# Microbial growth kinetics as a method to model and predict the development of starter cultures

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**Abstract:** A comparative study was conducted on the growth rate of lactic acid bacteria involved in starter cultures for the production of lactic acid products. Based on the data obtained, with respect to the rate of development, it is possible to predict their development as monocultures or as cultures in symbiotic relationship. In order to achieve the set objective, culture of lactic acid microorganisms was carried out in a bioreactor for 24 hours. Data on the rate of development in the different phases, represented by the acid formation curve, are reported for the following strains: *Lactobacillus delbrueckii* ssp. *bulgaricus* S22; *Streptococcus thermophilus* S1; *Lacticaseibacillus casei* ssp. *rhamnosus* AS15; *Lactobacillus casei* ssp. *shirota* 51C. The conclusions drawn allow the correct and predictable use of the investigated strains of lactic acid bacteria in starter cultures for the production of lactic acid products.

## 1 Introduction

Lactic acid microorganisms are the main group used in the production of dairy products for consumption. They are a group of gram-positive bacteria united by morphological, metabolic and physiological characteristics [1]. They can be generally described as gram-positive, non-spore-forming cocci or rods that produce lactic acid as a major end product during the fermentation of carbohydrates in milk and cause its coagulation.

The boundaries of this group of bacteria have often been debated, but historically the taxonomy is: Lactobacillus, *Leuconostoc*, *Pediococcus* и *Streptococcus* who form the core of the group [2].

*Lactobacillus bulgaricus,* originally known as the Bulgarian bacillus was known as *Thermobacterium bulgaricum* [3], later marked as *Lactobacillus bulgaricus* (also known at the moment as *Lb. delbrueckii* subsp. *bulgaricus*). It belongs to the group of homofermentative lactobacilli fermenting lactic acid-producing lactose [4]. Is known, that strains of *Lb. bulgaricus* produce proteolytic enzymes in yoghurt production [5-11]. The proteolytic properties of *Lb. bulgaricus* are the basis for strain selection to create symbiotic starters for dairy products.

*Streptococcus thermophilus* is one of the most widely used lactic acid bacteria in dairy products such as yogurt, other fermented products, white brined cheese, yellow cheese [12]. It has been shown that a huge amount of microorganisms from this strain are ingested by humans on a daily basis [13]. In that direction, *Str. thermophilus* is generally recognized as a safe strain by international food safety agencies [14]. The main role of *Str. thermophilus* in the lactic acid process is to provide acidification, fermentation of milk and lactic acid production.

*Lacticaseibacillus casei* ssp. *casei* is a commercial probiotic strain that helps increase the number of beneficial bacteria in the gut microbiota by balancing intestinal probiotic and harmful bacteria. It has also been shown to have positive effects in lowering cholesterol levels [15, 16]. Chinese dairy product yakult containing *L. casei* ssp. *shirota* shows antitumor, antibacterial and immunostimulant activity [17]. *L. casei* ssp. *shirota* can ameliorate chronic staph infection, increase cell activity and modify allergen-induced immune response in allergic rhinitis [18, 19]. In addition, *Lb. casei* ssp. *shirota* reduces biofilm formation by *Streptococcus mutans* on tooth surfaces by reducing biofilm acidogenicity and enamel lesion in depth to prevent cavity formation [20].

To study fermentation processes in depth, it's necessary to know the kinetics of the process. The growth kinetics of the microbial population, is a key function to determine the rate of development. Its study in lactic acid organisms is performed during or after the fermentation processes of the given bacteria. The main method to determine the kinetics during cultivation is mathematical modelling. Basically, different function systems are used to give a clear picture of the observed dependencies in a system [21]. Basically, structural and non-structural models are used to model kinetics [22]. Structural models consider the basic cellular structures,

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their composition and function and are characterized by an accurate description of fermentation processes, but are also temporarily very complex and require specialized computational software and a lengthy computational process.

In non-structural models, only the total concentration of cells or product is considered. These models do not include cell physiology, but they also accurately describe the lactic acid fermentation process [21. 22].

