



## Evaluation of Cell Growth and Substrate Consumption Kinetic of Five Different *Lactobacilli* in a Submerged Batch Whey Culture for Lactic Acid Production

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### ABSTRACT

Cell growth and lactose consumption profile of five *Lactobacilli*: *bulgaricus*, *casei*, *lactis*, *delbrueckii* and *fermentum* were investigated. Cell growth and substrate utilization in a batch submerged culture of whey with added lactose were evaluated. Fitness assessment of experimental data on the cell growth and lactose consumption by Monod and Logistic kinetic models was performed using the curve-fitting tool in Matlab software. *Lactobacillus delbrueckii* subsp. *bulgaricus* PTCC1737 and *Lactobacillus delbrueckii* subsp. *lactis* PTCC 1743 didn't fit with any of the two studied kinetic models. *Lactobacillus casei* subsp. *casei* PTCC1608 showed good consistency with Monod and not acceptable fitting with Logistic kinetic model. *Lactobacillus delbrueckii* subsp. *delbrueckii* PTCC1333 and *Lactobacillus fermentum* PTCC1744 had acceptable consistency with both studied models. *Lactobacillus casei* subsp. *casei* PTCC1608 showed the most consistency following Monod equation with  $R^2$ ,  $\mu_{max}$  and  $K_s$  of 0.965, 0.435 h<sup>-1</sup> and 27.05 g L<sup>-1</sup>, respectively. *Lactobacillus delbrueckii* subsp. *delbrueckii* PTCC1333 ( $R^2=0.926$ ) had the most desired capability with Logistic equation with  $\mu_{max}$  and  $X_{max}$  evaluated as 0.242 h<sup>-1</sup> and 4.84 g L<sup>-1</sup>, respectively.

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## 1. INTRODUCTION<sup>1</sup>

Lactic acid is a widely used natural organic acid in food, chemical and pharmaceutical industries. Lactic acid has two optical active forms, the D (-), levorotatory, and the L (+), dextrorotatory one. Both isomers exist in biological systems, but its esters and salts are levorotatory. Lactic acid can be obtained either from chemical synthesis or fermentation processes. Chemically synthesized lactic acid is usually a racemic mixture of two isomers. Fermentative processes are selective to produce pure isomers [1]. Poly lactic acid polymers also used as feedstock for production of biodegradable plastics. These are suitable alternative for synthetic polymers in food packaging and

pharmaceutical applications such as surgical sutures, controlled release of drugs and prostheses [2, 3].

Lactic acid bacteria refer to a large group of beneficial bacteria with similar properties, able to produce lactic acid as an end product of fermentation [4]. *Lactobacillus* is a genus of Gram-positive facultative anaerobic or microaerophilic rod-shape bacteria. They are a major part of the lactic acid bacteria which convert lactose and other sugars to lactic acid. According to metabolism, *Lactobacillus* species can be divided into three groups: obligate homo-fermentative, facultative hetero-fermentative and obligate hetero-fermentative. Some facts on *Lactobacilli* kinetic behavior are reported and also details about growth kinetics are discussed. Growth, substrate utilization and lactic acid production by *L. plantarum* NCDC 414 in juices of bitter melon (*Momordica charantia*), bottle gourd (*Lagenaria siceraria*) and carrot (*Daucus carota*) were studied using unstructured Gompertz and Logistic

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models. The viable cell counts increased from  $4 \times 10^5$  to  $7 \times 10^{10}$  CFU mL<sup>-1</sup> after 24 h of incubation. The lactic acid concentration increased by about 4.5 times in 24 h and about 44% w/v reduction in sugar content during growth of *L. plantarum* [5] was observed. The biosorption property of *Saccharomyces cerevisiae* for chromium uptake was also investigated in an immobilized cell bioreactor [6]. The kinetics of biomass and lactic acid production as well as substrate consumption of *L. casei* var. *rhamnosus* cultured in whey was investigated. Results showed a strong exponentially product dependent inhibition resulting in low lactic acid concentrations [7]. A kinetic model was developed that describes growth and lactic acid production rates of *L. helveticus* in pH-controlled batch cultures in whey permeate-yeast extract medium as a function of four variables: sugar, nitrogen substrate and lactic acid concentrations, and pH [8]. Kinetic model and effects of pH, substrate, and oxygen in lactic acid production from lactose by *L. plantarum* was studied. Lactose slightly inhibited cell growth in the exponential growth phase but there were no effects on the stationary and death phases [9]. In another research, the effect of pH on growth and carbohydrate metabolism of *L. fermentum* IMDO 130101 and non-equilibrium thermodynamic model of the temperature dependent biological growth of living systems were also investigated [10, 11]. Some other works studied kinetics of fermentative microbial processes such as glucoamylase [12], chitosan [13] and xylanase production by *Aspergillus niger* [14].

