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Fermentative Lactic Acid Production by *Lactobacilli*: Moser and Gompertz Kinetic Models

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ABSTRACT: Lactic acid production in a batch submerged fermentation process by five *Lactobacilli*: *bulgaricus*, *casei*, *lactis*, *delbrueckii* and *fermentum* in lactose fortified whey culture were investigated. Kinetic behavior of *Lactobacilli* growth rate and lactose utilization was studied based on the Moser and Gompertz kinetic models. Trendline tool in Excel software was applied for fitness assessment of the experimental data to investigate the kinetic models. *Lb. bulgaricus* had shown the best cell production yield of 0.119 $g.g^{-1}$ of consumed lactose. Also, maximum lactic acid production yield of 0.602 $g.g^{-1}$of consumed lactose was obtained by *Lb. bulgaricus*. *Lb. bulgaricus* (R^2=0.954, μ_{max}=0.5 and K_s=9.385) and *Lb. casei* (R^2=0.956, μ_{max}=0.580 and K_s=18.2) have shown acceptable consistency with Moser kinetic model. Moser kinetic model isn't a desired model to describe the cell growth and substrate consumption behavior of *Lb. fermentum*, *Lb. delbrueckii* and *Lb. lactis*. None of the investigated strains have shown acceptable consistency with Gompertz kinetic model, therefore, this model isn't known as a good model to describe the cell growth and substrate utilization behavior of *Lactobacilli*.

Keywords: *Fermentation, Gompertz, Lactic acid, Lactobacilli, Moser.*

Introduction

Lactic acid is a main chemical substance with numerous roles in different biochemical and food industries. Lactic acid has two optical isomers. One is known as L-(+)-lactic acid and the other, D-(−)-lactic acid. In food industry, lactic acid is found in some products such as sour milk, koumiss, yogurt, kefir, some cottage cheeses and kombucha (Cock and Stouvenel, 2006). In recent years, poly lactic acid has been introduced as a biodegradable food packaging biopolymer. Lactic acid can be obtained from anaerobic fermentation of simple carbohydrates by lactic acid bacteria. *Lactobacillus* is a genus of gram-positive facultative anaerobic bacteria divided into three groups: obligate homo-fermentative, facultative hetero-fermentative and obligate hetero-fermentative (Wee *et al*., 2006). These groups of bacteria also known as probiotic organism are involved in functional foods (Korbekandi *et al*., 2015; Aghajani *et al*., 2012) with antagonistic activity against some food-borne disease bacteria such as *salmonella spp.*, *E. coli*, *Listeria monocytogenes* and *Clostridium. Perfringens* (El-Kholy *et al*., 2014). *Lactobacilli* have higher activity as compared to *bifidobacteria*; another one of the two main probiotic bacteria in fermented

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milk products (Moayednia & Mazaheri, 2011). Some of lactic acid bacteria such as *Lactobacillus plantarum* and *Lactobacillus reuteri* isolated from sourdough were able to hydrolyse phytic acid by phytase production. This is a valuable capability to be useful in bread processing technology (Didar & Haddad Khodaparast, 2011).

Kinetic behavior of *Lactobacilli* was studied by some previous researchers. Cock and Stouvenel (2006) evaluated lactic acid production by *Lactococcus lactis* subs *lactis* isolated from the leaves of sugar cane plants. Their findings demonstrate that up to 35 $g.L^{-1}$ lactic acid was obtained in fermentation at 32ºC, with 60 $g.L^{-1}$ of glucose and a pH of 6.0 (Cock and Stouvenel, 2006). Different *Lactobacilli* have been investigated for lactic acid production yield and productivity. Some reports are presented for lactic acid production yield and productivity as 0.91 $g.g^{-1}$ and 5.6 $g.L^{-1}h^{-1}$, respectively by *Lactobacillus casei* NRRL B-441 (Hujanen and Linko, 1996), also 0.96 $g.g^{-1}$ and 5.1 $g.L^{-1}h^{-1}$, respectively by *Enterococcus faecalis* RKY1 (Yun *et al*., 2003), as well as 0.77 $g.g^{-1}$ and 0.8 $g.L^{-1}h^{-1}$, respectively by *Lactobacillus pentosus* ATCC 8041 (Bustos *et al*., 2004). Amrane (2005) investigated the growth kinetic and lactic acid production for *Lactobacillus helveticus* on whey permeate. He characterized and described five separate phases during *Lb. helveticus* growth. *Lactobacillus plantarum* kinetic growth was studied by Gupta *et al*. (2011). The results showed that increasing the agitation speed raised the cell growth and decreased lactic acid production. Maximum lactic acid production 2.5 $g.L^{-1}$ was obtained in a relatively anaerobic process without any agitation (Gupta *et al*., 2011). Investigating the kinetics of *Lactobacillus plantarum* growth indicated that the presence of malic acid can increase the specific growth rate from 0.2 to 0.34 h^{-1} with maximum biomass production (Passos *et al*., 2003). In our knowledge, there isn't any documented reported on *Lactobacilli* kinetics with Moser and Gompertz kinetic models.