According to the current views of a mathematical model in determining the kinetics of a process, there are the following requirements for the microorganism under study: 1. To be at population level; 2. To contain a minimum number of experimentally determined constants and parameters, which should be biologically meaningful; 3. To reflect all important processes of a given fermentation and their relationships; 4. Be capable of defining a pathway to improve, regulate, control and scale up the process [21] .

Since the main observable is the variation of the biomass concentration of the cultured lactic acid bacteria, logistic models are used to model the kinetics, which contain a clear biological meaning [21. 22].

In view of the need to know the microbial growth kinetics of lactic acid microorganisms, a comparative study of the following lactic acid bacteria was done in this study: *Lactobacillus delbrueckii* subsp. *Bulgaricus* S22*; Streptococcus thermophilus* S1*; Lacticaseibacillus casei* ssp. *rhamnosus* AS15*; Lacticaseibacillus casei* ssp. *shirota* 51C. Appropriate mathematical equations and models have been selected to correctly determine their rates of development.

## 2 Materials and methods

### 2.1 Microorganisms

The following species of lactic acid bacteria were used in the present work: *Lactobacillus delbrueckii* ssp. *bulgaricus* S22; *Streptococcus thermophilus* S1*; Lacticaseibacillus casei* ssp. *rhamnosus* AS13*; Lacticaseibacillus casei* ssp. *shirota* 51C. They were provided from the collection of the Department of Technology of Milk and Dairy Products at University of Food Technology-Plovdiv.

### 2.2 Nutrient environments

#### *2.2.1 MRS (deMan, Rogosa, Sharpe)–agar (Biokar-France)*

Ingredients (g/dm$^3$): casein peptone - 10; yeast extract - 4; meat extract - 10; glucose - 20; $K_2HPO_4$ - 2; sodium acetate - 5; diammonium citrate - 2; $MgSO_4$ – 0.2; $MnSO_4$ - 0.04; Tween 80 -1 cm$^3$/dm$^3$; pH adjusted to 5.7 sterilization - 15 min on 121°C.

#### *2.2.2 M17-agar (Biokar-France)*

Ingredients (g/dm$^3$): tryptone – 2.50; meat peptone – 2.50; soybean meal peptone – 5.00; yeast extract – 2.50; meat extract – 5.00; lactose – 5.00; sodium glycerol phosphate – 19.00; $MgSO_4$ – 0.25; ascorbic acid – 0.50; agar-agar – 15. pH -7.1 ± 0.2. Sterilization for 15 min on 121°C.

#### *2.2.3 Fermentation environment*

Ten % reconstituted dry milk, sterilized at 121°C for 15 min.

### 2.3 Determination of the number of active viable cells.

The number of viable cells was determined by the 10-fold dilution method according [23].

### 2.4 Titratable acidity determination

The titratable acidity was determined according to the requirements [24].

### 2.5 Cultivation of the strains under study

Cultivation of the studied strains was performed in a bioreactor with mechanical agitation and a working volume of 1.5 dm$^3$ in 10% sterile reconstituted milk at 42 ± 1 and 37 ± 1°C.

### 2.6 Modelling of growth kinetics

To model the kinetics of the process, the classical and viodesign logistic curve models (eq. 1 and 2) were used [22, 25]. To model the kinetics of acid formation, the modified power model was used (model 3) [26]. Logistic models (1) and (2) were solved numerically using the 4th order Runge-Kutta method, the identification of the parameters of models (1) and (2) was done using the Solver function in Excel, by minimizing the square of the difference between the experimental data and those obtained from the corresponding model [27].

