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Kinetic Studies In Production Of Lactic Acid From Waste Potato Starch Using Lactobacillus casei

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Abstract: Kinetic studies in production of lactic acid from waste potato starch using *Lactobacillus casei* is made in this investigation. Also the kinetic and stoichiometric parameters such as μ_{max}, K_S, α, β, $Y_{P/S}$ and $Y_{X/S}$ are studied and analyzed. *Lactobacillus casei*, is a homofermentative lactic acid bacteria and capable of producing L (+) lactic acid. In this investigation three batch fermentation experiments in fermentor are performed at 37^{o}C and pH 6.5. Seed culture that was produced in shake flask fermentations was used as the inoculum for the fermentor. Three sets of fermentation experiment are carried out by varying the initial substrate concentration with 100, 75 and 50 g/l. The maximum lactic acid production is found to be 52, 49 and 30 g/l for the initial substrate concentration of 100, 75 and 50 g/l respectively. The lactic acid concentration is found to increase with increase in cell mass concentration. Hence lactic acid fermentation of waste potato waste starch by *Lactobacillus casei* is a growth associated kinetic pattern. The maximum specific growth rate, μ_{max} is found to be 0.1146, 0.1148 and 0.1349 h^{-1} for the initial substrate concentrations of 100 g/l, 75 g/l and 50 g/l respectively. The Monod constant, K_S is found to be 4.1036, 2.5100 and 2.2585 g/l for the initial substrate concentration of 100 g/l, 75 g/l and 50 g/l respectively. The productivity of lactic acid fermentation is found to be increased with an increase in initial substrate concentration. It observed that 0.8500, 1.000 and 0.6905 g of lactic acid are produced per liter per hour for the initial substrate concentrations of 100 g/l, 75 g/l and 50 g/l respectively.
Keywords: Lactic acid, *Lactobacillus casei*, Potato Starch, Growth Associated Kinetic Pattern.

1.Introduction

Due to the wide application of Lactic acid in the food, cosmetic, pharmaceutical and chemical industries[1,2,3,4,5], the interest in lactic acid production in large scale has increased significantly[6].

The available literature for L-lactic acid production have mainly focused on the use of such as glucose, sucrose, maltose, or xylose[2,7,8] which are pure and directly fermentable substrates. As a result the production cost of L-lactic acid significantly and the process is less economic for industrial applications. Starchy, cellulosic materials, and molasses[1,2,9,10] are suitable alternate to reduce the production cost of L-lactic acid significantly.

Lactic acid can be produced by either chemical synthesis or by fermentation. The latter has proven to be the better alternative as it is more energy efficient[6]. Simultaneous saccharification and fermentation (SSF) is a possible method to substantially decrease the glucose inhibition since the glucose could be utilized promptly during this process. Furthermore, separate reactors are replaced by a single reactor, which could save the capital investment. In simultaneous

saccharification and fermentation, the cellulose first has to be hydrolyzed to glucose and then the various products can be obtained by subsequent fermentation. Therefore, if the microorganisms used for fermentation are compatible with the cellulase system, the simultaneous saccharification and fermentation process could be more efficient[11].

Lactobacillus. casei is an anaerobic microorganism having homofermentative nature and well known to be an L(+)-lactic acid producer. Consequently, the microorganism grows better in a static culture where the fermentation conditions are anaerobic[12]. *Lactobacillus casei* is a efficient lactic acid producing bacteria having remarkable phenotypic and genotypic variability[12,13,14,15,16] that colonize diverse ecological niches and have the broad commercial applications[15]. Furthermore, *L. casei* is acidotolerant with an optimum pH of 5.5 and is relatively insensitive to product inhibition by lactic acid[16].

The ability of lactic acid producing bacteria *Lactobacillus casei* by simultaneous saccharification and fermentation is studied and reported[6]. The enzymes α-amylase and glucoamylase are used in this investigation. The effect of amount potato waste substrate, concentration of enzyme mixture, yeast extract, NH_4Cl and inoculum size on the production of lactic acid is analyzed statistically using Box-Behnken Design. The amount of amount potato waste substrate, concentration of enzyme mixture, yeast extract, NH_4Cl and inoculum size are optimized statistically using Box-Behnken Design for high production of lactic acid.

To understand the behavior of the system, modeling is required. Models are used to define the biological, chemical and physical basis of the process. A kinetic model is a set of relationships between biomass growth, substrate utilization and product formation. Kinetic models predict how fast the microorganisms can grow and use substrates or make products. In practice, kinetic data are collected with respect to time in small-scale reactors and then used to scale-up the process along the mass transfer data.

Under the optimized condition of 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size, the present investigation is aimed to study the kinetic modeling to explain the lactic acid production from waste potato starch by *Lactobacillus casei*. Also it is aimed to study and analyze the kinetic and stoichiometric parameters such as μ_{max}, K_S, α, β, $Y_{P/S}$ and $Y_{X/S}$ for the production of lactic acid for waste potato starch using *Lactobacillus casei*.

2.Materials And Methods

2.1-Microorganism And Inoculum Preparation

Lactic acid producing culture *Lactobacillus casei* (MTCC 1423) National Collection of Industrial Microorganism, National Chemicals Laboratory, Pune, India is used in this present study. MRS agar medium is used to maintain the culture and subcultured in every two weeks. A temperature of 37°C is used for inoculum preparation[17]. Lactobacillus MRS Agar (as shown in Table 1) is recommended for cultivation of all Lactobacillus species.

2.2-Enzymes

Commercial amylases, α-amylase (2000 IU/mL) and glucoamylase (4000 IU/mL) (National Scientific Suppliers, India) are used for hydrolysis of potato starch.

2.3-Fermentation Medium

The medium consisted of potato waste in distilled water enriched with yeast extract and $NH_4Cl.CaCO_3$ (60%, w/w of starch) is added for burring. The medium is autoclaved at 121°C for 15 min and the enzymes are added to the medium along with the inoculum (24-hr-old). The inoculated flaks are incubated at 37°C for 60 hr.