In this article, kinetic behavior, cell growth and substrate consumption trends of five different *Lactobacilli* have been investigated based on Moser and Gompertz models. In each case, key kinetic parameters were also determined.

Materials and Methods

- Lactobacilli and inoculums

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 were obtained from Iranian Research Organization for Science and Technology. MRS culture was applied for inoculum preparation at 37°C for 48 h.

- Lactic acid production

Anaerobic fermentation process was carried out in 250 mL shaking flask containing 100 mL of de-proteinized sterile whey enriched with ($g.L^{-1}$): lactose, 50; yeast extract, 10; sodium acetate, 5; KH_2PO_4, 2; $MgSO_4$, 0.2; $MnSO_4$, 0.05; $FeSO_4$, 0.03; and peptone, 10. Each sterile culture was inoculated with 2.5 mL bacterial inoculum and then incubated in a shaker incubator at 37°C with 50 rpm agitation speed for 50 hours.

- Cell dry weight

Cell dry weight was assayed using a spectrophotometer (Shimadzu, 1601, Japan) at a wavelength of 480 nm. Cell dry weight calibration curve was determined for each strain separately. 15 mL of each standard sample was passed through a cellulose acetate filter with 0.45 micron pore size. Washed filters were dried at 100°C for 24 h. Cell dry weight was calculated based on the difference between the initial and the final filter weights.

- Kinetic models

Moser (equation 1) and Gompertz (equation 2) equations were used for bacterial cell growth modeling.

$$\mu = \mu_{\max} \frac{S^n}{k_s + S^n} \qquad (1)$$

$$X(t) = K \exp(\log(\frac{X(0)}{K}) \exp(-\alpha t)) \qquad (2)$$

In equations and relations of kinetic models, μ and μ_{max} are the specific growth rate and the maximum specific growth rate of bacteria, respectively in term of h^{-1}, S is the limiting substrate (lactose) concentration in term of $g.L^{-1}$, K_s is the semi-saturated coefficient in term of $g.L^{-1}$ and X is the biomass concentration in term of $g.L^{-1}$. In Gompertz model, K is the carrying capacity, i.e. the maximum size that can be reached with the available nutrients and α is a constant related to the proliferative ability of the cells (same as μ_{max} in Moser model).

Results and Discussion

- Cell growth characteristics

Growth behavior of five different species of *Lactobacilli* for incubation period of 50 hours was evaluated. The average lag phase for the investigated strains was determined near to 5 hours and exponential growth phase was evaluated for 25 to 45 hours depending on the strain. All strains were stayed in the stationary phase for approximate 30 h. Table 1 presents maximum cell dry weight for all studied strains obtained at different incubation times. *Lb. bulgaricus* had shown the best cell production yield of 0.119 $g.g^{-1}$ of consumed lactose. In the second place, *Lb. fermentum* was evaluated with 0.117 g biomass production for each gram of utilized lactose. *Lb. lactis* cell production yield (0.114 $g.g^{-1}$ of consumed lactose) is approximate near to both above mentioned strains. Cell production yield for *Lb. caesi* and *Lb. delbrueckii* is 11.8% and 22.7% less than *Lb. bulgaricus*, respectively. *Lb. bulgaricus* also had the best cell productivity equal to 0.17 $g.L^{-1}h^{-1}$. This is 42% more than the recorded productivity for *Lb. casei*. Cell productivity of *Lb. delbrueckii*, *Lb. lactis* and *Lb. fermentum* is in order of 55.3%, 52.3% and 60.6% less than *Lb. bulgaricus*.