$$\frac{dX_b}{d\tau} = \mu_{max}\left(1 - \frac{X_b}{X_{bm}}\right)X_b \qquad (1)$$

$$\frac{dX_b}{d\tau} = \mu_{max}\left(1 - \frac{X_b}{X_{bm}}\right)^n X_b \qquad (2)$$

$$K_{Tb} = 1 + (q_{pm} \cdot \tau)^c \qquad (3)$$

While the parametric identification of model (3) was performed in the Curve Expert Professional software by nonlinear regression, where: where: $\mu_{max}$ is the maximum specific growth rate, h$^{-1}$; $X_b$, and $X_{bm}$ are the current and final biomass concentration in dimensionless form, respectively; biomass, lactic acid

amount, final biomass concentration and lactic acid in dimensionless form; n - parameter taking into account the influence of the composition of the fermentation medium, the cultivation conditions on the development of the strain, and also the influence of the accumulating lactic acid on the cells, respectively indicating the resistance (sensitivity) of the cells to the increasing concentration of the product; $K_{Tb}$, dimensionless titratable acidity; $q_{pm}$, maximum specific rate of acid formation; c, an index determining the change in the shape of the $K_{Tb}(\tau)$ curve or the change in the rate of lactic acid accumulation over time in general, and also characterizing the post- acid- forming ability of the strain.

## 3 Results and discussion

The ability of selected lactic acid bacteria strains to grow in reconstituted skim milk, which is the most widely used fermentation medium in production conditions, was investigated. For this purpose, batch cultivation processes with strains *Lactobacillus delbrueckii ssp. bulgaricus* S22, *Streptococcus thermophilus* S1; *Lacticaseibacillus casei* ssp. *rhamnosus* AS15; *Lactobacillus casei* ssp. *shirota* 51C in a bioreactor with mechanical agitation at the optimal temperature for the strain. The dynamics of biomass variation during the fermentation process up to the point of milk coagulation was monitored. The results of these studies are presented in Fig. 1. 2, 3 and 4.

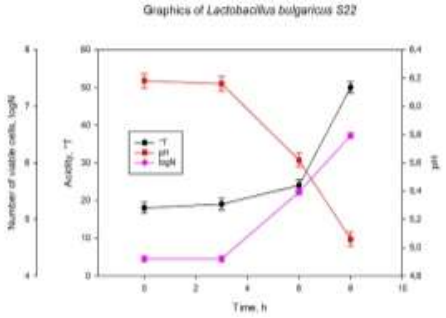


**Fig. 1.** Growth and acid-forming ability of the selected strain *Lactobacillus delbrueckii* ssp. *bulgaricus* S22 cultivated in a laboratory bioreactor with mechanical stirring at 37°C

From the data presented in Fig. 1 - 4, it can be seen that the shortest lag phase, compared to the other strains studied is represented by *Streptococcus thermophilus* S1. which lasts about 0.5 h, after which the culture enters exponential growth which continues until the onset of milk coagulation, which occurs in 3.4 h from the start of fermentation at a titratable acidity of 58°T and pH 4.67 (Fig. 3), with an active cell concentration of $1.0.10^8$ cfu/cm$^3$ at the time of coagulation.

The short lag phase of *Streptococcus thermophilus* S1 indicates its high adaptability to the fermentation medium and culture conditions.

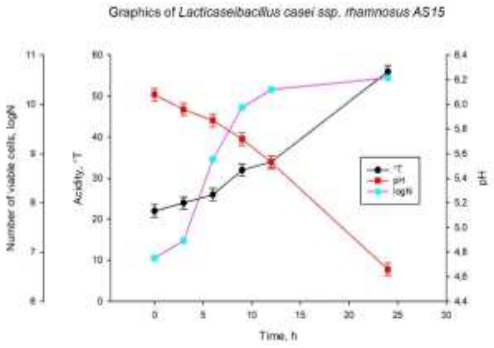


**Fig. 2.** Growth and acid-forming ability of the selected strain *Lacticaseibacillus casei* ssp. *rhamnosus* AS15 cultivated in a laboratory bioreactor with mechanical stirring at 37°C

