lactic acid fermentation medium enhances the production of lactic acid significantly. It has been observed that there is also a gradual increase in the yield of lactic acid with gradual stepping up of the compound II, i. e., 5-aminoorotic acid till the maximum yield of lactic acid is reached which is 8.6267162% higher in comparison to control fermentor flasks, i. e., 9.2999988g/100 ml at 5.0×10^{-5}M molar concentration of the compound 5-aminoorotic acid in 6 days of optimum incubation period.

It has been observed that the compound 5-aminoorotic acid is a very important active organic molecule and its biological activities may be attributed to the active >C = O groups associated with six membered hetero organic molecule and -CO-NH-CO-linkage. Since no clear evidence could be put forward regarding its activity and stimulating properties of lactic acid fermentation process the compound 5-aminoorotic acid is considered to influence critically some metabolic enzymatic pathways intimately concerned with the lactic acid fermentation by using the bacterial strain of *Lactobacillus bulgaricus* NCIM-2359. It has been discussed earlier those barbiturates and most of its derivative compounds possesses most effective >C=O groups and-NH-CO-NH-linkage.

The influence of mandelic acid:



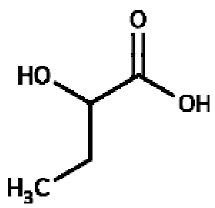
Mandelic acid

The data given in the table-3 shows that the compound mandelic acid has inhibitory effect on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359.

It has been observed that there is a gradual increase in the yield of lactic acid with the stepping up of mandelic acid up to the molar concentration of 5.0 x 10^{-5}M but after this concentration a big fall in the production of lactic acid has been noticed.

It has been observed that at concentrations in between 5.0 x 10^{-5}M, 6.0 x 10^{-5}M and 7.0 x 10^{-5}M the yield of lactic acid has been recorded to be 2.4227289% and 1.2227504% and 0.8064285% higher respectively in comparison to control fermentor flasks, i. e., 7.9347015 g/100ml in 6 days of optimum incubation period.

The influence of 2-hydroxybutyric acid:



2-hydroxybutyric acid

The data recorded in the table-4 shows that 2-hydroxybutyric acid is not beneficial instead much inhibitory for lactic acid fermentation by*Lactobacillus bulgaricus* NCIM-2359

Conclusion:

A comparative assessment of the different active organic molecules on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 can be had from the table-5 given below.

Table - 5

Study of the influence of 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea,

5-aminoaorotic acid, mandelic acid 2-hydroxybutyric acid on lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 in 6 days of optimum incubation period

AOM Used	Optimum concentration of the AOM used.	Max. yield of lactic acid* in control flasks in g/100ml	Max. yield of lactic acid* in the presence of AOM in g/100ml	% of lactic acid increase or decrease in 6 days of incubation pd.
1	6.0 x 10^{-5}M	7.9115967	8.8380566	(+) 11.7101507
2	5.0 x 10^{-5}M	7.8926956	8.6267162	(+) 9.2999988
3	4.0 x 10^{-5}M	7.9347015	8.1497429	(+) 2.7101384
4	4.0 x 10^{-5}M	7.8869820	8.0062844	(+) 1.5126495

* Each value represents mean of three observations

(+) Values indicates % increase in the yield of lactic acid.

Experimental deviation ($\pm$) 2.5 to 3.5%.

1. 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea

2. 5-aminoaorotic acid

3. mandelic acid

4. 2-Hydroxybutyric acid

Thus, it may be summarised that 1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl) methyl]urea and 5-aminoaorotic acid enhances the lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 at all concentrations used; while mandelic acid and 2-hydroxybutyric acid antagonises the course of lactic acid fermentation by *Lactobacillus bulgaricus* NCIM-2359 at their higher concentrations used, i.e., 4.0 × 10^{-5}M and 4.0 × 10^{-5} respectively retarding thereby the yield of lactic acid.

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