Hujanen and Linko (1996) obtained cell production yield and productivity of 0.91 $g.g^{-1}$ and 5.6 $g.L^{-1}h^{-1}$, respectively for *Lb. casei* NRRL B-441 (Hujanen and Linko, 1996) and Bustos *et al*., (2004) obtained yield of 0.77 $g.g^{-1}$ and productivity of 0.8 $g.L^{-1}h^{-1}$ for *Lb. pentosus* ATCC 8041 (Bustos *et al*., 2004). The yield for *Lb. bulgaricus* (the best growth strain in this research) was considerably less than the reported values; therefore, the quality of substrate and selection of species of organism might influence the yield. The main reason might be due to the existence of high mineral concentration in the whey. Cell productivity of *Lb. bulgaricus* was less than *Lb. casei* NRRL B-441 as reported by Hujanen and Linko (1996) and of course more than *Lb. pentosus* ATCC 8041 (Bustos *et al*., 2004).

Lactic acid production yield and productivity for five studied strains are presented in Table 2. Maximum lactic acid production yield of 0.602 $g.g^{-1}$of consumed lactose was obtained for *Lb. bulgaricus*. In the second place, *Lb. casei* was introduced with 0.586 g lactic acid production for each gram of consumed lactose. Lactic acid production yield for *Lb. lactis* was obtained as 0.437 $g.g^{-1}$ of consumed lactose. Lactic acid production yield for *Lb. delbrueckii* and *Lb. fermentum* is 41.7% and 40.53% less than *Lb. bulgaricus*, respectively. *Lb. bulgaricus* also had the best lactic acid productivity equal to 0.511 $g.L^{-1}h^{-1}$. This is only 1.4% more than the recorded productivity for *Lb. casei*. Lactic acid productivity of *Lb. delbrueckii*, *Lb. lactis* and *Lb. fermentum* is in order of 50.3%, 31.5% and 59.7% less than *Lb. bulgaricus*.

Table 1. Yield and productivity of biomass production for five different *Lactobacilli*

Strain	Maximum cell dry weight ($g.L^{-1}$)	Incubation time (h)	Yield ($g.g^{-1}$)	Productivity ($g.L^{-1}h^{-1}$)
Lb. casei	4.3	36	0.105	0.119
Lb. bulgaricus	5.1	30	0.119	0.17
Lb. delbrueckii	3.2	42	0.092	0.076
Lb. lactis	3.9	48	0.114	0.081
Lb. fermentum	3.5	52	0.117	0.067

Table 2. Yield and productivity of lactic acid production for five different *Lactobacilli*

Strain	Maximum lactic acid ($g.L^{-1}$)	Incubation time (h)	Yield ($g.g^{-1}$)	Productivity ($g.L^{-1}h^{-1}$)
Lb. casei	24.2	48	0.586	0.504
Lb. bulgaricus	26.6	52	0.602	0.511
Lb. delbrueckii	13.2	52	0.351	0.254
Lb. lactis	14.7	42	0.437	0.350
Lb. fermentum	10.7	52	0.358	0.206

- *Moser kinetic*

Experimental data on lactose and cell dry weight concentrations at exponential growth phase were used to determine the consistency of five studied strains with Moser kinetic model. Figure 1 shows the fitted plot for experimental data on substrate utilization and cell growth to Moser kinetic model for five investigated *Lactobacilli*.

The exponential phase of growth curve of *Lactobacillus* species in a batch culture is defined by Malthus law as stated in equation 3. Separation of variables as applied in equation 3 and integration using suitable initial condition ($X=X_0$ at $t=t_0$) resulted in equation 4. 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^{-1} and 6 hours, respectively.

$$\frac{dX}{dt} = \mu X \tag{3}$$

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

Kinetic constant coefficients (μ_{max}, K_s) were determined using curve fitting method. Specific cell growth rate values were calculated according to the cell dry weight as biomass concentration (X) and average lactose concentration as the limiting substrate concentration (S_{ave}) for the exponential growth phase. Power plot for fitting experimental data for the growth of *Lactobacillus* species using Moser kinetic model is presented in Figure 1.