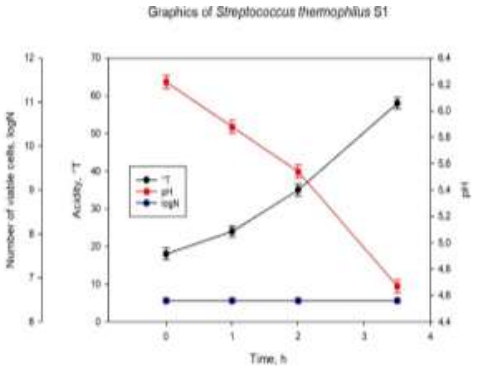


**Fig. 3.** Growth and acid-forming ability of the selected strain *Streptococcus thermophilus* S1 cultivated in a laboratory bioreactor with mechanical stirring at 37°C

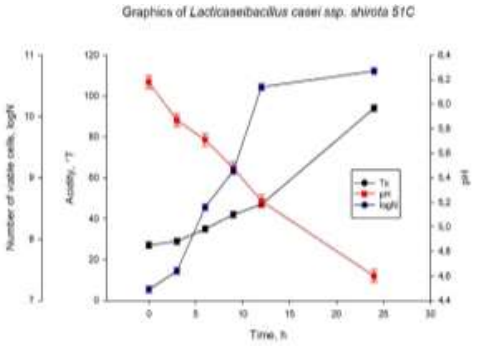


**Fig. 4.** Growth and acid-forming ability of the selected *Lacticaseibacillus casei* ssp. *shirota* 51C cultivated in a laboratory bioreactor with mechanical stirring at 37°C

The strains *Lactobacillus delbrueckii* ssp. *bulgaricus* S22, *Lacticaseibacillus casei* ssp. *rhamnosus* AS15*; Lacticaseibacillus casei* ssp. *shirota* 51C are characterized by a longer lag phase, which is 3 h from the beginning of the process, after which all three strains studied enter exponential growth, with a continuous increase in the concentration of active cells. For *Lactobacillus delbrueckii* ssp. *bulgaricus* S22 (Fig. 1)

this growth continued until milk coagulation, which occurred at the 8th h from the start of the fermentation process at a titratable acidity of 50°T and pH 4.94, and the concentration of active cells reached was $3.0 \times 10^6$ cfu/cm$^3$. For strains *Lacticaseibacillus casei* ssp. *rhamnosus* AS15*; Lacticaseibacillus casei* ssp. *shirota* 51C a continuous increase in the concentration of active cells was observed until the 24th h from the start of the process, where maximum values were reached of $3.5 \times 10^{10}$ and $5.5 \times 10^{10}$ cfu/cm$^3$ for strains *Lacticaseibacillus casei* ssp. *rhamnosus* AS15 and *Lacticaseibacillus casei* ssp. *shirota* 51C (Fig. 2 and Fig. 4). The titratable acidity at the end of the fermentation process reached 56 and 94°T and pH 4.66 and 4.38 for the respective strains (Fig. 2 and Fig. 4). A complete study of the cultivation process is not possible without knowledge of its kinetics, which is essential for scaling up the process to industrial conditions and its management. Therefore, the growth and acid-forming kinetics of the studied strains intermittently cultured in a laboratory bioreactor with mechanical agitation was modelled. The kinetic comparison of individual strains cultured in the same medium and processes need to start with the same concentration of active clathrates and the same initial acidity, which is difficult to achieve. Therefore, the biomass and titratable acidity in the mathematical models are presented in dimensionless form, which facilitates mathematical processing and eliminates the influence of dimensionality on the results obtained from the models, since all initial data start from the same origin-1 [28]. Parameter identification of the models was performed and the results are presented in Table 1 and Table 2.