2.4-Analysis

Samples are withdrawn in equal time interval of incubation period and treated with 1 M H_2SO_4 to release the lactic acid from medium as it is formed as calcium lactate with buffering agent, CaCO3. Lactic acid extracted out from medium and the extract is diluted to the required level with distilled water and the amount of total lactic acid is estimated according to the colorimetric method of Barker and Summerson[18] and is expressed as mg/mL of the fermentation medium. The amount of reducing sugar is determined by the 3, 5 dinitro salicylic acid method[19]. Starch is estimated by Nampoothiri et al description[20] using aqueous iodine solution as reagent. The color development is measured using UV spectrophotometer (Elico Limited) at 620 nm.

Table 1: The composition of MRS Medium

Ingredients	gm/L
Protease peptone	10
Beef extract	10
Yeast extract	5
Dextrose	20
Polysorbate 80	1
Ammonium citrate	2
Sodium acetate	5
Magnesium sulphate	0.1
Manganese sulphate	0.05
Dipotassium phosphate	2
Agar	12
Final pH (at 25°C)	6.5±0.2

3.Results And Discussions

3.1-Fermentation Experiments

The kinetic modeling is studied for the production of lactic acid using *Lactobacillus casei* from waste potato starch. Three sets of fermentation experiment are carried out by varying the initial substrate concentration with 100, 75 and 50 g/l. Statistical analysis for these three initial substrate concentration are made to optimize the experimental variables for the production of lactic acid using *lactobacillus casei* from waste potato starch by Box-Behnken Design[6]. The maximum lactic acid production is found to be 52, 49 and 30 g/l respectively for the initial substrate concentration of 100, 75 and 50 g/l. All sets of experiments are carried out at the optimum temperature and pH of 37^{o}C and 6.5 respectively. In this investigation, the kinetic and stoichiometric parameters such as μ_{max}, K_S, α, β, $Y_{P/S}$ and $Y_{X/S}$ have been studied and analyzed.

The lag phase is observed in 0 – 12 hours, the exponential phase is found in 12 – 40 hours, deceleration phase is found in 40 – 45 hours, the stationary phase is found in 45 – 55 hours and the death phase is found in above 55 hours for the fermentation of 100 g/l of initial substrate concentration. The same microbial growth curve is observed but time date alone is changed with changing the initial substrate concentration. For the case of 75 g/l of initial substrate concentration fermentation, it is observed that the lag phase is observed in 0 – 8 hours, the exponential phase is found in 8 – 35 hours, deceleration phase is found at 35 hours, the stationary phase is found in 35 – 45 hours and the death phase is found in above 45 hours. Similarly for the case of 50 g/l of initial substrate concentration fermentation, it is observed that the lag phase is observed in 0 – 4 hours, the exponential phase is found in 4 – 30 hours, deceleration phase is found at 30 hours, the stationary phase is found in 30 – 40 hours and the death phase is found in above 40 hours. The lactic acid concentration is found to increase with increase in cell mass concentration. Hence lactic acid fermentation is a growth associated kinetic pattern.

Figure 1 shows the experimental data for modeling at 100 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size. The effect of fermentation time on biomass concentration and Lactic acid production is also represented in Figure 1. Figure 2 shows the relationship between cell growth and substrate concentration for the initial substrate concentration and Figure 3 shows the relationship of growth rate with lactic acid production for the initial substrate concentration of 100 g/l potato waste.

The experimental data for modeling at 100 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size is presented in Table 2, Table 3 and Table 4.

The fermentation with 100 g/l of initial substrate concentration is completed in 55 hours. The biomass concentration is reached to 68 g/l at the end of the fermentation. At the stationary phase of 55 hours, 95% lactose was utilized for lactic acid production.

Similarly, with the initial substrate concentration of 75 g/l and 50g/l, the fermentation is completed in 42 hours. The biomass concentrations are reached to 64 g/l and 43 g/l at the end of the fermentation for the initial substrate concentration of 75 g/l and 50g/l respectively. Similarly after 42 hours and 36 hours of fermentation, all lactose was utilized as soon as the stationary phase was attained in both the case of initial substrate concentration of 75 g/l and 50g/l. 93.3% and 90% of lactose was utilized in the case of 75 g/l and 50g/l of initial substrate concentrations.

Figure 4 shows the experimental data for modeling at 75 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size. The effect of fermentation time on biomass concentration and Lactic acid production is also represented in Figure 4. Figure 5 shows the relationship between cell growth and substrate concentration for the initial substrate concentration and Figure 6 shows the relationship of growth rate with lactic acid production for the initial substrate concentration of 75 g/l potato waste.

Table 5, Table 6 and Table 7 provides the experimental data for modeling at 75 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme

mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size.

Figure 7 shows the experimental data for modeling at 50 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size. The effect of fermentation time on biomass concentration and Lactic acid production is also represented in Figure 7. Figure 8 shows the relationship between cell growth and substrate concentration for the initial substrate concentration and Figure 9 shows the relationship of growth rate with lactic acid production for the initial substrate concentration of 50 g/l potato waste.

The experimental data for modeling at 50 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size is presented in Table 8, Table 9 and Table 10.

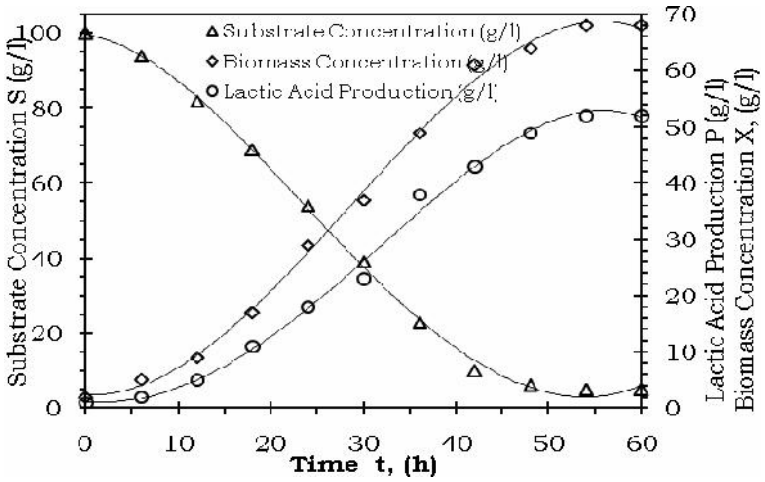

Figure 1: Experimental data for modeling at 100 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size.

