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Studies on production of lactic acid from various wastes using lactobacillus rhamnosus and lactococcus lactis subsp lactis

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Abstract

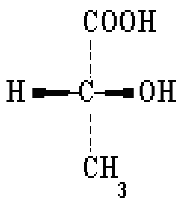
Lactic acid can be produced by fermentation of organic matters present in various wastes with the addition of various carbon and nitrogen sources using *Lactobacillus* species. Organic wastes form kitchen garbage and food-processing industries are especially rich in carbon and moisture content. The present study investigates the treatment of the organic wastes using microbiological process for effective usage of waste and to develop value added products from it. The organic wastes used in this processes are domestic wastes, vegetable wastes, fruit wastes, bakery wastes and whey. The microorganisms used in the synthesis of lactic acid are *Lactobacillus rhamnosus* and *Lactococcus lactis subsp lactis*. The fermentation study includes the effect of various wastes, effect of carbon and nitrogen sources with optimal waste medium, various concentrations of optimal carbon and nitrogen source and effect of time for free cells and their immobilized form on lactic acid production and biomass growth for both the organisms. The optimal production of lactic acid was determined as 35.45 g/l and bacterial growth was 1.34 g/l from whey by *Lactobacillus rhamnosus*. Optimal carbon source was mannitol, which yields 38.3 g/l of lactic acid and 1g/l of microbial growth. The study on effect of various concentrations of mannitol resulted a higher yield of lactic acid production at a concentration of 12 g/l with the production of 40.85 g/l. whereas the biomass yield was about 1.12 g/l at this optimum concentration.12g/l of peptone as nitrogen source effectively yields 53.5g/l of lactic acid and cell growth of 1.56 g/l. The results are promising for higher yield of lactic acid to be produced using *Lactobacillus* species compared to *Lactococcus lactis subsp lactis.*

Key words: Carbon sources, Lactic acid, *Lactococcus lactis subsp lactis* ,*Lactobacillus rhamnosus* , and Nitrogen sources.

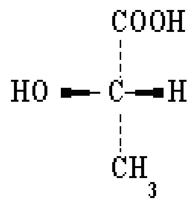
1. INTRODUCTION

Lactic acid is one of the most widely used organic acids in food industry. Recently, lactic acid has gained much interest because it can be used as a raw material for the production of biodegradable polymers with applications in medical, pharmaceutical, and food-packaging industries. Lactic acid , is a valuable industrial chemical used as an acidulant, preservative in the food industry, pharmaceutical, leather and textile industries as well as a chemical feedstock. Lactic acid bacteria have the property of producing lactic acid. The genera *Bacillus, Leuconostoc, Pediococcus and Streptococcus* are important members of this group. The species which have been used for lactic acid production are *L. sporogenes, L. acidophilus, L. plantarum, L. casei, L. brevis, L. delbruckii, L. lactis.* During these days, food-processing industry generates large volumes of carbohydrate wastes, which are ideal substrates for lactic acid production. Lactic acid is then converted into useful, high-value products such as lactic acid derivatives, which include biodegradable plastics, oxy chemicals, “green” solvents, and specialty chemicals[1].
The general equation for lactic acid fermentation is $C_6H_{12}O_6$ ------> 2 $C_3H_6O_3$. The levels of optical isomeric forms of lactic acid produced depend upon the nature of the culture [2]. The

structural configurations of these isomers are as follows :



D(-) levorotatory lactic acid



L(+) dextrorotatory lactic acid

Generally the optimum pH and temperature for a batch culture of *Lactobacillus sp*. was found to be 6.0 and 36°C, respectively. For *L. acidophilus* pH was 6.5 or 8.0 and temperature was 37 °C, *Lactobacillus pentosus* the pH was 7.6 and temperature was 32 °C. *Lactobacillus delbrueckii* utilized industrial corn cob waste from xylose manufacturing and the yield of lactic acid from glucose were 48.7 g l^{-1} and 95.2% respectively. *Lactobacillus delbrueckii* NRRL B445 yielded lactic acid from pulp mill solid waste ranged from 86 to 97% [3]. Efficient production of lactic acid was obtained from raw starch by *Streptococcus bovis* 148[4]. *Lactobacillu sake* & *Lactobacillus casei* yielded the lactic acid conversion of 48% and 56% respectively however lactic acid conversion increased to 71% by co- inoculation of both the strains from soya bean stalk. Lactic acid production was investigated using filamentous fungus *Rhizopus arrhizus* and the yield was only 75% and 61% results from the glucose medium. *Rhizopus arrhizus* produced lactic acid from waste potato starch[5]. All bacteria used for lactic acid production was more efficient in MRS broth[6].