**Table 1.** Kinetic parameters of classical and modified logistic curve model

| **Strains** | **Kinetic parameters** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Classic logistic curve model** | | | | | **Modified logistic curve model** | | | | | |
| | $\mu_{max}$, h$^{-1}$ | β, $X_b$ /cm$^3$.h | $X_{bк}$ | $R^2$ | e | $\mu_{max}$, h$^{-1}$ | β, $X_b$ /cm$^3$.h | $X_{bк}$ | n | $R^2$ | e |
| *L. rhamnosus* | 0.115 | 0.066 | 1.75 | 0.9979 | 0.097 | 0.115 | 0.066 | 1.73 | 0.9555 | 0.9985 | 0.098 |
| *L. shirota* | 0.103 | 0.066 | 1.57 | 0.9968 | 0.071 | 0.094 | 0.059 | 1.58 | 0.9703 | 0.9977 | 0.073 |
| *L. bulgaricus* | 0.132 | 0.067 | 1.97 | 0.9977 | 0.085 | 0.110 | 0.060 | 1.83 | 0.6126 | 0.9965 | 0.095 |
| *St. thermophilus* | 0.158 | 0.089 | 1.78 | 0.9997 | 0.047 | 0.113 | 0.069 | 1.63 | 0.5340 | 0.9998 | 0.048 |

***Table 2.*** *Kinetic parameters in the modified power model*

| | $q_{max}$, h$^{-1}$ | c | $R^2$ | e |
|---|---|---|---|---|
| *L. rhamnosus* | 0.057 | 1.41 | 0.9995 | 0.040 |
| *L. shirota* | 0.072 | 1.65 | 0.9986 | 0.038 |
| *L. bulgaricus* | 0.138 | 5.82 | 0.9998 | 0.038 |
| *St. thermophilus* | 0.488 | 1.67 | 0.9991 | 0.025 |

From the data presented in the tables, it is evident that the models used are characterized by high correlation coefficients, which range from 0.9965 to 0.9998, as well as low identification error values, ranging from 0.023 to 0.098. The high correlation coefficients as well as the low values of the identification error indicate that the models adequately and accurately describe the experimental results and can be used to predict the fermentation process in the defined cultivation time interval as well as the post-acid-burst process.

From the data presented in Table 1. it is evident that the coefficient of intra-population competition for all four strains studied assumes a low value, which varies in the range of 0.059 to 0.089 $X_b$/cm$^3$.h in the respective models. This indicates a lack of competition of the cells of the strains studied with each other, often leading to an increase in the amount of dead cells. The logistic curve models used gave relatively high maximum growth rates for the strains studied. From the data presented in Table 1 it can be seen that for strain *Lacticaseibacillus casei* ssp. *rhamnosus* AS15 the two models gave the same value of maximum specific growth rate, 0.115 h$^{-1}$. This strain was characterized by high values of the parameter n, 0.9555, indicating that the strain requires the addition of growth factors to improve its growth in the fermentation medium. Furthermore, the high value of n indicates that the cells of this strain are sensitive to lactic acid accumulation. Theoretically, the maximum biomass concentration in the dimensionless form predicted by the two models is commensurate and is 1.75 and 1.73, which values are close to the experimentally determined value of 1.68. This further confirms that the models describe the experimental results with high accuracy and can be used to predict and calculate the biomass concentration at different time points of the fermentation process. For strain *Lacticaseibacillus casei* ssp. *shrirota* 51C are observed lower maximum specific growth rates of 0.103 and 0.097 h$^{-1.}$ respectively, and also a high value of the n-factor– 0.9703. This indicates that this strain is the most sensitive to lactic acid accumulation compared to all others, and also needs mandatory addition of growth factors to the medium to improve strain development.

In confirmation of this, the lower theoretically calculated maximum biomass value in dimensionless form of 1.57 and 1.58 can be noted. These values are again close to the experimentally determined value of 1.50, indicating that for this strain both models used describe the experimental results with high accuracy. The high sensitivity of the cells of this strain to lactic acid makes it necessary to encapsulate this strain in acid-resistant capsules if it is to be used as a probiotic or to combine it with weakly acid-forming strains in starter cultures, in order to maintain a high concentration of active cells during the storage period of the finished fermented products. From the data presented in Table 1 it is evident that for strain *Lactobacillus delbrueckii* ssp. *bulgaricus* S22 the classical logistic curve model yields a higher maximum specific growth rate of 0.132 $h^{-1.}$ compared to the modified model where μmax is 0.110 $h^{-1}$. This strain showed the highest values of the theoretical maximum biomass concentration in the dimensionless form, 1.97 and 1.83, which were close to the experimental value at the time of coagulation, 1.50, and which would be reached if the optimum pH was maintained during the fermentation process. For this strain, low value of the parameter n of 0.6126 was observed, indicating that the fermentation medium and culture conditions were optimal for the strain, and furthermore the cells of the *Lactobacillus delbrueckii* ssp. *bulgaricus* S22 have a low sensitivity to increasing lactic acid, suggesting high survival in the gastrointestinal tract and enabling this strain to be used in starter cultures to produce probiotic fermented lactic acid products.