In this article, kinetic behavior of five different *Lactobacilli* was investigated based on Monod and Logistic kinetic models. Some important kinetic parameters were determined and the consistency of the cell growth and substrate uptake behavior with the noted equations was identified using Matlab software in the form of statistical parameters.

## 2. MATERIALS AND METHODS

### 2. 1. Microorganisms and Inoculum Preparation

*Lactobacillus* species were prepared from Iranian Research Organization for Science and Technology. The species were: *Lactobacillus casei* subsp. *casei* PTCC1608, *Lactobacillus delbrueckii* subsp. *delbrueckii* PTCC1333, *Lactobacillus delbrueckii* subsp. *bulgaricus* PTCC1737, *Lactobacillus fermentum* PTCC1744 and *Lactobacillus delbrueckii* subsp. *lactis* PTCC 1743. The separated stock culture of each strain was prepared on MRS culture at 37°C for 48 h and then stored in a refrigerator at 4°C. Fermentation process was performed in separate laboratory shaking flasks containing deproteinized sterile whey as a batch submerged culture. The culture media was enriched with some nutrients including (g L<sup>-1</sup>): lactose, 50; yeast

extract, 10; sodium acetate, 5; KH<sub>2</sub>PO<sub>4</sub>, 2; MgSO<sub>4</sub>, 0.2; MnSO<sub>4</sub>, 0.05; FeSO<sub>4</sub>, 0.03 and peptone, 10.

### 2. 2. Culture Preparation

The media for batch fermentation process in a 250 mL shaking flask was 100 mL of deproteinized and enriched sterile whey. Before autoclaving, the medium pH was adjusted to 6.5 using a solution of 2M NaOH or 2N HCl. To prevent undesired reactions, deproteinized whey and the enrichment medium were autoclaved separately at 121°C for 15 min and then mixed together under sterile conditions to obtained 100 mL final culture medium in each flask.

### 2. 3. Batch Submerged Fermentation

2.5 mL of each *Lactobacillus* inoculum was inoculated into 100 mL of prepared and sterilized culture medium in each flask and the flasks were incubated in an incubator shaker at 37°C with 150 rpm agitation speed for 50 h. At this period, samples were removed at proper 5 h intervals to assay lactic acid, lactose and cell dry weight concentrations [15].

### 2. 4. Analytical Methods

Cell dry weight was assayed using a spectrophotometer (Shimadzu, 1601, Japan) at a wavelength of 480 nm. Standard dilute solutions of bacterial cell were prepared from stationary phase of cell growth. To determine cell dry weight calibration curve, 15 mL of each standard sample was passed through a cellulose acetate filter with 0.45 micron pore size. Filters were then washed with distilled water and dried at 100°C for 24 h. Cell dry weight was calculated based on the difference between the initial and the final filter weights. For each *Lactobacillus* species, a separate standard curve of cell dry weight versus adsorption value was recorded and applied to determine cell dry weight in actual experimental samples.

Lactic acid and lactose concentrations were analyzed by high performance liquid chromatography (HPLC, Perkin Elmer 200, Shimadzu, Japan) with AminexHEX-87H column. The mobile phase consisted of 5 mM sulfuric acid solution at 40°C and used at a flow rate of 0.6 mL min<sup>-1</sup> [16].

### 2. 5. Kinetic Model

Monod (Equation (1)) and Logistic (Equation (2)) equations are un-structured kinetic models based on substrate and biomass concentration, respectively, which were chosen for two parameters modeling.

$$\mu = \mu_{\max} \frac{S}{K_s + S} \quad (1)$$

$$\mu = \mu_{\max} \left[ 1 - \frac{X}{X_m} \right] \quad (2)$$

where  $\mu$ ,  $\mu_{\max}$ ,  $K_s$ ,  $S$ ,  $X$  and  $X_m$  are the specific growth rate, the maximum specific growth rate of bacterial strain, the semi-saturated coefficient, the limiting substrate (lactose) concentration, cell dry weight and the maximum cell dry weight with dimensions of  $\text{h}^{-1}$ ,  $\text{h}^{-1}$ ,  $\text{g L}^{-1}$ ,  $\text{g L}^{-1}$ ,  $\text{g L}^{-1}$  and  $\text{g L}^{-1}$ , respectively.