Lactic acid bacteria (LAB) which are used throughout the world for manufacture of a wide variety of traditional fermented foods [7], are beneficial probiotic organisms that contribute to improved nutrition, microbial balance, and immuno-enhancement of the intestinal tract, as well as lower cholesterol along with that it will produce antimicrobial substances, sweeteners, sugar polymers, useful enzymes and aromatic compounds .Lactic acid bacteria foods and supplements have been found to modulate inflammatory and hypersensitivity responses, an observation thought to be at least in part due to the regulation of cytokine function. Clinical studies suggest that they can prevent reoccurrences of Inflammatory Bowel Disease in adults, as well as improve milk allergies and decrease the risk of atopic eczema in children. *L. rhamnosus* appears to protect the urogenital tract by excreting biosurfactants to inhibit the adhesion of vaginal and urinary pathogens. *Lactobacillus* also releases acids, bacteriocins, and hydrogen peroxide which inhibit the pathogen growth. *Lactobacillus Rhamnosus* has also been shown beneficial in the prevention of rotavirus diarrhea in children. The use of *Lactobacillus rhamnosus* for probiotic therapy does present a risk of sepsis. *L. rhamnosus* adheres to the mucous membrane of the intestine and may help to restore the balance of the GI micro flora; promote gut-barrier functions; diminish the production of carcinogenic compounds by other intestinal bacteria; and activate the innate immune response and enhance adaptive immunity, especially during infections [8].

2. MATERIALS AND METHODS

2.1 Material:

All the chemicals used are of analytical grade and samples were prepared using deionized water. *Lactobacillus rhamnosus* MTCC1408, and *Lactococcus lactis subsp lactis* MTCC440 were purchased from MTCC (Microbial Type Culture collection, Chandigarh, India).

2.2 Methods:

Lactococcus lactis subsp lactis MTCC440 were cultured on medium containing skim milk (10%), tomato juice (10%) and yeast extract agar slants at pH of 7 for 48 hours at $37^{0}C$ under aerobic conditions. Subculturing is done once in 30 days.

Lactobacillus rhamnosus MTCC1408 were cultured on MRS (Man, Rogosa and Sharpe medium) slants adjusted to pH 6.2-6.6 and incubated at $37^{0}C$ for 48 hours under aerobic conditions. Sub culturing was done once in 30 days.

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2.2.1 Media composition

Composition of MRS medium (Adjusted pH to 6.2-6.6)

Peptone	- 10.0 g
Beef extract	- 10.0 g
Yeast extracts	- 5.0 g
Glucose	- 20.0 g
Tween 80	- 1.0 ml
Na_2HPO_4	- 2.0 g
Sodium acetate	- 5.0 g
Triammonium citrate	- 2.0 g
$MgSO_4$, $7H_2O$	- 0.2 9
$MnSO_4$, $4H_2O$	- 0.2 g
Agar	- 15.0 g
Distilled water	- 1.0 l

Composition of Potato Dextrose Broth medium (Adjusted pH to 5.6)

Potato (scrubbed and diced)	- 200.0 g
Dextrose	- 20.0 g
Distilled water	- 1.0 l
Agar	- 15 g

2.2.2 Fermentation in various wastes

The various wastes such as domestic wastes, bakery wastes, fruit wastes, vegetable wastes and whey were used as fermentation medium.The domestic wastes containing kitchen garbage waste, agricultural wastes, gardening waste and stationary waste were collected from house hold and the organic matter were selected from the waste. These organic matters of about 1 kg was ground along with 1100 ml of distilled water and filtered to get the filtrate which is used as the fermentation medium.

Similarly vegetable waste, fruit waste and bakery waste were collected from household. One kg of each waste was ground along with 1100 ml of distilled water and filtered to obtain the filtrate which is used as the fermentation medium. All the above wastes will have different chemical compositions of carbohydrates, lignin, cellulose, hemicelluloses, protein and lipid. Whey was obtained from the household, since it was the by-product while preparing sweets and paneer.