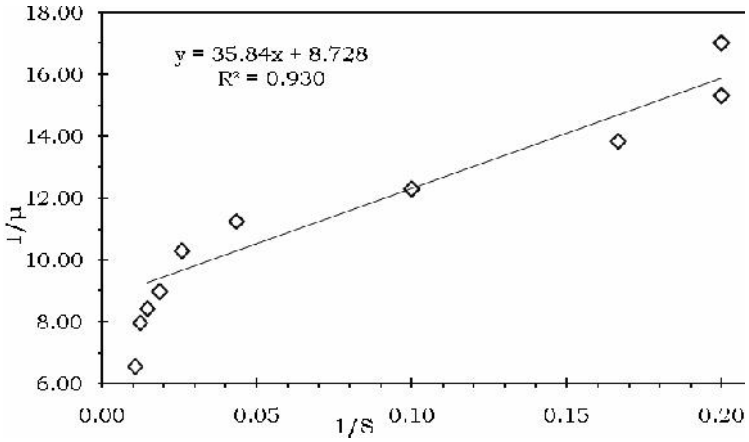

Figure 2: Relationship between cell growth and substrate concentration for the initial substrate concentration of 100 g/l potato waste

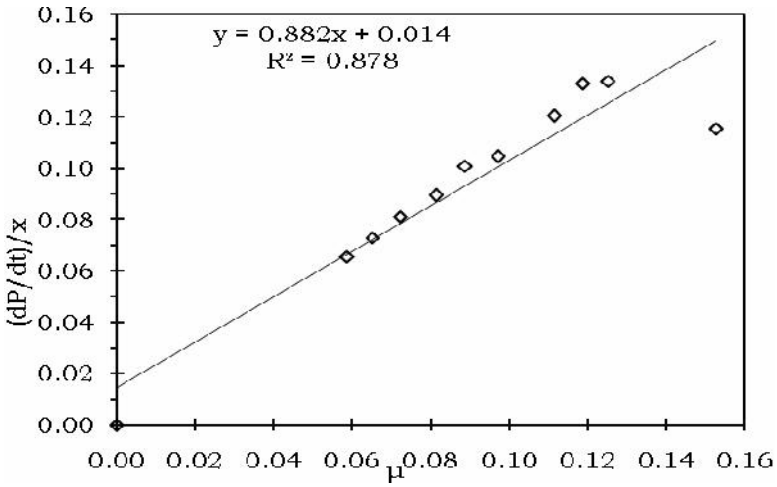

Figure 3: Relationship growth rate with lactic acid production for the initial substrate concentration of 100 g/l potato waste

Table 2: Experimental data for modeling at 100 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6 × 10^9 cfu/100ml of inoculum size.

Time (hr)	Biomass Conc (X) (g/l)	Substrate Conc (S) (g/l)	Lactic Acid Prod (P) (g/l)
0	2	100	1
6	5	94	2
12	9	82	5
18	17	69	11
24	29	54	18
30	37	39	23
36	49	23	38
42	61	10	43
48	64	6	49
54	68	5	52
60	68	5	52

Table 3: Biomass Concentration (X), substrate Concentration (S) during the course of fermentation using liquid waste potato starch for the initial substrate concentration of 100 g/l potato waste

Time (hr)	Biomass Concentration X (g/l)	Substrate Concentration S (g/l)	μ	1/μ	1/S
0	2	100	0.0000	0.0000	0.0100
6	5	94	0.1527	6.5478	0.0106
12	9	82	0.1253	7.9781	0.0122
18	17	69	0.1189	8.4108	0.0145
24	29	54	0.1114	8.9747	0.0185
30	37	39	0.0973	10.2817	0.0256
36	49	23	0.0889	11.2545	0.0435
42	61	10	0.0814	12.2887	0.1000
48	64	6	0.0722	13.8497	0.1667
54	68	5	0.0653	15.3130	0.2000
60	68	5	0.0588	17.0145	0.2000

Table 4: Biomass Concentration (X), Lactic Acid Production (P) during the course of fermentation using liquid waste potato starch for the initial substrate concentration of 100 g/l potato waste

Time (hr)	Lactic Acid Production P (g/l)	dP/dt	Biomass Concentration X (g/l)	(dP/dt)/X	μ
0	1	0.0000	2	0.0000	0.034
6	2	0.5776	5	0.1155	0.026
12	5	1.2071	9	0.1341	0.111
18	11	2.2647	17	0.1332	0.121
24	18	3.4925	29	0.1204	0.068
30	23	3.8671	37	0.1045	0.048
36	38	4.9512	49	0.1010	0.029
42	43	5.4627	61	0.0896	0.019
48	49	5.1891	64	0.0811	0.009
54	52	4.9756	68	0.0732	0.004
60	52	4.4781	68	0.0659	0.002

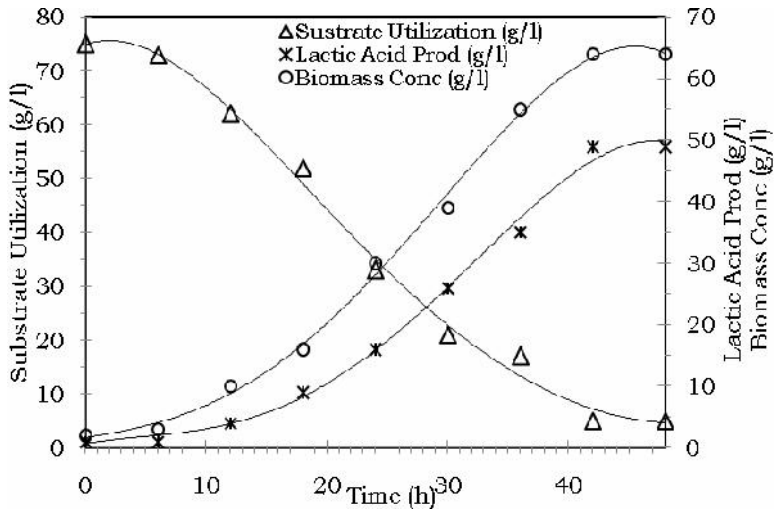

Figure 4: Experimental data for modeling at 75 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size.