Lb. fermentum showed an acceptable fitness with Moser kinetic model with R^2=0.968 and maximum specific cell growth rate (μ_{max}) of 0.667 h^{-1} but its Moser semi-saturated coefficient (K_s) was the greatest obtained value of 5132 $g.L^{-1}$. Therefore, Moser kinetic model isn't a good desired model to describe the cell growth and substrate consumption behavior of *Lb. fermentum*. *Lb. delbrueckii* had the highest μ_{max} equal to 0.769 h^{-1} with regard to the curve-fitting results, indicated suitable cell growth rate at the applied conditions. On the other hand, with a good consistency (R^2=0.925), its K_s parameter was too high (195.7 $g.L^{-1}$). Thus, Moser kinetic model isn't a proper model for this strain too. *Lb. bulgaricus* and *Lb. casei* showed acceptable consistency with Moser kinetic model. R^2 for these strains were obtained 0.954 and 0.956, respectively. Their μ_{max} were 0.5 and 0.580 h^{-1} and K_s were 9.385 and 18.2 ($g.L^{-1}$), respectively. The results indicated that the consistency of *Lb. lactis* with Moser kinetic model is less than all other investigated strains. For this strain, R^2 was determined as 0.841.

Vasudha and Hari (2014) investigated the Gompertz and Logistic kinetic models for *Lb. plantarum* NCDC 414. Their results showed that after 24 h of incubation, the viable cell counts increased from 4×10^5 to 7×10^{10} CFU.mL^{-1}. In 24 h incubation time, lactic acid concentration also increased by about 4.5 folds and 44% w/v of substrate consumption occurred during growth of *Lb. plantarum* (Vasudhu and Hari, 2014). In this work, significant lactic acid production was observed for the period of growth and stationary phases. In addition, the cell dry weight increased by about 10 to 15 folds in 24 h. Alvarez *et al*. (2010) studied the kinetics of cell growth, lactic acid production and substrate utilization of *Lb. casei* var. *rhamnosus*. 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 (Alvarez *et al*., 2010).

