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*Article*

# Lactic Acid Production by *Lactiplantibacillus plantarum* AC 11S—Kinetics, and Modeling

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**Abstract:** Lactic acid production from lactose by *Lactiplantibacillus plantarum* AC 11S was studied. The influence of initial substrate concentration was investigated, and optimal pH and temperature were determined. An unstructured mathematical model was developed comprising equations for bacterial growth, substrate consumption, and product formation. The model was solved with different terms for specific growth rates considering substrate and/or product inhibition. The best bacterial growth and lactic acid production were achieved at pH = 6.5 and 30 °C. Production of lactic acid was mainly growth-associated and at substrate concentration over 15 g/L a considerable product inhibition was observed. The parameters of different models were determined and compared. While the modified Gompertz equation gave the best fit for biomass growth, a variant of the logistic equation including a term for product inhibition described in the best way the entire set of equations comprising bacterial growth, substrate consumption, and product formation at different initial substrate concentrations.

**Keywords:** lactic acid; *Lactiplantibacillus plantarum*; lactose; growth; mathematical modeling

## 1. Introduction

Lactic acid bacteria (LAB) are a multitudinous microbial group of microorganisms with diverse beneficial applications in the food and pharmaceutical industry. LAB share the same characteristics of metabolism and physiology, with strain-specific functionality and potential. Their main common feature is the production of lactic acid as an end-product of the fermentation of various carbohydrates. From a taxonomic point of view, they comprise a large variety of Gram-positive, catalase-negative, and anaerobic non-sporulation bacteria belonging to the *Lactobacillaceae* family, with different G+C *Firmicutes* groups. They are producers of organic acids, polyols, bacteriocins, exopolysaccharides (EPS), and aromatic compounds, among others [1]. Several LAB species are widely accepted as probiotics, with functional characteristics and beneficial healthy effects based on their metabolic activity.

*Lactiplantibacillus plantarum* can be found in various ecological niches - in dairy products, fermented vegetables and cereals, meats, fish, silage, wine, gastrointestinal, urogenital and vaginal tracts [2]. It is one of the most exploited LABs for producing various postmetabolites. The *L. plantarum* is well known for its versatility in substrate utilization [3]. Various *L. plantarum* strains are capable of utilizing different monosaccharides either hexoses or pentoses [4], as well as different lignocellulosic hydrolysates from rice straw [5], corncobs [6], sugarcane bagasse [7], *Opuntia ficus indica* waste [8], algal biomass [3] among others. These species are considered the most advantageous among LAB in the production of lactic acid (LA) from lactose and it is also capable of utilizing other nutrients presented in whey [9].

*L. plantarum* has a larger genome than other LAB species, (size of about 3.3 Mbp and more than 3000 genes) which indicates its strong adaptability, high versatility, enormous diversity in phenotypic properties, metabolic capacity, and industrial applications [10]. According to Zhang et al. [11.] *L. plantarum* group represents the evolutionary link between heterofermentative and

homofermentative LAB. Though *L. plantarum* is phylogenetically related to heterofermentative *lactobacilli,* it shares different metabolic features with homofermentative *lactobacilli*. *L. plantarum* has been widely used as a model species for ecological, genetic, and metabolic studies in lactobacilli. The major commercial use of *L. plantarum* is as a starter culture for various food fermentations and in recent years as a probiotic culture [11].

*L. plantarum* is considered to belong to the group of facultatively heterofermentative *lactobacilli*. Via the Embden–Meyerhof–Parnas pathway, hexoses are almost completely converted to lactic acid. On the other hand, pentoses are converted to acetic and lactic acids via the 6-phosphogluconate/phosphoketolase pathway by the induction of phosphoketolase [12]. *L. plantarum* like other LAB possesses specific nutritional requirements and thus, a complex growth media, which must contain peptides, vitamins, nucleic, fatty, and amino acids, besides the carbon source is needed. Another particular characteristic of the process of lactic acid production is the end-product inhibition. Due to the lactic acid accumulation, the fast acidification of the fermentation medium leads to low substrate utilization, low conversion, and low final product concentration.

In the process of a comprehensive investigation of lactic acid production, kinetic studies are an indispensable step. A proper mathematical model is very useful in understanding, describing, evaluating, and predicting the fermentation process. Mathematical models help disclose the relationship between cell growth, substrate consumption, and product formation and can be used for control optimization and scale-up of a fermentation process. Mathematical models have been classified according to different criteria [13]. One of these criteria distinguishes between structured and unstructured mathematical models. Structured models are more accurate and take into account cell functions, structure, and composition. Unstructured models, on the other hand, treat the cells as a black box, a sole component without involving any cells' physiological characteristics. Although the structured models are more detailed and precise, providing a better understanding of the system, unstructured models are widely used, because of their applicability and simplicity in describing bacterial kinetics. Different models have been used in the modeling of lactic acid production from various substrates. Some of them are summarized in the works of Zacharof & Lovitt [13], Bouguettoucha et al. [14], and Gordeev et al. [15].