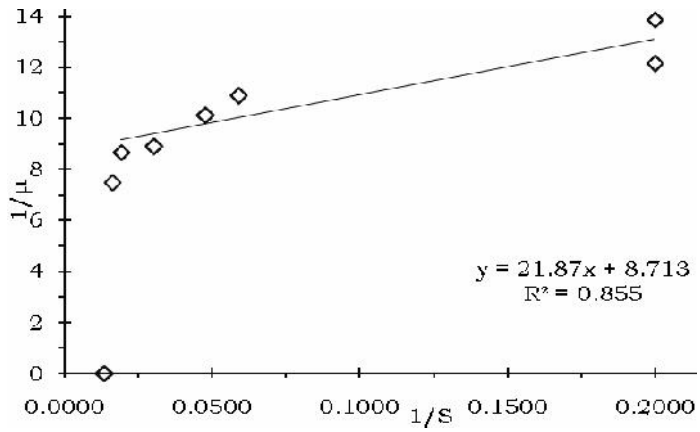

Figure 5: Relationship between cell growth and substrate concentration for the initial substrate concentration of 75 g/l potato waste

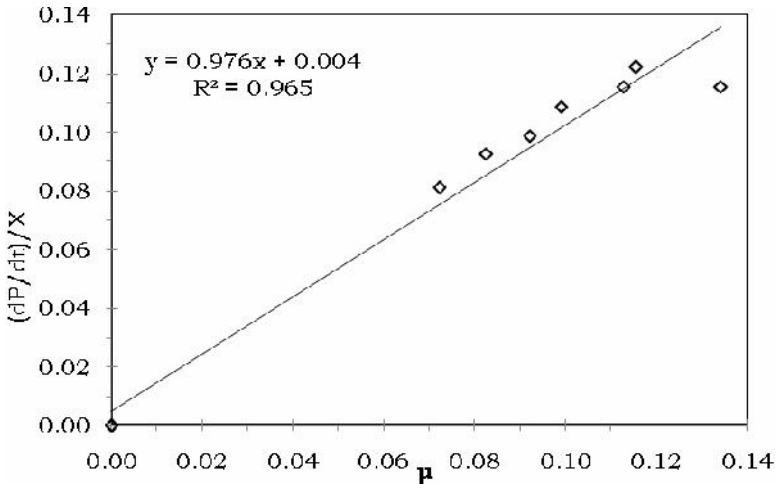

Figure 6: Relationship growth rate with lactic acid production for the initial substrate concentration of 75 g/l potato waste

Table 5: Experimental data for modeling at 75 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6 × 10^9 cfu/100ml of inoculum size.

Time (hr)	Biomass Conc (X) (g/l)	Substrate Conc (S) (g/l)	Lactic Acid Prod (P) (g/l)
0	2	75	1
6	3	73	1
12	10	62	4
18	16	52	9
24	30	33	16
30	39	21	26
36	55	17	35
42	64	5	49
48	64	5	49

Table 6: Biomass Concentration (X), substrate Concentration (S) during the course of fermentation using liquid waste potato starch for the initial substrate concentration of 75 g/l potato waste

Time (hr)	Biomass Concentration X (g/l)	Substrate Concentration S (g/l)	μ	1/μ	1/S
0	2	75	0.0000	0.0000	0.0133
6	3	73	0.0000	0.0000	0.0137
12	10	62	0.1341	7.4553	0.0161
18	16	52	0.1155	8.6556	0.0192
24	30	33	0.1128	8.8620	0.0303
30	39	21	0.0990	10.0991	0.0476
36	55	17	0.0921	10.8619	0.0588
42	64	5	0.0825	12.1181	0.2000
48	64	5	0.0722	13.8493	0.2000

Table 7: Biomass Concentration (X), Lactic Acid Production (P) during the course of fermentation using liquid waste potato starch for the initial substrate concentration of 75 g/l potato waste

Time (hr)	Lactic Acid Production P (g/l)	dP/dt	Biomass Concentration X (g/l)	(dP/dt)/X	μ
0	1	0.0000	2	0.160	0.0000
6	1	0.0000	3	0.193	0.0000
12	4	1.1552	10	0.125	0.1341
18	9	1.9531	16	0.097	0.1155
24	16	3.4657	30	0.078	0.1128
30	26	4.2355	39	0.072	0.0990
36	35	5.4318	55	0.052	0.0921
42	49	5.9304	64	0.035	0.0825
48	49	5.1891	64	0.025	0.0722

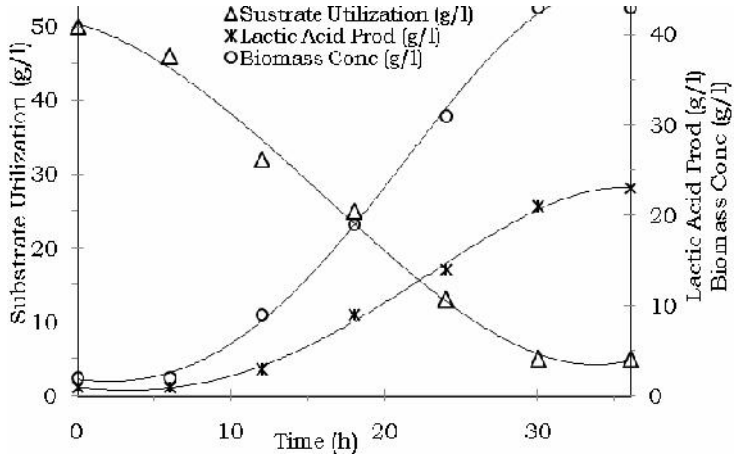

Figure 7: Experimental data for modeling at 50 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size.