A similar trend was observed for *Streptococcus thermophilus* S1. where again the classical logistic curve model gave a higher maximum specific growth rate of 0.158 $h^{-1.}$ compared to the modified strain, where $\mu_{max}$ occupied a value of 0.113 $h^{-1}$. The theoretical maximum biomass concentrations in the dimensionless form for this strain are 1.78 and 1.53, respectively, which would be reached in pH maintenance cultivation. From the data presented in Table 1. it can be seen that the parameter n also assumes low values of 0.5340, indicating that the fermentation medium and culture conditions for this strain are also optimal and also the cells of Streptococcus *thermophilus* S1 show low sensitivity to lactic acid accumulation and it can be combined in starter cultures with more pronounced acid-forming strains.

It is of high interest to determine the acid-binding capacity of the strains studied, which is relevant for their technological application [29]. For this reason, mathematical modelling of the kinetics of the acid-forming ability of the strains was carried out and the main kinetic parameters of acid formation were determined. The results of these studies are presented in Table 2. From the data presented in the table, it can be seen that the modified power model used is characterized by high correlation coefficient values, which range from 0.9986 to 0.9998 for the respective strains, and low identification error, from 0.025 to 0.040, indicating that the model used accurately and adequately describes the acid formation process. The data presented in Table 2 show that the lowest maximum rate of acid formation was not observed in strain *Lacticaseibacillus casei* ssp. *rhamnosus* AS15, where $q_{max}$ is 0,054 $h^{-1.}$ and also, the lowest value of parameter c - 1.41.

**Table 3.** Biomass in dimensionless form by the classical and modified logistic curve model for the selected strains (A, B, C, D)

A

| Time, h | *Streptococcus thermophilus* S1 | | |
|---|---|---|---|
| | Biomass in dimensionless form | | |
| | Experiential | Classic model | Modified model |
| 0 | 1.00 | 1.00 | 1.00 |
| 3 | 1.04 | 1.07 | 1.07 |
| 6 | 1.13 | 1.14 | 1.14 |
| 8 | 1.24 | 1.22 | 1.22 |

B

| Time, h | *Lactobacillus bulgaricus* S22 | | |
|---|---|---|---|
| | Biomass in dimensionless form | | |
| | Experiential | Classic model | Modified model |
| 0 | 1.00 | 1.00 | 1.00 |
| 3 | 1.23 | 1.19 | 1.21 |
| 6 | 1.27 | 1.37 | 1.41 |
| 8 | 1.51 | 1.47 | 1.53 |

C

| Time, h | *Lacticaseibacillus casei* ssp. *shirota* 51C | | |
|---|---|---|---|
| | Biomass in dimensionless form | | |
| | Experiential | Classic model | Modified model |
| 0 | 1.00 | 1.00 | 1.00 |
| 3 | 1.04 | 1.11 | 1.10 |
| 6 | 1.19 | 1.20 | 1.20 |
| 9 | 1.27 | 1.28 | 1.28 |
| 12 | 1.46 | 1.34 | 1.34 |
| 24 | 1.50 | 1.49 | 1.50 |

D

| Time, h | *Lacticaseibacillus casei* ssp. *rhamnosus* AS15 | | |
|---|---|---|---|
| | Biomass in dimensionless form | | |
| | Experiential | Classic model | Modified model |
| 0 | 1.00 | 1.00 | 1.00 |
| 3 | 1.05 | 1.14 | 1.15 |
| 6 | 1.29 | 1.27 | 1.28 |
| 9 | 1.44 | 1.38 | 1.39 |
| 12 | 1.50 | 1.47 | 1.48 |
| 24 | 1.68 | 1.67 | 1.67 |

This makes the strain a weak acid forming agent and consequently low consumption of neutralizing agent during its cultivation. *Lacticaseibacillus casei* ssp. *shirota* 51C also showed a low but higher maximum specific acid-forming rate of 0.075 $h^{-1}$ compared to strain *Lacticaseibacillus casei* ssp. *rhamnosus* AS15 and also a higher value of parameter c -1.65 compared to strain AS15.