The present work aims to investigate *Lactiplantibacillus plantarum* strain AC 11S, as a promising producer of lactic acid from lactose, to determine the optimum conditions for growth and production, and to compare some kinetics models describing the fermentation dynamics.

## 2. Materials and Methods

### 2.1. Strain, Growth Media, and Culture Conditions

*Lactiplantibacillus plantarum* AC 11S was isolated from a homemade white brined cheese [16]. It was cultured in de Man, Rogosa, and Sharpe broth (MRS Merck, Darmstadt, Germany) and stored at – 20°C in MRS broth, supplemented with glycerol 20% v/v. Before the experiments, the strain was pre-cultured twice in modified MRS broth with lactose. The strain was cultured on growth medium contained: lactose monohydrate - 11 g/L, yeast extract - 5.5 g/L, peptone from casein - 12.5 g/L, sodium acetate - 10 g/L, $KH_2PO_4$ - 0.25 g/L, $K_2HPO4$ - 0.25 g/L, $MgSO_4.7H_2O$ - 0.1 g/L, $MnSO4.7H2O$ - 0.05 g/L, $Fe_2(SO_4)_3$ - 0.05 g/L. All chemicals were p. a. grade (Fluka). The inoculum (10% v/v) was prepared from overnight culture in MRS broth with lactose monohydrate (11 g/L). All experiments were carried out in flasks (100 m growth medium in 300 mL Erlenmeyer flasks at 30°C with an initial pH of 6.5) using a WiseCube® WIS30 shaking incubator (Witeg Labortechnik GmbH, Germany).

### 2.2. The LAB Species Identification

The strain AC 11S was identified as *Lactiplantibacillus plantarum* by classical phenotypic methods Gram staining, oxidase (kit HiMedia, Mumbai, India), catalase tests, and carbohydrate fermentation test with 49 carbon sources (using API 50 CHL, bioMérieux, Marcy l'Etoile, France). The species affiliation was confirmed by Multiplex PCR amplification with primers targeting the *recA* gene, according to Torriani et al. [17]. The PCR analysis was performed on a PCR thermocycler (BioRad,

laboratories, Inc. group), using ready to use PCR mix (IllistraTM PuRe TaqTM Ready To GoTM PCR beads; Amersham Biosciences, Amersham, UK). The target DNAs were isolated from an overnight LAB culture of the strain AC 11S using the Gene Matrix Bacterial and Yeast Genomic DNA Purification Kit (EURx Ltd., Gdańsk, Poland), following the manufacturer's instructions. The PCR reaction conditions are as previously described [18].

### 2.3. In Vitro Assessment of Acid Tolerance of L. plantarum AC11S.

In vitro test, in 96-well U-bottomed, polystyrene microtiter plates (Corning, NY, USA) with a final volume of 150 µL, was carried out. The cells from 10 mL overnight culture were harvested by centrifugation (9000x g Hermle, Germany centrifuge) and washed twice with sterile saline. Washed cells re-suspended in 10 mL were adjusted to $6.0 \times 10^8$ CFU/mL (2x McFarland). The cells from 5 mL were harvested and re-suspended in 5 mL simulated gastric fluid with low pH - 1.5, NaCl 0.8% w/v, and pepsin 3 mg/mL (Sigma-Aldrich, Saint Louis, MO, USA). The resting 5 mL was used as a control re-suspended in sterile saline pH 6.5. They were incubated for 3 h at 30°C. After treatment, they were washed 2 times, with sterile saline. Following the treated culture, the control was used as inoculum (10% v/v) in modified MRS broth (with lactose 20 g/L) in 96-well microplates. Viability, after treatment was monitored spectrophotometrically (OD 600nm) during 24 h cultivation at 30 °C using Elisa Plate Reader (INNO, Seongnam-si, Korea). The results are presented as a mean of triplicate.

### 2.4. Analytical Procedures

For biomass determination, optical density (OD) was measured at 620 nm (UV-VIS spectrophotometer Milton Roy 401, Rochester, USA). From OD data, by using a previously prepared calibration curve, the biomass concentration was calculated. An HPLC system composed of a Knauer Smartline-100 pump, a Pekin-Elmer LC-25RI refractometric detector, and data processing software Eurochrome (Knauer) was used for lactic acid measurements. Aminex HPX-87H (Bio-Rad) column was used. A 0.005 M solution of $H_2SO_4$ at a flow rate of 0.6 mL/min was used as the mobile phase. For standard solutions preparation pure (98 % mass, Sigma), crystalline L (+)-lactic acid was used. All measurements were made in triplicate.