### 3. RESULTS AND DISCUSSION

#### 3.1. Cell growth, Lactose Consumption and Lactic Acid Production

Growth behavior of five different species of *Lactobacilli* for incubation period of 50 h was evaluated. During the course of fermentation, samples were taken at proper time intervals. The samples were analyzed for lactose consumption, lactic acid and cell dry weight. For all evaluated species of *Lactobacilli*, for *L. casei* subsp. *casei* PTCC1608, 79% of lactose content in the medium was consumed in the first 30 hours of incubation (lag and exponential growth phase). *L. delbrueckii* subsp. *bulgaricus* PTCC1737 consumed 85.4% of lactose in the same period. This value for *L. delbrueckii* subsp. *delbrueckii* PTCC1333, *L. delbrueckii* subsp. *lactis* PTCC 1743 and *L. fermentum* PTCC1744 was 63, 60.8 and 46.4%, respectively. While at stationary phase appeared, lactose concentration had limited changes and for instance the concentration of lactose for *L. casei* subsp. *casei* PTCC1608 slightly decreased from 10.5 to 8  $\text{g L}^{-1}$  in a 22 h time interval. In the other word, at stationary phase of cell growth profile, the consumed lactose was only 5% of initial lactose. This value for *L. delbrueckii* subsp. *bulgaricus* PTCC1737, *L. delbrueckii* subsp. *delbrueckii* PTCC1333, *L. delbrueckii* subsp. *lactis* PTCC 1743 and *L. fermentum* PTCC1744 was 3, 11.6, 12.8 and 13.4%, respectively.

For all investigated strains, an average lag phase of 5 h was observed. After normal lag phase, the exponential growth phase started and continued until 25 or even 45 h which depends on the strain of organism. An average of stationary phase was 30 h.

Maximum cell dry weight for *L. casei* subsp. *casei* PTCC1608, *L. delbrueckii* subsp. *bulgaricus* PTCC1737, *L. delbrueckii* subsp. *delbrueckii* PTCC1333, *L. delbrueckii* subsp. *lactis* PTCC 1743 and *L. fermentum* PTCC1744 was 4.3  $\text{g L}^{-1}$  in 36 h, 5.1  $\text{g L}^{-1}$  in 30 h, 3.2  $\text{g L}^{-1}$  in 42 h, 3.9  $\text{g L}^{-1}$  in 48 h and 3.5  $\text{g L}^{-1}$  in 52 h, respectively. A marvelous result is that the exponential growth phase of *L. delbrueckii* subsp. *bulgaricus* PTCC1737 was completed in 30 h of incubation while at the same conditions, the exponential growth phase of *L. fermentum* PTCC1744 lasted to 54 h of incubation. This suggests that special growth rate of *L. fermentum* PTCC1744 is significantly less than *L. delbrueckii* subsp. *bulgaricus* PTCC1737. In the case of lactic acid production, considerable differences among five different *Lactobacillus* species were determined.

While with *L. delbrueckii* subsp. *bulgaricus* PTCC1737 the obtained product yield was 0.602 (g lactic acid/ g consumed lactose), the yield for *L. delbrueckii* subsp. *delbrueckii* PTCC1333 was only 0.351 (g produced lactic acid/ g consumed lactose). Actually, the yield of lactic acid production of *L. delbrueckii* is 42% less than *L. delbrueckii* subsp. *bulgaricus*. Lactic acid production yields for *L. casei* subsp. *casei* PTCC1608, *L. delbrueckii* subsp. *lactis* PTCC 1743 and *L. fermentum* PTCC1744 were 0.586, 0.437 and 0.358, respectively. Berry et al. [17] investigated the growth and lactic acid production in batch culture of *L. rhamnosus* ATCC10863 in a defined medium and achieved a yield of 0.84 (g lactic acid/ g consumed substrate). Bustos et al. [18] found a production yield of 0.77 (g lactic acid/ g consumed substrate) from vine-trimming wastes by *L. pentosus* ATCC8041. The obtained yield was relatively less than the reported values; therefore, the quality of substrate and selection of species of organism may influence the yield. The main reason may be due to existence of high mineral concentration in the whey. Figure 1 shows the concentration profiles of lactose for five species of *Lactobacillus*. Maximum lactose consumption also belongs to *L. bulgaricus*.

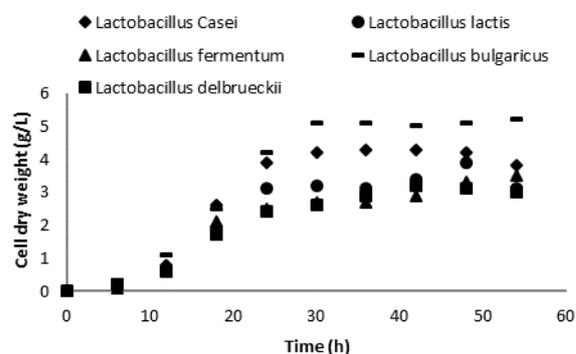


Figure 1. Comparative cell dry weight profiles for five species of *Lactobacillus* in a batch culture of whey at 37°C with 170 rpm agitation speed