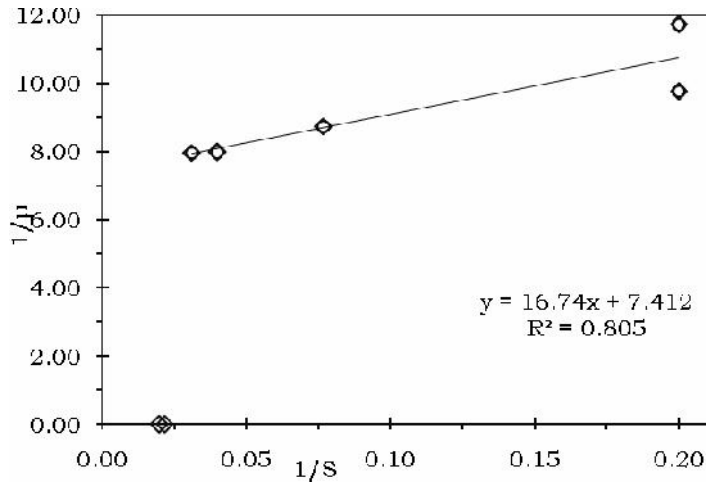

Figure 8: Relationship between cell growth and substrate concentration for the initial substrate concentration of 50 g/l potato waste

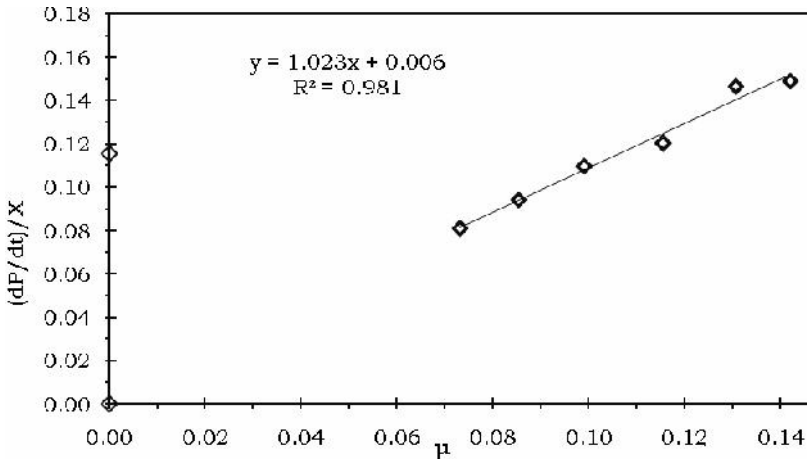

Figure 9: Relationship growth rate with lactic acid production for the initial substrate concentration of 50 g/l potato waste

Table 8: Experimental data for modeling at 50 g/l potato waste, 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size.

Time (hr)	Biomass Conc (X) (g/l)	Substrate Conc (S) (g/l)	Lactic Acid Prod (P) (g/l)
0	2	50	1
6	4	46	2
12	11	32	6
18	21	25	14
24	32	13	18
30	39	9	27
36	43	5	30
42	43	5	30

Table 9: Biomass Concentration (X), substrate Concentration (S) during the course of fermentation using liquid waste potato starch for the initial substrate concentration of 50 g/l potato waste

Time (hr)	Biomass Concentration X (g/l)	Substrate Concentration S (g/l)	μ	1/μ	1/S
0	2	50	0.0000	0.0000	0.0200
6	4	46	0.0000	0.0000	0.0217
12	11	32	0.1421	7.0390	0.0313
18	21	25	0.1306	7.6549	0.0400
24	32	13	0.1155	8.6560	0.0769
30	39	9	0.0990	10.0994	0.1111
36	43	5	0.0852	11.7336	0.2000
42	43	5	0.0731	13.6893	0.2000

Table 10: Biomass Concentration (X), Lactic Acid Production (P) during the course of fermentation using liquid waste potato starch for the initial substrate concentration of 50 g/l potato waste

Time (hr)	Lactic Acid Production P (g/l)	dP/dt	Biomass Concentration X (g/l)	(dP/dt)/X	μ
0	1	0.0000	2	0.00	0.0000
6	2	0.4621	4	0.12	0.0000
12	6	1.6424	11	0.15	0.1421
18	14	3.0789	21	0.15	0.1306
24	18	3.8538	32	0.12	0.1155
30	27	4.2846	39	0.11	0.0990
36	30	4.0625	43	0.09	0.0852
42	30	3.4822	43	0.08	0.0731

3.2-Determination Of Kinetic Parameters

Based on the experimental biomass and lactic acid concentration data, the biomass, and product yield coefficients ($Y_{X/S}$ and $Y_{P/S}$ respectively) are determined according to the standard methods and presented in **Table 11 and Table 12**.

The product yield on substrate, $Y_{P/S}$, changes with different initial substrate concentrations. For this reason, the average value of calculated product yield is taken as 0.6223 in the model development.

However, the biomass yield on substrate, $Y_{X/S}$ followed a different trend. The biomass yield decreased with the increase in the initial substrate concentration. The relationship between the biomass yield coefficient and the initial substrate concentration is represented in Figure 10. Similarly Figure 11 represents the relationship between the product yield coefficient, $Y_{P/S}$ and initial substrate concentration

Table 11 also presents the experimental α values (α_{exp}). α is the growth associated term parameter in the Leudeking - Piret Equation 1.

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X \quad ----(1)$$

I

n the study of Amrane and Prigent[21], more confident conclusions were reached when the coefficient of Leudeking - Piret equation, α is calculated from the following Equation 2.

$$\alpha = \frac{(P - P_o)}{(X - X_o)} \quad ----(2)$$

The α values in Table 11 are calculated by using the experimental lactic acid and biomass concentration data in Equation 1. In Figure 12, it is seen that α decreased with the increase in initial substrate concentration. It might be some experimental error. Based on this result, a linear relationship was assumed between α and initial substrate concentration.

The maximum specific growth rate (μ_{max}) is calculated using the following Monod Model Equation 3.

$$\mu = \frac{\mu_{max} S}{K_S + S} \quad ----(3)$$

The equation 3 can be linearized in double reciprocal form:

$$\frac{1}{\mu} = \frac{\frac{1}{\mu_{max}} K_S}{S} \frac{1}{\mu_{max}} \quad ----(4)$$

A plot of 1/μ versus 1/S, which is also known as a Lineweaver - Burk plot[22], yields a linear line with a slope of K_S/μ_{max} and the y axis intercept of $1/\mu_{max}$ as depicted in Figure 15.