All these five waste extracts were used as the fermentation medium. The two bacterial was inoculated in respective medium and incubated at pH of about 6.5 for 24 hours at 37_0C . In 1000 ml of the sterilized wastes medium, 1% (10 ml) of 24 hours bacterial innoculum was added and incubated at pH of about 6.5 for 24 hours at 370C. After 8 hour, the pH was maintained between 5.5-6 by addition of 2% $CaCO_3$. After fermentation the lactic acid concentration, biomass growth and substrate conversion was found for each organism in different wastes.

2.2.3 Study of various carbon sources

The optimal waste medium for the production of lactic acid was analysed for both organisms. Galactose, glucose, lactose, maltose and mannitol were selected as the carbon sources. The lactic acid concentration, biomass growth and substrate conversion for each organism were found in the best suited waste medium containing 10g/l of each carbon sources.

2.2.4 Study of various concentrations of the optimal carbon source

The carbon source which gives maximum yield of lactic acid for each organism was estimated. The various concentrations of the carbon sources such as 6, 8,10,12,14 g/l are added to the optimal waste medium and lactic acid productivity is analyzed.

2.2.5 Study of various nitrogen sources

The optimal waste medium for the production of lactic acid is analyzed for each organisms. Sodium nitrate, peptone, potassium nitrate, urea and yeast extract were selected as the nitrogen sources. The lactic acid concentration, biomass growth and substrate conversion for each organism were found in the best suited waste medium containing 10g/l of each nitrogen sources.

2.2.6 Study of various concentrations of the optimal nitrogen source

The various nitrogen sources which produces maximum yield of lactic acid for each microorganisms was estimated. The various

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concentrations of the nitrogen sources such as 6,8,10,12,14 g/l are added to the optimal waste medium and lactic acid productivity is analyzed.

2.2.7 Time profile of the microorganisms

The bacterial strains were grown on MRS agar plates by the method of four quadrants streaking using a sterile innoculum loop. For time profile study a single isolated colony was picked up from its plate from the help of a sterile inoculation loop and added to 10 ml of sterile MRS broth in a test tube to grow overnight (18h) in order to activate the colony taken from the plate.From the suspension of overnight grown bacterial culture, 10ml (1% innoculum) suspension was transferred in 1000 ml of sterile waste medium containing the best suited carbon or nitrogen sources in its optimal concentration.

3. RESULTSAND DISCUSSION

3.1 *Lactobacillus rhamnosus* MTCC1408 was inoculated in various wastes and MRS medium and incubated. Among the wastes as substrate, lactic acid production was found to be high in whey about 35.45 g/l and in the control MRS medium about 55.6 g/l. The biomass concentration was found to be high in whey about 1.34 g/l compared to other wastes and 1.59 g/l in MRS medium. Since the lactic acid production was comparatively high in whey than other wastes as illustrated in Table 1, further studies on effect of various carbon sources and nitrogen sources in lactic acid production was carried out with whey as substrate. Among the various carbon sources such as galactose, glucose, lactose, maltose and mannitol, Lactobacillus rhamnosus MTCC1408 showed higher yield of lactic acid about 38.3 g/l in mannitol . The biomass growth was found to be high in mannitol about 1 g/l as illustrated in Table 2. So the effect of various concentrations of mannitol were analysed and the higher yield of lactic acid production was found in 12 g/l concentration with the production of 40.85 g/l and the biomass yield was 1.12 g/l as illustrated in Table 3. The effect of various nitrogen sources such as sodium nitrate, peptone, potassium nitrate, urea and yeast extract on the production of lactic acid by *Lactobacillus rhamnosus* MTCC1408 were studied and found to be high in peptone about 51.3 g/l of lactic acid.The biomass yield was found to be high in peptone of 1.43 g/l as illustrated in the Table 4. So the lactic acid production on various concentrations of peptone was studied and found maximum yield of lactic acid was in the concentration of 12 g/l which resulted in a maximum yield of 53.5 g/l of lactic acid. The biomass growth was found to be high in 12 g/l of peptone and the biomass produced was about 1.56 g/l as illustrated in the Table 5 .On examining the time profile of *Lactobacillus rhamnosus* MTCC1408 the maximum lactic acid production and biomass growth was found at 48 hours and further increase in time showed no significant effect on lactic acid production and biomass yield, as shown in the Fig. 1 and Fig. 2.