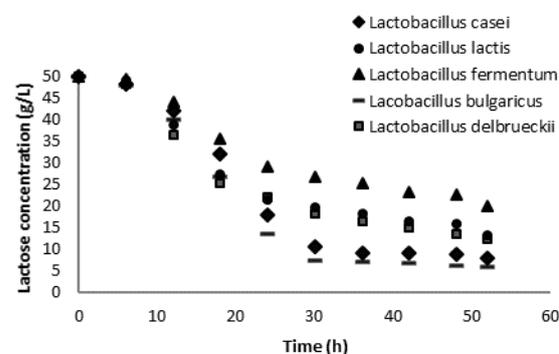


Figure 2. Comparative lactose concentration profiles for five species of *Lactobacillus* in a batch culture of whey at 37°C with 170 rpm agitation speed

**3. 2. Monod Kinetic** In case of kinetic data investigation, experimental data on lactose and biomass concentrations were used to determine suitable kinetic model for the exponential growth phase of *Lactobacilli* in batch culture. Figure 2 illustrates growth curves of 5 different species of *Lactobacillus*; maximum cell dry weight is devoted to *L. bulgaricus*. The exponential phase of growth curve of *Lactobacillus* species in a batch culture is defined by Malthus law as stated below:

$$\frac{dX}{dt} = \mu X \quad (3)$$

Applying separation of variables in Equation (3); then, integration using suitable initial condition ( $X=X_0$  at  $t=t_0$ ) resulted in the following equation:

$$\mu = \frac{\ln\left(\frac{X}{X_0}\right)}{t - t_0} \quad (4)$$

**TABLE 1.** Experimental data on cell growth and nutrient utilization and calculated values for specific growth rate in exponential growth phase of *L. delbrueckii* subsp. *bulgaricus* PTCC1737 in a batch culture

Time (h)	X (g. L <sup>-1</sup> )	S <sub>ave</sub> (g. L <sup>-1</sup> )	1/S <sub>ave</sub> (L. g <sup>-1</sup> )	μ (h <sup>-1</sup> )	1/μ (h)
0	0	48.9	0.02	0	0
6	0.2	45.7	0.022	0	0
9	0.5	41.8	0.024	0.305	3.278
12	0.9	37.1	0.027	0.250	4
15	1.6	30.35	0.033	0.231	4.329
18	2.5	23.5	0.042	0.210	4.762
21	3.4	16.95	0.059	0.189	5.291
24	4.2	15.15	0.066	0.169	5.917
27	4.8	12.05	0.083	0.151	6.622
30	5.1	7.15	0.140	0.135	7.407

**TABLE 2.** Experimental data on cell growth and nutrient utilization and calculated values for specific growth rate in exponential growth phase of *L. casei* subsp. *casei* PTCC1608 in a batch culture

Time (h)	X (g. L <sup>-1</sup> )	S <sub>ave</sub> (g. L <sup>-1</sup> )	1/S <sub>ave</sub> (L. g <sup>-1</sup> )	μ (h <sup>-1</sup> )	1/μ (h)
0	0	49.1	0.02	-	-
6	0.2	46.6	0.021	-	-
9	0.5	43.6	0.023	0.305	3.279
12	0.8	39.3	0.025	0.231	4.329
15	1.8	34.2	0.029	0.244	4.098
18	2.6	28.1	0.035	0.214	4.673
21	3.2	21	0.048	0.185	5.405
24	3.9	15.9	0.063	0.165	6.061
27	4	12.3	0.081	0.143	6.993
30	4.2	10.1	0.099	0.127	7.874
33	4.2	9.5	0.105	0.113	8.849
36	4.3	9.2	0.109	0.102	9.804

**TABLE 3.** Experimental data on cell growth and nutrient utilization and calculated values for specific growth rate in exponential growth phase of *L. delbrueckii* subsp. *lactis* PTCC 1743 in a batch culture

Time (h)	X (g. L <sup>-1</sup> )	S <sub>ave</sub> (g. L <sup>-1</sup> )	1/S <sub>ave</sub> (L. g <sup>-1</sup> )	μ (h <sup>-1</sup> )	1/μ (h)
0	0	49.6	0.020	-	-
6	0.2	46.8	0.021	0	-
9	0.4	41.6	0.024	0.231	4.329
12	0.6	36	0.028	0.183	5.464
15	1.2	30.3	0.033	0.199	5.025
18	1.9	25.9	0.039	0.188	5.319
21	2.5	23	0.043	0.168	5.952
24	3.1	20.7	0.048	0.152	6.579
27	3.2	19.8	0.050	0.132	7.576
30	3.2	19.2	0.052	0.115	8.696
33	3.1	18.5	0.054	0.101	9.901
36	3.1	17.8	0.056	0.091	10.989
39	3.3	16.9	0.059	0.085	11.765
42	3.4	16.2	0.062	0.079	12.658
45	3.6	15.9	0.063	0.074	13.513
48	3.9	15.8	0.063	0.071	14.084