The specific growth rate is calculated by the following first order exponential growth rate differential Equation 5.

$$\frac{dX}{dt} = \mu X \quad ----(5)$$

The above Equation 5 can be rewritten as Equation 6.

$$\mu = \frac{1}{X} \frac{dX}{dt} \quad ----(6)$$

Figure 2, Figure 5 and Figure 8 are shows the relationship between cell growth and substrate concentration for the initial substrate concentration of 100 g/l, 75 g/l and 50 g/l respectively. The reciprocal experimental μ values (1/μ) versus reciprocal of substrate values (1/S) are used to generate Figure 2, Figure 5 and Figure 8.

The slope of the linear line is K_S/μ_{max} and the x-axis intercept is $1/K_S$. According to least square equation, K_S is found to be 4.1063, 2.5100

and 2.2583 g/l for the initial substrate concentration of 100 g/l, 75 g/l and 50 g/l respectively. Similarly the μ_{max} is found to be 0.1146, 0.1148 and 0.1349 h^{-1} for the initial substrate concentrations of 100 g/l, 75 g/l and 50 g/l respectively. **Figure 13** shows the relationship between maximum specific growth μ_{max}, (h^{-1}) and initial substrate concentration (g/l).

3.3-Productivity Of The Fermentation Runs

The productivity values calculated with the experimental data in exponential phase were plotted against initial substrate concentrations. The productivity values were calculated by the following Equation 7.

$$\text{Productivity} = \frac{P_f - P_o}{t_f - t_o} \quad ----(7)$$

Figure 14 shows the productivity from experimental data for the initial substrate concentration of 100 g/l, 75 g/l and 50 g/l. This plot indicates that the productivity increases with an increase in amount of initial substrate concentration. It observed that 0.5800, 1.000 and 0.6905 g of lactic acid are produced per liter per hour for the initial substrate concentrations of 100 g/l, 75 g/l and 50 g/l respectively.

Table 11: Kinetic parameters on the initial substrate concentration

Initial Substrate concentration S_o (g/l)	Maximum specific growth μ_{max} (h^{-1})	Monod constant K_S (g/l)	Growth associated product formation α (h^{-1})	Non-Growth associated product formation β	Biomass yield on the utilized substrate $Y_{X/S}$ (g/g)	Product yield on the utilized substrate $Y_{P/S}$ (g/g)	Productivity $\frac{P_f - P_o}{t_f - t_o}$ (g/l h)
100	0.1146	4.1063	0.8820	0.0140	0.6947	0.5368	0.8500
75	0.1148	2.5100	0.9760	0.0040	0.8267	0.6857	1.0000
50	0.1349	2.2585	1.0230	0.0060	0.9111	0.6444	0.6905

Table 12: Experimental and Calculated values of ~

S No	Initial Substrate Concentration (g/l)	α_{exp} (h^{-1})	α_{cal} (h^{-1})
1	100	0.882	0.7727
2	75	0.976	0.7742
3	50	1.023	0.7073

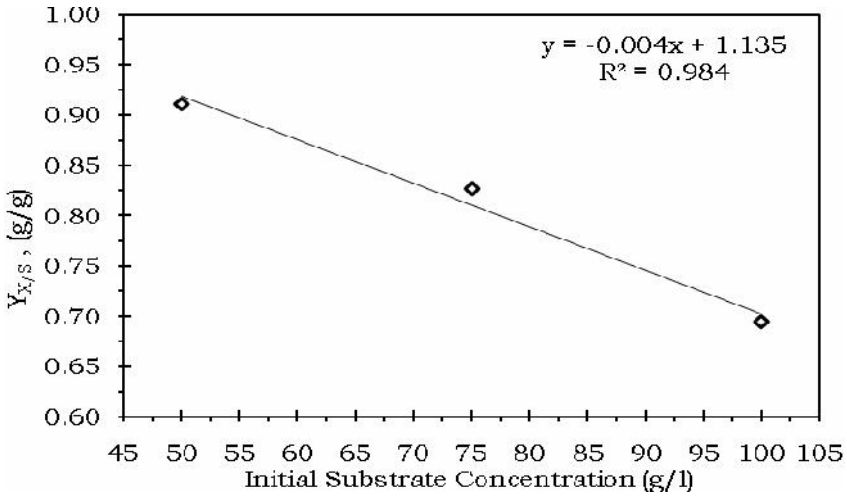

Figure 10: Relationship between the biomass yield coefficient, $Y_{X/S}$ and initial substrate concentration

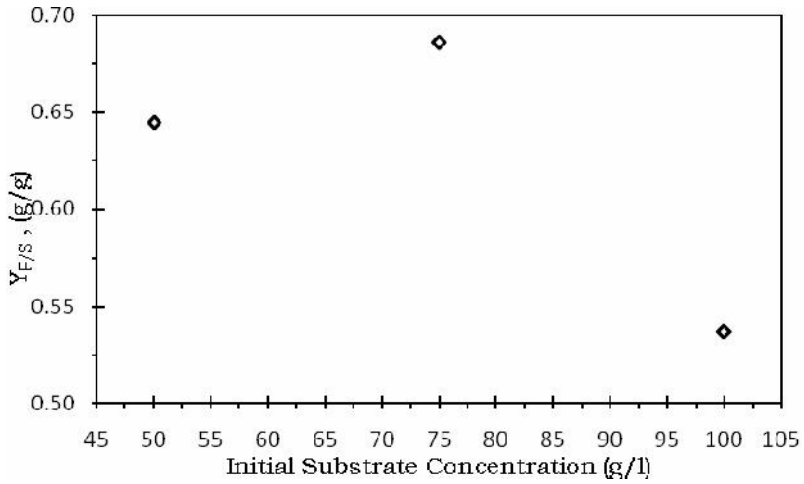

Figure 11: Relationship between the product yield coefficient, $Y_{P/S}$ and initial substrate concentration