This indicates that in strain *Lacticaseibacillus casei* ssp. *shirota* 51C the acid formation process will be more intense overall. With the highest maximum specific rate of acid formation was observed in strain *Streptococcus thermophilus* S1. where $q_{max}$ assumed a value of 0.488 $h^{-1.}$ but the value of the parameter c was 1.67, indicating that as lactic acid accumulates at the initial time, it will be synthesized at a higher intensity, but after a certain time, the acid-breaking process will proceed at a lower intensity until the limiting acidity is reached. This high

rate of lactic acid formation makes the strain suitable for the production of mozzarella-type cheeses where *Streptococcus thermophilus* S1 strains are the main starter cultures [29]. An intermediate but relatively high $q_{max}$ value was observed for strain *Lactobacillus delbrueckii* ssp. *bulgaricus* S22, where it was 0.138 $h^{-1.}$ a parameter with the highest value assumed compared to the other strains studied, 5.82. This makes the strain a strong acid-former in which lactic acid biosynthesis will proceed with high intensity throughout the process, and also a high post-acid-forming ability of the strain is expected.

**Table 4.** Acidity in a dimensionless form of the respective strains measured experimentally and by the modified power model (A, B, C, D)

A

| Time, h | *Lacticaseibacillus casei* ssp. *rhamnosus* AS15 | |
|---|---|---|
| | Acidity in a dimensionless form | |
| | Experiential | Stage model |
| 0 | 1.00 | 1.00 |
| 3 | 1.09 | 1.08 |
| 6 | 1.18 | 1.22 |
| 9 | 1.45 | 1.39 |
| 12 | 1.55 | 1.58 |
| 24 | 2.55 | 2.54 |

B

| Time, h | *Lacticaseibacillus casei* ssp. *shirota* 51C | |
|---|---|---|
| | Acidity in a dimensionless form | |
| | Experiential | Stage model |
| 0 | 1.00 | 1.00 |
| 3 | 1.07 | 1.08 |
| 6 | 1.30 | 1.25 |
| 9 | 1.52 | 1.49 |
| 12 | 1.74 | 1.79 |
| 24 | 3.48 | 3.47 |

C

| Time, h | *Lactobacillus bulgaricus* S22 | |
|---|---|---|
| | Acidity in a dimensionless form | |
| | Experiential | Stage model |
| 0 | 1.00 | 1.00 |
| 3 | 1.01 | 1.01 |
| 6 | 1.33 | 1.33 |
| 8 | 2.78 | 2.78 |

D

| Time, h | *Streptococcus thermophilus* S1 | |
|---|---|---|
| | Acidity in a dimensionless form | |
| | Experiential | Stage model |
| 0 | 1.00 | 1.00 |
| 3 | 1.06 | 1.01 |
| 6 | 1.33 | 1.33 |
| 8 | 2.78 | 2.78 |

A comparison of experimental data and those from mathematical models of growth and acid-forming kinetics was made, and the results of these studies are presented in Tables 3 and 4.

From the data presented in the tables, it can be seen that the model results agree very similar with the experimental results.

## 4 Conclusion

The studied strains *Lactobacillus delbrueckii ssp. bulgaricus* S22, *Streptococcus thermophilus* S1. *Lacticaseibacillus casei* ssp. *rhamnosus* AS15 and *Lacticaseibacillus casei* ssp. *shirota* 51C possess valuable technological characteristics that make them suitable for use as starter cultures for the production of lactic acid products. The study of the kinetics of their development provides the scientific information necessary to formulate guidelines for manufacturers related to their application in practice.

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