3.2 *Lactococcus lactis subsp lactis* MTCC440 was inoculated in various wastes and with MRS medium and then incubated. Among the various wastes, lactic acid production was found to be high in whey of about 8.28 g/l and in the control MRS medium of about 42.2 g/l. The biomass concentration was found to be high in whey of about 0.74 g/l compared to other waste and 1.34 g/l in MRS medium, as illustrated in the Table 6. Since the lactic acid production was comparatively high in whey than other wastes, further studies on effect of various carbon sources and nitrogen sources was studied with whey as substrate.Among the various carbon sources such as galactose, glucose, lactose, maltose and mannitol, *Lactococcus lactis subsp lactis* MTCC440 showed higher yield of lactic acid about 13.2 g/l in glucose . The biomass growth was found to be high in glucose of about 0.97 g/l as illustrated in the Table 7. So the effect of various concentrations of glucose was further studied and found higher yield of lactic acid production was in 6 g/l concentration with the production of 24.2 g/l. The biomass yield was found to be high in 6 g/l of about 1.15 g/l as illustrated in the Table 8.

The effect of various nitrogen sources such as sodium nitrate, peptone, potassium nitrate, urea and yeast extract on the production of lactic acid by *Lactococcus lactis subsp lactis* MTCC440 was studied and found to be high in sodium nitrate about 9.4 g/l of lactic acid and the biomass growth was found to be high in sodium nitrate about 0.95 g/l as illustrated in the Table 9. So the lactic acid production on various concentrations of sodium nitrate was studied and maximum yield of lactic acid was found in the concentration of 10 g/l, which resulted in a maximum yield of 9.4 g/l of lactic acid. The biomass growth was high in 10 g/l of sodium nitrate and the biomass produced was about 0.95 g/l as illustrated in the Table 10.On examining the time profile of *Lactocoscuss lactis subsp lactis* MTCC440 the maximum lactic acid production and biomass growth was found at 48 hours as shown in the Fig. 3 and Fig. 4.

3.3 Immobilized *Lactobacillus rhamnosus* MTCC1408 and *Lactococcus lactis subsp lactis* MTCC440

The immobilized cells were allowed for 5 runs of batch fermentation. During the first batch of fermentation ,the lactic acid production was high and that increased further in second & third run of batch fermentation for both *Lactobacillus rhamnosus* and *Lactococcus lactis subsp lactis*. But the production was significantly decreased during fourth and fifth run of batch fermentation for both the species as illustrated in Fig. 5.

4. CONCLUSIONS

Based on the finding of the present study, it is concluded that the lactic acid production using *Lactobacillus rhamnosus* and *Lactococcus lactis subsp lactis* was high when whey was used as substrate. An additional supplement of glucose at a concentration of 6 g/l and sodium nitrate in a concentration of 10 g/l improved the production yield of lactic acid in case of *Lactococcus lactis subsp lactis* . Whereas *Lactobacillus rhamnosus* showed higher yield of lactic acid with mannitol as carbon source and with peptone as nitrogen source at an optimum concentration of 12 g/l. About 48 hours of fermentation time gave good yield of lactic acid for both the strains and on immobilization *Lactobacillus rhamnosus* foundto have better productivity of lactic acid compared to *Lactococcus lactis subsp lactis.* Hence, these strains could be good candidates for bioconversion of renewable sources for lactic acid production.

Nomenclature

S_0 - Initial feed concentration, g/l
S - Final substrate concentration, g/l
X - Biomass concentration, g/l
P - Product concentration, g/l
$Y_{X/S}$ - Substrate yield coefficient, g/g
$Y_{P/S}$ - Product yield coefficient, g/l
SC - Substrate conversion, %
DM - Domestic waste
BW - Baker waste
FM - Fruit Waste
VM - Vegetable waste

Table 1

Lactic acid production by *Lactobacillus rhamnosus* MTCC1408 from various wastes and MRS medium

Kinetic parameters	D W	B W	FW	VW	Whey	MRS
S_0 (g/l)	18.9	17.5	18.1	18.3	17.3	29.8
S (g/l)	15	13.6	14.1	13.9	12.7	14.1
S_0 - S (g/l)	3.9	3.89	4.02	4.38	4.59	15.7
SC (%)	26	22.2	22.2	23.9	26.6	52.7
X (g/l)	0.8	1.11	1.18	1.21	1.34	1.59
P (g/l)	16	12.5	16.9	24.4	35.5	55.6
Yx/s (g/g)	0.21	0.28	0.29	0.28	0.29	0.10
Y_P/s (g/l)	4.16	3.22	4.20	5.58	7.72	3.54