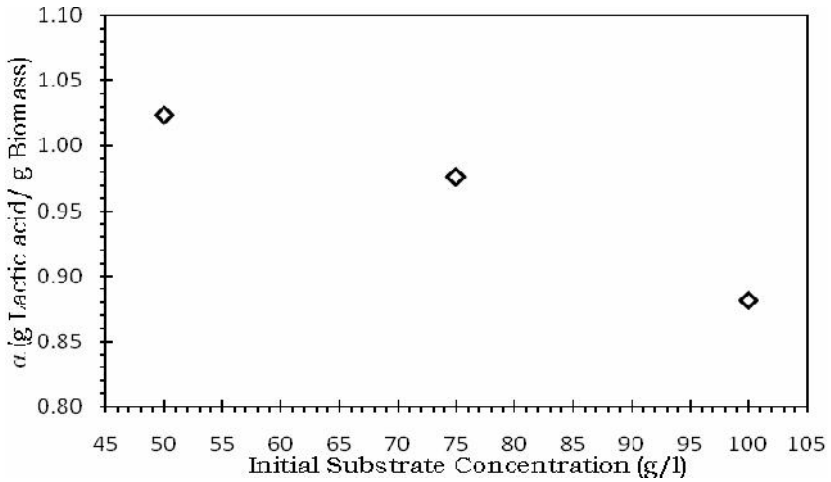

Figure 12: Relationship between experimental ~ and initial substrate concentration

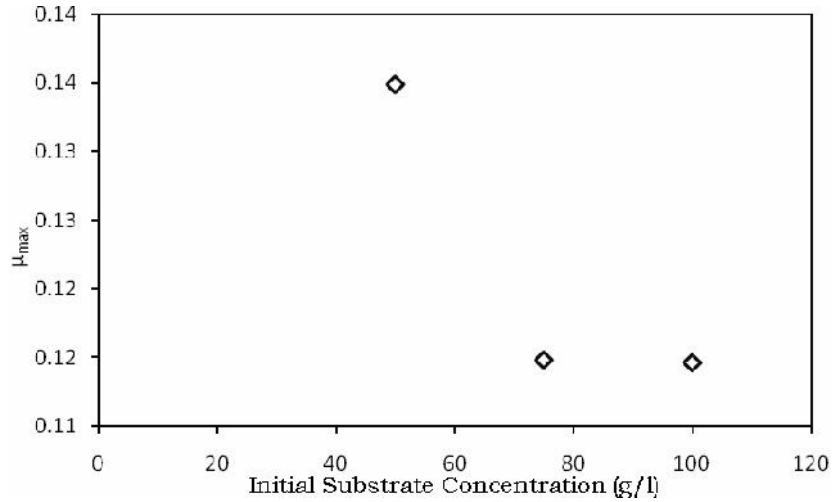

Figure 13: Relationship between maximum specific growth μ_{max}, (h^{-1}) and initial substrate concentration (g/l).

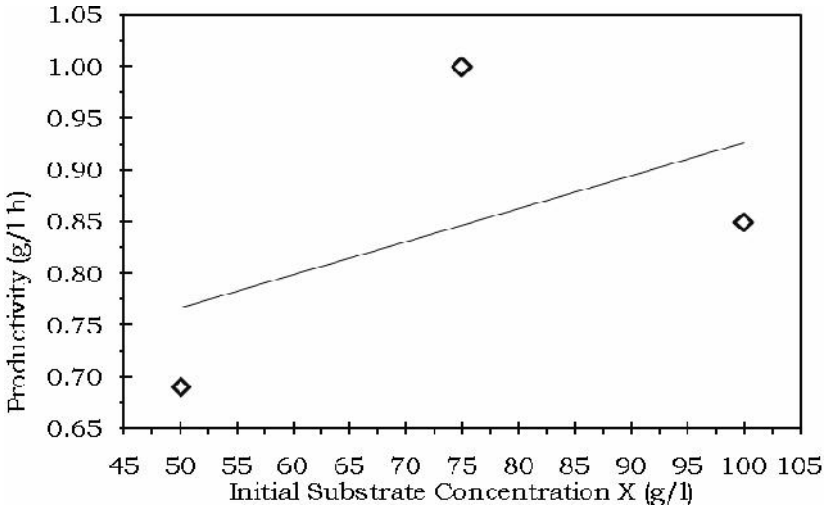

Figure 14: Productivity from experimental data for the Initial Substrate Concentration of 100 g/l, 75 g/l and 50 g/l.

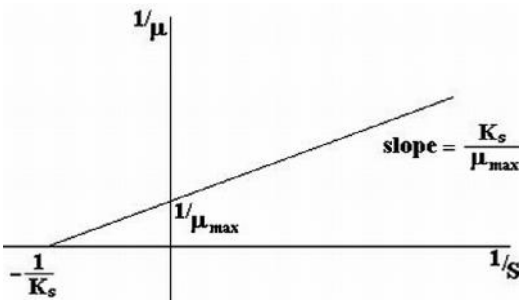

Figure 15: Double reciprocal of Line weaver - Burk plot

Conclusion

The kinetic modeling of fermentation of waste potato waste starch by *Lactobacillus casei* to produce lactic acid is studied under the optimized condition of 12 g/l of yeast extract, 10ml/l of enzyme mixture, 3 g/l of ammonium chloride and 6×10^9 cfu/100ml of inoculum size. Biomass growth, product formation and substrate consumption are used as the kinetic parameters in this investigation.

The maximum lactic acid production is found to be 52, 49 and 30 g/l respectively for the initial substrate concentration of 100, 75 and 50 g/l. All sets of experiments are carried out at the optimum temperature and pH of 37°C and 6.5 respectively. The lactic acid concentration is found to increase with increase in cell mass concentration. Hence lactic acid fermentation of waste potato waste starch by *Lactobacillus casei* is a growth associated kinetic pattern.