**TABLE 4.** Experimental data on cell growth and nutrient utilization and calculated values for specific growth rate in exponential growth phase of *L. delbrueckii* subsp. *delbrueckii* PTCC1333 in a batch culture

Time (h)	X (g. L <sup>-1</sup> )	S <sub>ave</sub> (g. L <sup>-1</sup> )	1/S <sub>ave</sub> (L. g <sup>-1</sup> )	μ (h <sup>-1</sup> )	1/μ (h)
0	0	49.05	0.020	-	-
6	0.2	44.9	0.022	0	-
9	0.4	39.05	0.026	0.231	4.329
12	0.6	33.75	0.030	0.183	5.464
15	1.1	28.2	0.035	0.189	5.291
18	1.7	24.55	0.041	0.178	5.618
21	2	22.95	0.043	0.153	6.536
24	2.4	20.85	0.048	0.138	7.246
27	2.5	18.9	0.053	0.120	8.333
30	2.6	17.75	0.056	0.107	9.346
33	2.7	16.85	0.059	0.096	10.417
36	2.9	16.15	0.062	0.089	11.236
39	3.1	15.5	0.064	0.083	12.048
42	3.2	15.1	0.066	0.077	12.987

Kinetic constant coefficients ( $\mu_{max}$ ,  $K_s$ ) were determined using the curve fitting method. Specific cell growth rate values were calculated according to cell dry weight as biomass concentration ( $X$ ) and average lactose concentration as limiting substrate concentration ( $S_{ave}$ ) for the exponential growth phase. Experimental and calculated values are summarized in Tables 1 to 5 for five different species of *Lactobacillus*. Specific cell growth rate was calculated by Equation (4). The values for initial biomass concentration and lag phase time delay ( $X_0$  and  $t_0$ ) were considered 0.2 g L<sup>-1</sup> and 6 h, respectively.

**TABLE 5.** Experimental data on cell growth and nutrient utilization and calculated values for specific growth rate in exponential growth phase of *L. fermentum* PTCC1744 in a batch culture

Time (h)	X (g. L <sup>-1</sup> )	S <sub>ave</sub> (g. L <sup>-1</sup> )	1/S <sub>ave</sub> (L. g <sup>-1</sup> )	μ (h <sup>-1</sup> )	1/μ (h)
0	0	49.65	0.020	-	-
6	0.1	47.9	0.021	0	-
9	0.3	45.35	0.022	0.366	2.732
12	0.7	42.05	0.024	0.324	3.086
15	1.6	37.75	0.026	0.308	3.247
18	2.1	33.75	0.030	0.254	3.937
21	2.3	30.5	0.033	0.209	4.784
24	2.5	28.6	0.035	0.179	5.586
27	2.6	27.45	0.036	0.155	6.452
30	2.7	26.4	0.038	0.137	7.299
33	2.6	25.65	0.039	0.121	8.264
36	2.7	24.9	0.040	0.110	9.091
39	2.8	23.85	0.042	0.101	9.901
42	2.9	23.05	0.043	0.093	10.753
45	3.2	22.75	0.044	0.089	11.236
48	3.3	21.35	0.047	0.083	12.048
52	3.5	20.1	0.050	0.077	12.987

**TABLE 6.** A comparison of Monod kinetic constants for five different species of *Lactobacilli*

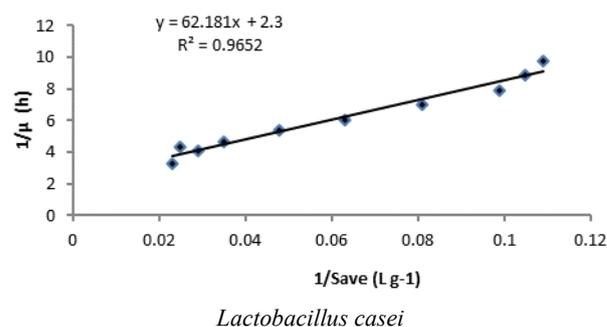
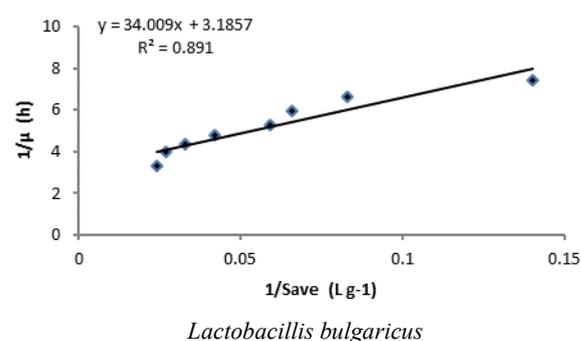
Strain	R <sup>2</sup>	μ <sub>max</sub> (h <sup>-1</sup> )	K <sub>s</sub> (g. L <sup>-1</sup> )
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> PTCC1737	0.891	0.314	10.68
<i>L. casei</i> subsp. <i>casei</i> PTCC1608	0.965	0.435	27.05
<i>L. delbrueckii</i> subsp. <i>lactis</i> PTCC 1743	0.857	0.331	80.6
<i>L. delbrueckii</i> subsp. <i>delbrueckii</i> PTCC1333	0.931	0.535	111.22
<i>L. fermentum</i> PTCC1744	0.951	0.134	54.46