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Table 2

Effect of various carbon sources on lactic acid production and biomass yield

Kinetic parameters	Galactose (g/l)	Glucose (g/l)	Lactose (g/l)	Maltose (g/l)	Mannitol (g/l)
S_0 (g/l)	18.52	17.82	18.30	17.41	18.75
S (g/l)	14.41	13.67	13.77	12.92	14.12
S_0 - S (g/l)	4.11	4.15	4.53	4.49	4.63
SC (%)	22.19	23.2	24.75	25.78	24.69
X (g/l)	0.86	0.89	0.95	0.93	1.0
P (g/l)	23	26.4	36.5	36.2	38.2
Yx/s (g/g)	0.209	0.214	0.2097	0.207	0.215
Y_P/s (g/l)	5.596	6.361	8.057	8.062	8.25

Table 3

Effect of various concentrations of mannitol

Kinetic parameters	6 (g/l)	8 (g/l)	10 (g/l)	12 (g/l)	14 (g/l)
S_0 (g/l)	18.51	18.63	18.75	18.88	18.97
S (g/l)	14.12	14.08	14.12	14.1	14.56
S_0 - S (g/l)	4.39	4.55	4.63	4.78	4.35
SC (%)	23.7	24.42	24.69	25.31	22.93
X (g/l)	0.94	0.97	1	1.12	0.89
P (g/l)	37	37.5	38.2	40.85	35
Yx/s (g/g)	0.21	0.213	0.215	0.234	0.205
Y_P/s (g/l)	8.43	8.241	8.25	8.546	8.045

Table 4

Effect of various nitrogen sources on lactic acid production and biomass yield

Kinetic parameters	Sodium nitrate (g/l)	Peptone (g/l)	Potassium nitrate (g/l)	Urea (g/l)	Yeast extract (g/l)
S_0 (g/l)	18.4	17.8	18.9	18.3	17.8
S (g/l)	14.1	12.3	14.57	14.2	13.1
S_0 - S (g/l)	4.34	5.54	4.29	4.05	4.75
SC (%)	23.6	31.1	23.1	22.2	26.7
X (g/l)	1.03	1.43	0.97	0.89	1.11
P (g/l)	25	51.8	21.8	19	13.4
Yx/s (g/g)	0.24	0.26	0.23	0.22	0.23
Y_P/s (g/l)	5.76	9.35	5.08	4.69	8.29

Table 5

Effect of various concentrations of peptone

Kinetic parameters	6 (g/l)	8 (g/l)	10 (g/l)	12 (g/l)	14 (g/l)
S_0 (g/l)	17.53	17.68	17.82	17.97	18.12
S (g/l)	12.42	12.48	12.28	12.28	12.81
S_0 - S (g/l)	5.11	5.2	5.54	5.69	5.31
SC (%)	29.15	29.41	31.07	31.66	29.30
X (g/l)	0.87	1.01	1.43	1.56	1.31
P (g/l)	31.5	38.7	51.8	53.5	45.2
Yx/s (g/g)	0.17	0.194	0.258	0.274	0.246
Y_P/s (g/l)	6.164	7.44	9.35	9.40	8.512

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Table 6

Lactic acid production by *Lactococcus lactis subsp lactis* MTCC440 from various wastes and MRS medium

Kinetic parameters	D W	B W	FW	VW	Whey	MRS
S_0 (g/l)	18.9	17.8	18.1	18.3	17.3	29.8
S (g/l)	15.2	14.1	14.1	14.6	13.2	16.1
S_0 - S (g/l)	3.70	3.68	3.98	3.75	4.03	13.7
SC (%)	19.6	20.6	22.0	20.5	23.4	45.9
X (g/l)	0.65	0.63	0.73	0.67	0.74	1.34
P (g/l)	3.3	2.35	7.52	4.26	8.28	42.2
Yx/s (g/g)	0.18	0.17	0.18	0.18	0.19	0.10
Y_P/s (g/l)	0.89	0.64	1.89	1.14	2.05	3.23