The kinetic parameters of lactic acid productions were determined by series of batch fermentation experiments with three different initial substrate concentrations of 100, 75 and 50 g/l. The maximum specific growth rate, μ_{max} is found to be 0.1146, 0.1148 and 0.1349 h^{-1} for the initial substrate concentrations of 100 g/l, 75 g/l and 50 g/l respectively. The Monod constant, K_S is calculated from the Line weaver - Burk plot and found to be 4.1063, 2.5100 and 2.2585 g/l for the initial substrate concentration of 100 g/l, 75 g/l and 50 g/l respectively. The products on substrate yield values are very close in different fermentations, so the average value of 0.6223 was used in models. The productivity of lactic acid fermentation is found to be increase with an increase in initial substrate concentration. It observed that 0.8500, 1.000 and 0.6905 g of lactic acid are produced per liter per hour for the initial substrate concentrations of 100 g/l, 75 g/l and 50 g/l respectively.

References

1 Dumbrepatil A, Adsul M, Chaudhari S, Khire J, Gokhale D (2008). Utilization of molasses sugar for lactic acid production by Lactobacillus delbrueckii subsp. delbrueckii mutant Uc-3 in batch fermentation. Appl. Environ. Microbiol. 74: 333–335.

2 Limin Wanga, Bo Zhao, Bo Liu, Chunyu Yang, Bo Yua, Qinggang Li, Cuiqing Mab, Ping Xu, Yanhe Maa (2010). Efficient production of L-lactic acid from cassava powder by Lactobacillus rhamnosus. Bioresource Technology. 101: 7895–7901.

3 Gegios A, Amthor R, Dixon B M, Egesi C, Mallowa S, Nungo R, Gichuki S, Mbanaso A,

Manary M J (2010). Children consuming cassava as a staple food are at risk for inadequate zinc, iron, and vitamin A intake. Plant Foods Hum. Nutr. doi:10.1007/s11130-010-0157-5.

4 Bo Zhao, Limin Wanga, Fengsong Li, Dongliang Hua, Cuiqing Mab, Yanhe Maa, Ping Xu (2010). Kinetics of D-lactic acid production by Sporolactobacillus sp. strain CASD using repeated batch fermentation. Bioresource Technol. 101: 6499–6505.

5 Sara L Walton, Kenneth M Bischoff, Adriaan R P, van Heiningen, G Peter van Walsum (2010). Production of lactic acid from hemicellulose extracts by Bacillus coagulans MXL-9. J Ind Microbiol Biotechnol. 37: 823–830.

6 Palaniraj R, P. Nagarajan (2012). Statistical analysis of experimental variables for the production of lactic acid using *lactobacillus casei* from waste potato starch by box-behnken design. Int. J. ChemTech Res. 131 4(2): 1 - 40 (Article in Press)

7 Patel M A, Ou M S, Harbrucker R, Aldrich H C, Buszko M L, Ingram L O, Shanmugam K T (2006). Isolation and characterization of acid-tolerant, thermophilic bacteria for effective fermentation of biomass-derived sugars to lactic acid. Appl. Environ. Microbiol. 72: 3228–3235.

8 Ilmen M, Koivuranta K, Ruohonen L, Suominen P, Penttila M (2007). Efficient production of L-lactic acid from xylose by Pichia stipitis. Appl. Environ. Microbiol. 73: 117–123.

9 Ohkouchi Y, Inoue Y (2006). Direct production of L-lactic acid from starch and food wastes using Lactobacillus manihotivorans LMG 18011. Bioresour. Technol. 97: 1554–1562.

10 Romani A, Yanez R, Garrote G, Alonso J L (2008). SSF production of lactic acid from cellulosic biosludges. Bioresour. Technol. 99: 4247–4254.

11 Wang Q, D Zou, H Ma, Y Ji, X. Wangc (2010). Simultaneous Saccharification and Fermentation of Corn Straw to Lactic Acid. Chem. Biochem. Eng. Q. 24 (3): 371–376.

12 Vaccari G, Gonalez-Varay R A, Campi A L, Dosi E, Brigidi P, Matteuzzi D (1993). Fermentative production of L-lactic acid by *Lactobacillus casei* DSM 20011 and product recovery using ion exchange resins. Appl Microbiol Biotechnol. 40: 23-27.

13 Chooklin S, L. Kaewsichan, J. Kaewsrichan (2011). Potential Use of *Lactobacillus casei* TISTR 1500 for the Bioconversion of Palmyra Sap to Lactic acid. Journal of Sustainable Energy & Environment. 2: 83-87

14 Cai H, Thompson R, Budinich MF, Broadbent, JR, Steele JL (2009). Genome sequence and comparative genome analysis of *Lactobacillus casei*: insights into their niche-associated evolution., Genome Biol Evol. 1: 239-257.

15 Kinoshita H, Uchida H, Kawai Y, Kitazawa H, Miura K, Shiba K, Horii A, Saito T (2007). Quantitative evaluation of adhesion of *Lactobacilli* isolated from human intestinal tissue to human colonic mucin using surface plasmon resonance. J Appl Microbiol. 102: 116-123.

16 Bruno Barcena JM, Ragout AL, Cordoba PR, Sineriz F (1999). Continuous production of L(+)-lactic acid by *Lactobacillus casei* in two-stage systems. Appl Microbiol Biotechnol. 51: 316-324.

17 Rojan P J, Sukumaran R K, Nampoothiri K M, Pandey A (2007). Statistical optimization of simultaneous saccharification and l(+)-lactic acid fermentation from cassava bagasse using mixed culture of lactobacilli by response surface methodology. Biochem. Eng. J. 36 (3), 262–267.

18 Barker S B, Summerson W H (1941). The colorimetric determination of lactic acid in biological materials. J. Biol. Chem. 138: 535 – 554.

19 Miller G L (1959). Use of dinitrosaliclic acid reagent for determination of reducing suger, Anal. Chem, 1959, 31, 426 – 429.

20 Nampoothiri K M, Singhania R R, Sabarinath C, Pandey A (2003). Fermentative production of gellan using *Sphingomonas paucimobillis.* Proc. Biochem. 38: 1513 – 1519.

21 Amrane A, Prigent Y (1997). Growth and lactic acid production coupling for *Lactobacillus helveticus* cultivated on supplemented whey: influence of peptidic nitrogen deficiency. J. Biotechnol. 55: 1-8.

22 Bailey J E, Ollis D F (1986). Biochemical Engineering Fundamentals. McGraw-Hill, Inc., USA. p.385.

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