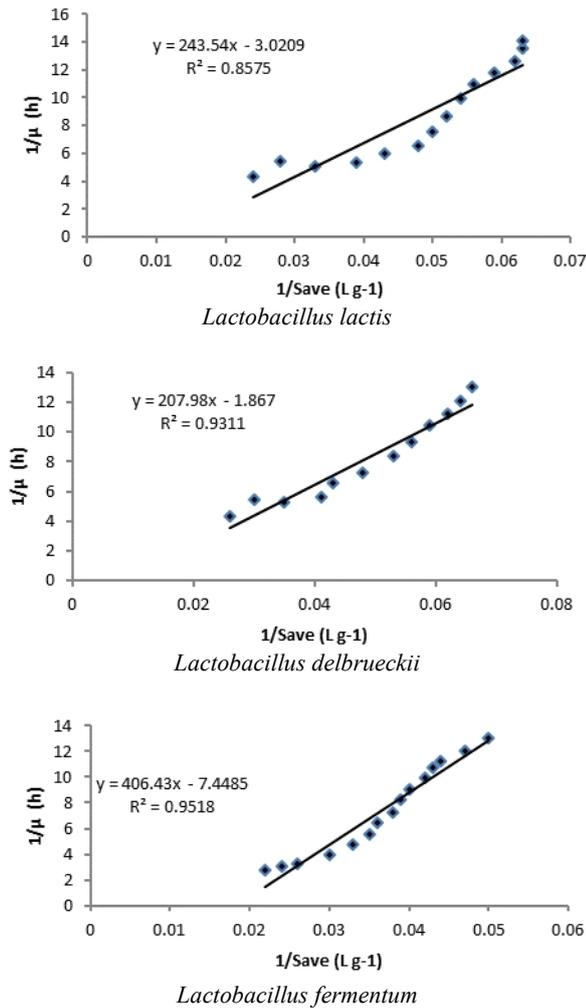
Line weaver-Burk linear plot for fitting experimental data for the growth of *Lactobacillus* species using Monod kinetic model is presented in Figure 3. With regard to obtained R-square values, *L. casei* subsp. *casei* PTCC1608 (R<sup>2</sup>=0.965) showed the most desired capability with Monod equation among the investigated strains. For this strain, maximum specific cell growth rate (μ<sub>max</sub>) and Monod semi-saturated coefficient (K<sub>s</sub>) were obtained as 0.435 h<sup>-1</sup> and 27.05 g L<sup>-1</sup>, respectively (Table 6). *L. delbrueckii* subsp. *delbrueckii* PTCC1333 had the highest μ<sub>max</sub> equal to 0.535 h<sup>-1</sup> based on the curve-fitting results, indicating suitable cell growth rate at the applied conditions (Table 6). On the other hand, with a good consistency (R<sup>2</sup>=0.931), its K<sub>s</sub> parameter was too high (111.12 g L<sup>-1</sup>). Thus, Monod kinetic model isn't a good desired model to describe the cell growth and substrate consumption behavior of that particular strain. *L. fermentum* PTCC1744 also showed good consistency with Monod kinetic model (R<sup>2</sup>= 0.951). Its

μ<sub>max</sub> and K<sub>s</sub> values were 0.134 h<sup>-1</sup> and 54.46 g L<sup>-1</sup>, respectively (Table 6).

Results showed that the experimental data of the cell growth and substrate consumption in batch submerged cultures of *L. delbrueckii* subsp. *bulgaricus* PTCC1737 (R<sup>2</sup>= 0.891) and *L. delbrueckii* subsp. *lactis* PTCC 1743 (R<sup>2</sup>=0.857) did not have an acceptable agreement with Monod kinetic model compared to three mentioned strains. Maximum specific growth rate (μ<sub>max</sub>) for these two strains were 0.314 and 0.331 h<sup>-1</sup>, respectively.