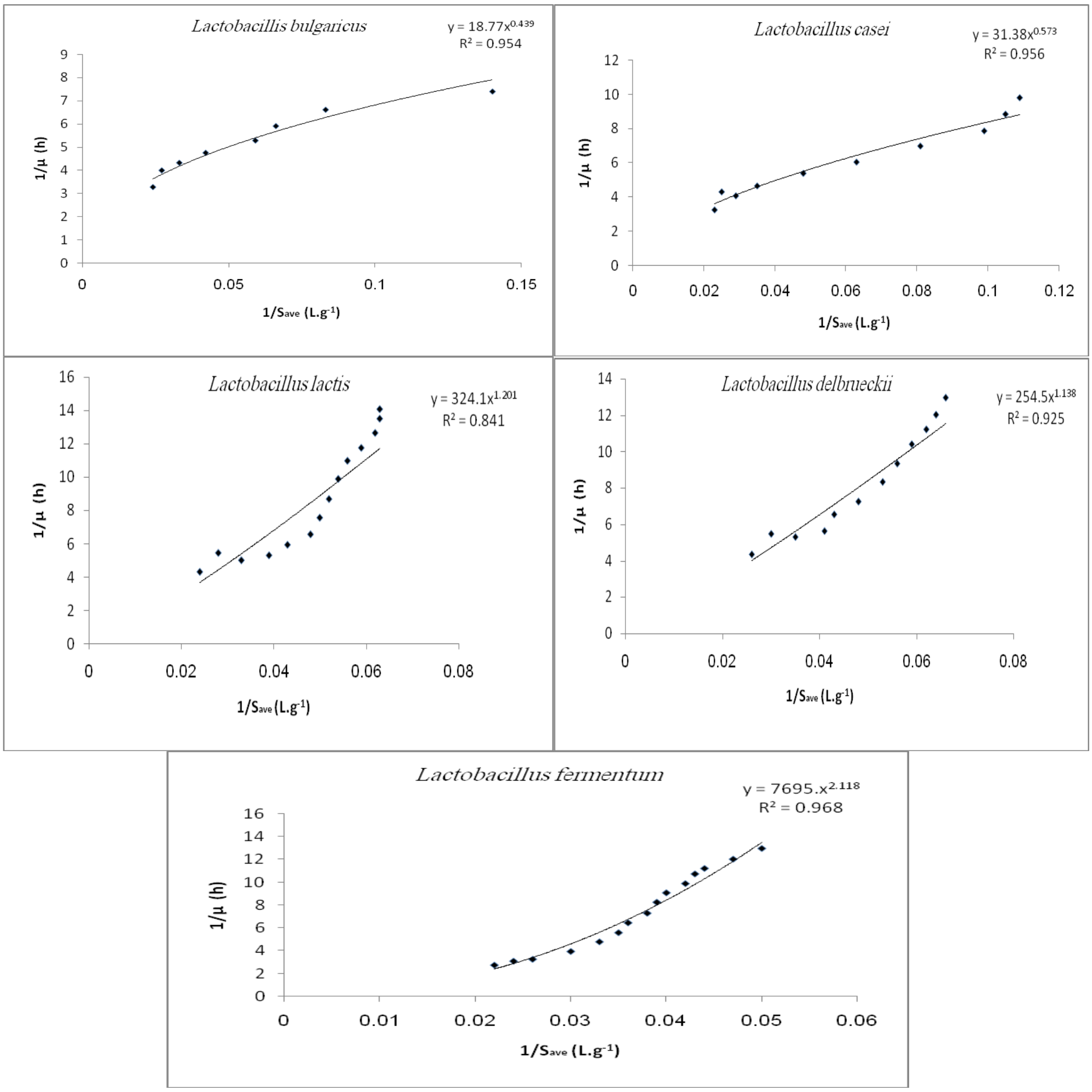

Fig. 1. The power plot to fit the experimental data on substrate utilization and cell growth for Moser kinetic model for five studied *Lactobacilli* in a submerged batch culture medium of lactose fortified whey

Table 3. A comparison of Moser kinetic constants for five different species of *Lactobacilli*

Strain	R^2	μ_{max} (h^{-1})	K_s ($g.L^{-1}$)
Lb. delbrueckii subsp. *bulgaricus* PTCC1737	0.954	0.5	9.385
Lb. casei subsp. *casei* PTCC1608	0.956	0.580	18.200
Lb. delbrueckii subsp. *lactis* PTCC 1743	0.841	0.5	162.05
Lb. delbrueckii subsp. *delbrueckii* PTCC1333	0.925	0.769	195.7
Lb. fermentum PTCC1744	0.968	0.667	5132

- *Gompertz model*

Power plot for fitting experimental data for the growth of *Lactobacillus* species with Gompertz kinetic model is presented in Figure 2. Based on the calculated kinetic parameters (Table 4), all the investigated strains did not show acceptable consistency with Gompertz kinetic model. *Lb. bulgaricus* (R^2=0.885) had the most desired capability with Gompertz equation among the investigated strains and for this strain, maximum specific cell growth rate (μ_{max}) was obtained as 1.719 h^{-1}. *Lb. delbrueckii* had the highest μ_{max} equal to 1.793 h^{-1}. Based on the results, Gompertz kinetic model is not a good and desired model to describe the cell growth and substrate consumption behavior of *Lactobacilli*.

Conclusion

This is the first report on the cell growth and substrate utilization kinetic of five different *Lactobacilli* with respect to Moser and Gompertz kinetic models. *Lb. bulgaricus* was defined as the best strain in fields of biomass and lactic acid production yield. Moser kinetic model is a suitable model to describe cell growth and substrate utilization trends for *Lb. bulgaricus* and *Lb. lactis*. While Gompertz was not introduced as a proper model to describe the cell growth and substrate consumption behavior of *Lactobacilli*.