Table 7

Effect of various carbon sources lactic acid production and biomass yield

Kinetic parameters	Galactose (g/l)	Glucose (g/l)	Lactose (g/l)	Maltose (g/l)	Mannitol (g/l)
S_0 (g/l)	18.52	17.82	18.30	17.41	18.8
S (g/l)	14.38	13.24	14.09	13.22	14.7
S_0 - S (g/l)	4.14	4.58	4.21	4.19	4.02
SC (%)	22.35	25.70	23	24.06	21.4
X (g/l)	0.78	0.97	0.87	0.83	0.71
P (g/l)	9.2	13.2	9.6	9.6	8.2
Yx/s (g/g)	0.188	0.212	0.206	0.198	0.18
Y_P/s (g/l)	2.22	2.88	2.28	2.29	2.03

Table 8

Effect of various concentrations of glucose

Kinetic parameters	6 (g/l)	8 (g/l)	10 (g/l)	12 (g/l)	14 (g/l)
S_0 (g/l)	17.63	17.71	17.82	17.95	18.07
S (g/l)	12.44	12.88	13.24	13.61	3.8
S_0 - S (g/l)	5.19	4.83	4.58	4.34	4.27
SC (%)	29.43	27.27	25.70	24.17	23.63
X (g/l)	1.15	1.05	0.97	0.83	0.80
P (g/l)	24.2	15.5	13.2	11.6	10.8
Yx/s (g/g)	0.22	0.217	0.212	0.192	0.187
Y_P/s (g/l)	4.662	3.209	2.88	2.672	2.529

Table 9

Effect of various nitrogen sources lactic acid production and biomass yield

Kinetic parameters	Sodium nitrate (g/l)	Peptone (g/l)	Potassium nitrate (g/l)	Urea (g/l)	Yeast extract (g/l)
S_0 (g/l)	18.38	17.82	18.855	18.3	17.78
S (g/l)	14.23	14.01	14.875	14.4	13.86
S_0 - S (g/l)	4.15	3.81	3.98	3.87	3.92
SC (%)	22.57	21.38	21.10	21.2	22.04
X (g/l)	0.95	0.69	0.84	0.72	0.79
P (g/l)	9.4	5.4	8.2	5.6	7.4
Yx/s (g/g)	0.228	0.181	0.211	0.19	0.20
Y_P/s (g/l)	2.265	1.417	2.06	1.44	1.887

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Table 10

Effect of various concentrations of sodium nitrate

Kinetic parameters	6 (g/l)	8 (g/l)	10 (g/l)	12 (g/l)	14 (g/l)
S_0 (g/l)	18.19	18.25	18.383	18.60	18.68
S (g/l)	14.23	14.23	14.23	14.79	14.98
S_0 - S (g/l)	3.96	4.02	.15	3.81	3.72
SC (%)	21.77	22.02	22.57	20.49	19.91
X (g/l)	0.86	0.90	0.95	0.83	0.76
P (g/l)	9.1	9.2	9.4	8.5	8.2
Yx/s (g/g)	0.217	0.223	0.229	0.218	0.204
Y_P/s (g/l)	2.297	2.288	2.265	2.230	2.204

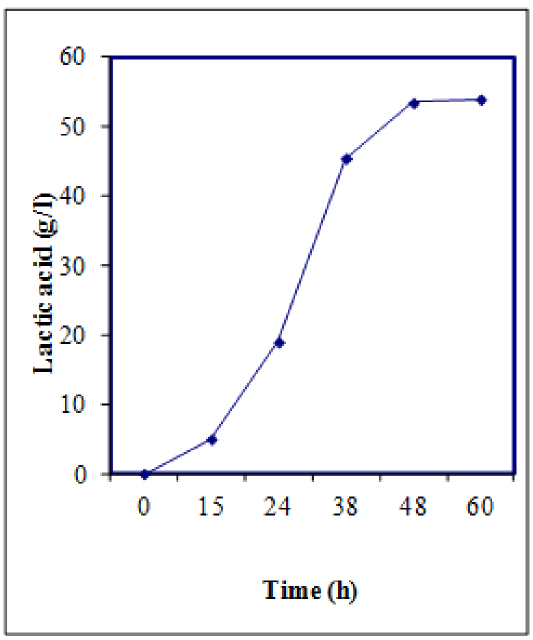

Fig.1: Effect of fermentation time on lactic acid production using *lactobacillus rhamnosus* MTCC1408 in whey + peptone (12 g/l) +mannitol(12 g/l)