Vasudha and Hari [5] studied the unstructured Gompertz and Logistic kinetic modeling of growth and lactic acid production by *L. plantarum* NDCD 414 for the fermentation of vegetable juices. After 24 h of incubation, the viable cell counts increased from 4 × 10<sup>5</sup> to 7 × 10<sup>10</sup> CFU mL<sup>-1</sup>. The lactic acid concentration also increased by about 4.5 folds in 24 h and about 44% w/v reduction in sugar consumption was observed during growth of *L. plantarum* [5]. In this work, significant lactic acid production occurred for the period of growth and stationary phases. In addition, the cell dry weight increased by about 10-15 folds in 24 h. Alvarez et al. [7] characterized the kinetics of biomass production, lactic acid production and substrate consumption of *L. casei* var. *rhamnosus* cultured in deproteinized milk whey. Their results showed a strong exponentially dependent product inhibition affected at low lactic acid concentrations. They found that lactic acid production rate was partially associated with biomass growth [7].





**Figure 3.** The Line weaver-Burk linear plot for  $\frac{1}{\mu}$  versus  $\frac{1}{S-S_0}$  to fit the experimental data of substrate utilization and cell growth to Monod kinetic model for five studied *Lactobacillus* in a submerged batch culture medium

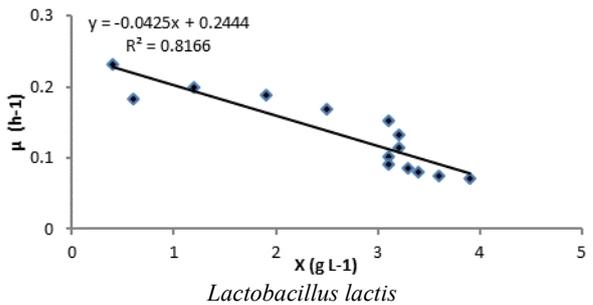
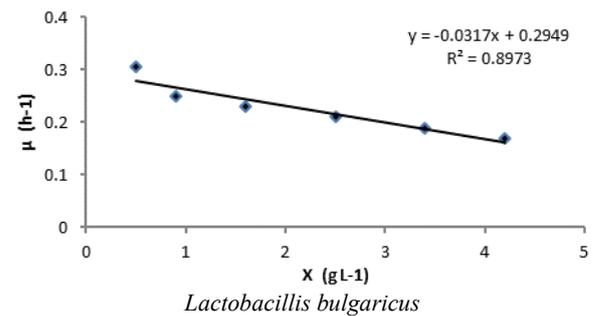
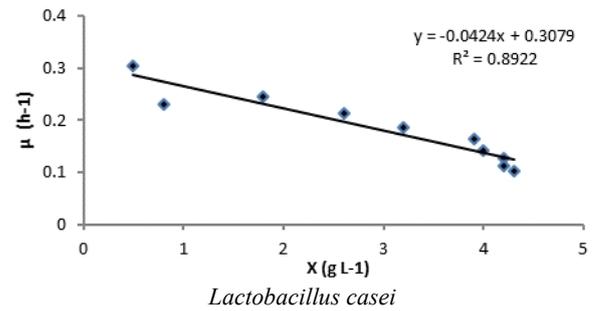
**3. 3. Logistic Model**

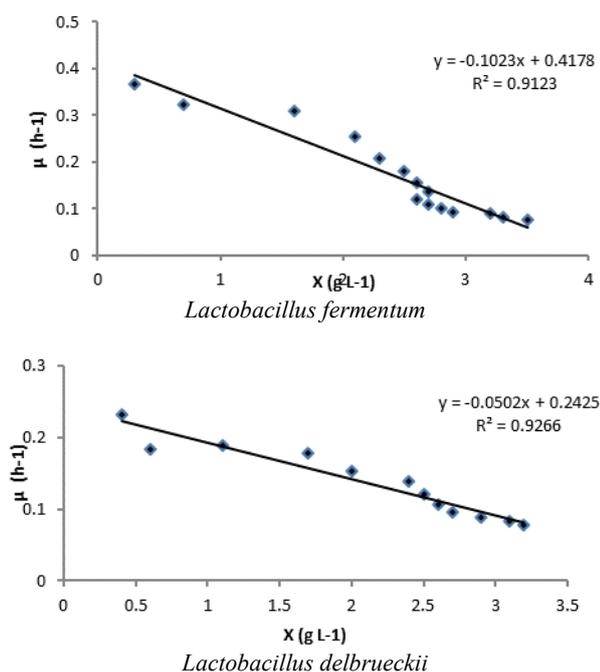
Figure 4 presents Line weaver-Burk linear plot for fitting experimental data for the growth of *Lactobacillus* species using Logistic kinetic model. R-square values showed, *L. delbrueckii* subsp. *delbrueckii* PTCC1333 ( $R^2=0.926$ ) had the most desired agreement with Logistic equation among the investigated strains. For this strain, maximum specific cell growth rate ( $\mu_{max}$ ) and maximum cell dry weight were evaluated as  $0.242 \text{ h}^{-1}$  and  $4.84 \text{ g L}^{-1}$ , respectively (Table 7). *L. fermentum* PTCC1744 with the highest  $\mu_{max}$  equal to  $0.417 \text{ h}^{-1}$  and a good consistency ( $R^2=0.912$ ), showed an acceptable consistency with Logistic equation (Table 7). Based on the results, Logistic kinetic model isn't a good desired model to describe the cell growth and substrate consumption behavior of *L. delbrueckii* subsp. *bulgaricus* PTCC1737, *L. casei* subsp. *casei* PTCC1608 and *L. delbrueckii* subsp. *lactis* PTCC 1743. While, *L.*