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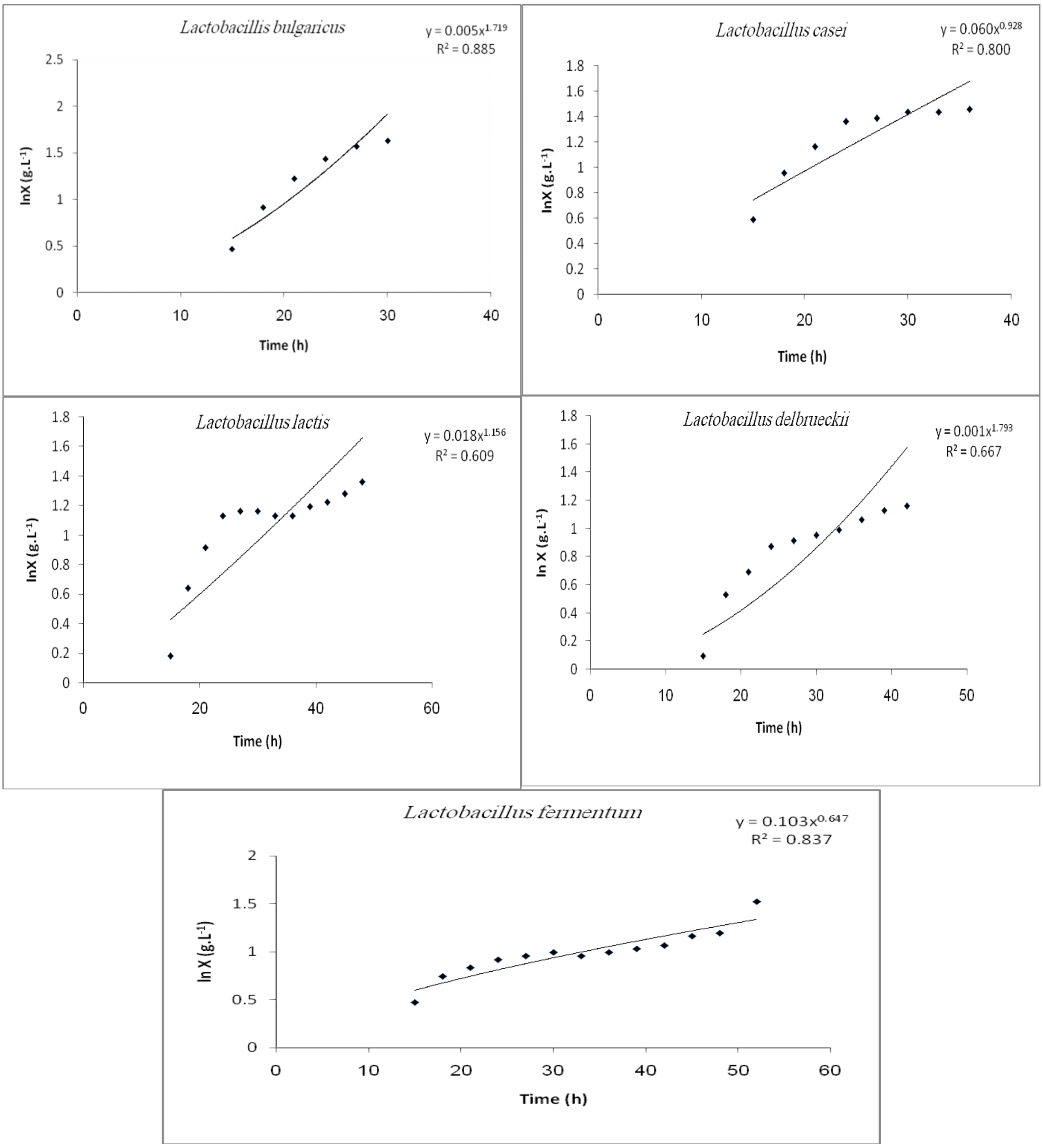

Fig. 2. The power plot to fit the experimental data on substrate utilization and cell growth for Gompertz kinetic model for five studied *Lactobacilli* in a submerged batch culture medium of lactose fortified whey

Table 4. A comparison of Gompertz kinetic constants for five different species of *Lactobacilli*

Strain	R^2	μ_{max} (h^{-1})
Lb. delbrueckii subsp. *bulgaricus* PTCC1737	0.885	1.719
Lb. casei subsp. *casei* PTCC1608	0.800	0.928
Lb. delbrueckii subsp. *lactis* PTCC 1743	0.609	1.156
Lb. delbrueckii subsp. *delbrueckii* PTCC1333	0.667	1.793
Lb. fermentum PTCC1744	0.837	0.647

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