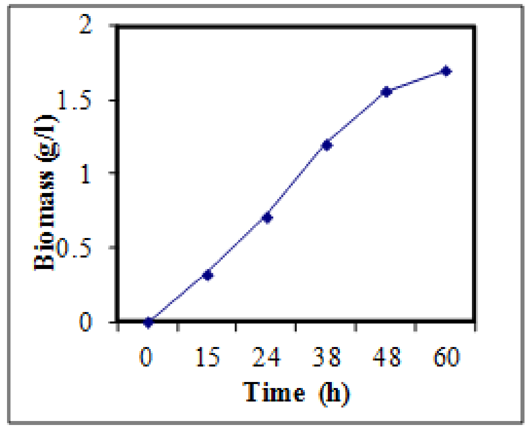

Fig. 2: Effect of fermentation time on biomass yield using *lactobacillus rhamnosus* MTCC1408 in whey + peptone (12 g/l) + mannitol(12 g/l)

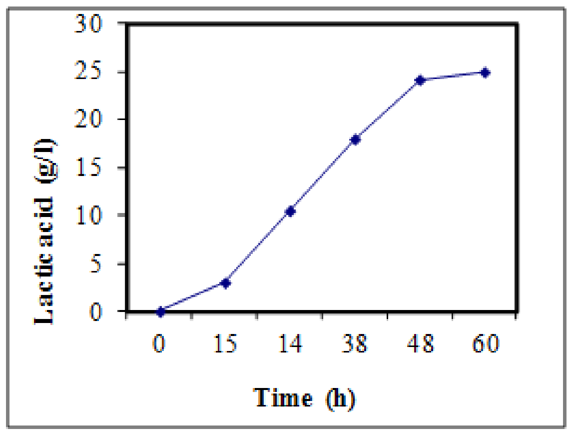

Fig. 3: Effect of fermentation time on lactic acid production using *lactococcus lactis subsp lactis* MTCC440 in whey + glucose (6 g/l) +sodium nitrate (10 g/l)

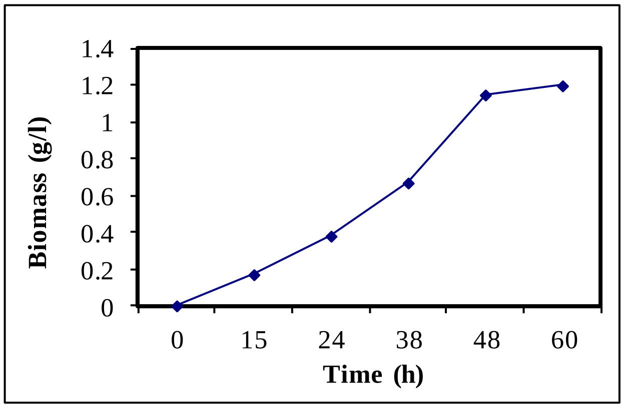

Fig. 4: Effect of fermentation time on biomass yield using *lactococcus lactis subsp lactis* MTCC440 in whey + glucose (6 g/l) +sodium nitrate (10 g/l)

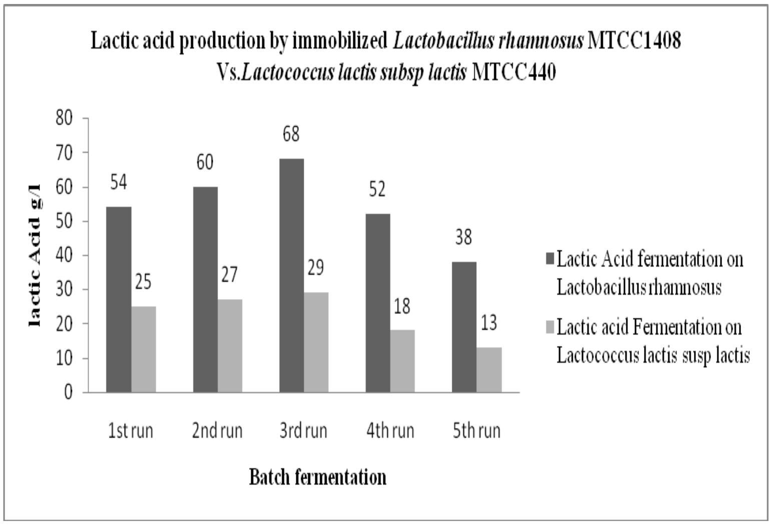

Fig. 5: Lactic acid production by immobilized *Lactobacillus rhamnosus* MTCC1408 and *Lactococcus lactis subsp lactis* MTCC440

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