*delbrueckii* subsp. *bulgaricus* PTCC1737 had higher  $X_{max}$  ( $9.48 \text{ g L}^{-1}$ ) compared to other investigated strains (Table 7). *L. delbrueckii* subsp. *bulgaricus* PTCC1737 and *L. delbrueckii* subsp. *lactis* PTCC 1743 didn't fit with any of the two studied kinetic models. *L. casei* subsp. *casei* PTCC1608 showed good consistency with Monod and not acceptable fitting with Logistic kinetic model. *L. delbrueckii* subsp. *delbrueckii* PTCC1333 and *L. fermentum* PTCC1744 had acceptable consistency with the both studied models.

**TABLE 7.** A comparison of Logistic kinetic constants for five different species of *Lactobacilli*

Strain	R <sup>2</sup>	$\mu_{max}$ (h <sup>-1</sup> )	X <sub>m</sub> (g·L <sup>-1</sup> )
<i>L. delbrueckii</i> subsp. <i>bulgaricus</i> PTCC1737	0.897	0.294	9.48
<i>L. casei</i> subsp. <i>casei</i> PTCC1608	0.892	0.307	7.31
<i>L. delbrueckii</i> subsp. <i>lactis</i> PTCC 1743	0.816	0.244	5.81
<i>L. delbrueckii</i> subsp. <i>delbrueckii</i> PTCC1333	0.926	0.242	4.84
<i>L. fermentum</i> PTCC1744	0.912	0.417	4.10





**Figure 4.** The Line weaver-Burk linear plot for  $\mu$  versus X to fit the experimental data of substrate utilization and cell growth to Logistic kinetic model for five studied *Lactobacillus* in a submerged batch culture medium

#### 4. CONCLUSION

This is the first report on the cell growth and substrate utilization kinetic of *Lactobacillus* with respect to Monod and Logistic kinetic models. *L. delbrueckii* subsp. *bulgaricus* PTCC1737 and *L. delbrueckii* subsp. *lactis* PTCC 1743 didn't fit with any of the two studied kinetic models. *L. casei* subsp. *casei* PTCC1608 showed good consistency with Monod and not acceptable fitting with Logistic kinetic model. *L. delbrueckii* subsp. *delbrueckii* PTCC1333 and *L. fermentum* PTCC1744 had acceptable consistency with both studied models.

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## Evaluation of Cell Growth and Substrate Consumption Kinetic of Five Different *Lactobacilli* in a Submerged Batch Whey Culture for Lactic Acid Production

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روند رشد سلول و مصرف سوبسترا برای پنج سویه مختلف از لاکتوباسیلوس شامل سویه‌های بولگاریکوس، کازئی، لاکتیس، دلبروکی و فرمنتوم بررسی شده است. رشد سلول و مصرف سوبسترا بر روی محیط کشت آب‌پنیر با لاکتوز در سیستم ناپیوسته آزمون شده است. داده‌های تجربی با استفاده از ابزار برازش منحنی در نرم‌افزار مت‌لب با مدل‌های سینتیکی مونود و لجستیک مطابقت داده شدند. لاکتوباسیلوس بولگاریکوس و لاکتوباسیلوس لاکتیس با هیچ کدام از دو مدل مطابقت نداشتند. لاکتوباسیلوس کازئی با مدل مونود مطابقت خوبی داشته اما با مدل لجستیک سازگار نبوده است. لاکتوباسیلوس دلبروکی و لاکتوباسیلوس فرمنتوم با هر دو مدل مطابقت داشته اند. لاکتوباسیلوس کازئی با رگرسیون، بیشینه شدت رشد ویژه و ثابت اشباع مونود به ترتیب برابر با ۰/۹۶۵، ۰/۴۳۵ بر ساعت و ۲۷/۰۵ گرم در لیتر، بیشترین تطابق را با مدل سینتیکی مونود نشان داده است. لاکتوباسیلوس دلبروکی با رگرسیون ۰/۹۲۶ در بین سویه های بررسی شده، بیشترین مطابقت را با مدل لجستیک با بیشینه شدت رشد ویژه و بیشینه وزن خشک سلولی به ترتیب برابر با ۰/۲۴۲ بر ساعت و ۴/۸۴ گرم در لیتر داشته است.

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