### 2.5. Fermentation

All experiments for lactic acid production were carried out in MRS broth with lactose as a carbon source in 300 mL flasks at 30 °C and 6.5 initial pH of the medium (except those for optimum pH and temperature determination).

### 2.6. Modeling

The main goal in a fermentation process is complete substrate utilization, leading to a maximum product yield. The development of a proper mathematical model and correct determination of model parameters will permit to conduct the fermentation in its optimal conditions. The mathematical model should contain equations describing cell growth, substrate consumption, and product formation and their interrelationship. In an unstructured mathematical model, only the total cell concentration is considered. In general, the bacterial growth rate can be described as follows:

$$\frac{dX}{dt} = \mu X - k_d X \tag{1}$$

where $X$ is the cell concentration, g/L; $t$ is time; $\mu$ is the specific growth rate, h$^{-1}$ and $k_d$ is the specific cell death rate. In many cases, the cell death was not observed and the second term can be omitted.

The simplest model representing the specific growth rate as a function of substrate concentration is the Monod equation:

$$\mu = \mu_{max}\left(\frac{S}{k_S + S}\right) \tag{2}$$

where $\mu_{max}$ is the maximum specific growth rate, $h^{-1}$; $S$ is the initial substrate concentration, g/L; $K_S$ – the substrate saturation constant, g/L. The Monod equation describes the increase of cell concentration with time and considers limiting substrate concentration. It is widely accepted that lactic acid fermentation is limited by substrate and inhibited by the product. Many authors insert different terms in the Monod equation to take inhibition effects into account. Some of them are presented in Table 1.

**Table 1.** Modified Monod models describing cell growth.

| No | Model name | Equation of biomass specific growth rate | Ref. |
|:---:|:---:|:---:|:---:|
| 1 | Verhulst | $\mu = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)^n$    if $n=1$ <br><br> $\mu = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)$ | [19] |
| 2 | Altıok | $\mu = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)^{n_1}\left(1 - \frac{P}{P_{max}}\right)^{n_2}$ if $n_1, n_2 = 1$ <br><br> $\mu = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)\left(1 - \frac{P}{P_{max}}\right)$ | [20] |
| 3 | Aiba | $\mu = \mu_{max}\left(\frac{S}{k_S + S}\right)e^{-k_P P}$ | [21] |
| 4 | Andrews | $\mu = \mu_{max}\left(\frac{S}{k_S + S + S^2/k_i}\right)$ | [22] |
| 5 | Akerberg | $\mu = \mu_{max}\left(\frac{S}{k_S + S + S^2/k_i}\right)(1 - k_P P)^n$ | [23] |
| 6 | Monod-Jerusalimsky | $\mu = \mu_{max}\left(\frac{S}{k_S + S}\right)\left(\frac{k_P}{k_P + P}\right)$ | [24] |
| 7 | Hinshelwood | $\mu = \mu_{max}\left(\frac{S}{k_S + S}\right)(1 - k_P P)$ | [25] |

Other authors have used different sigmoidal models for the description of microbial growth. The most utilized are the logistic equation and the modified Gompertz model:

$$\ln\left(\frac{X}{X_0}\right) = \frac{a}{\left[1 - exp(b - ct)\right]} \tag{3}$$

$$\ln\left(\frac{X}{X_0}\right) = a\,exp\left[-exp(b - ct)\right] \tag{4}$$

These models do not include the consumption of the substrate and describe only the number of cells.

With the aim to give a biological meaning to the parameters $a$, $b$, and $c$ Zwietering [26] reparametrized the above equations to:

$$X(t) = X_0 + \frac{A}{1 + exp\left[\frac{4\mu_{max}}{A}(\lambda - t) + 2\right]} \tag{5}$$

$$X(t) = X_0 + A\,exp\left\{-exp\left[\frac{\mu_{max}e}{A}(\lambda - t)\right] + 1\right\} \tag{6}$$

The Luedeking – Piret equation [27] is the most used for the description of lactic acid formation. It takes into account the fact that the rate of product accumulation $dP/dt$, depends on bacterial growth $dX/dt$, as well as on the bacterial density $X$.

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X \tag{7}$$

$\alpha$ and $\beta$ are coefficients related to growth and non-growth product formation.

The rate of substrate consumption is closely related to the cell's growth rate and the rate of product formation. This relationship is usually given by the following equation:

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{dX}{dt} - \frac{1}{Y_{P/S}}\frac{dP}{dt} - m_s X \tag{8}$$

where $Y_{X/S}$ and $Y_{P/S}$ are yield coefficients for biomass on substrate and product on substrate respectively, g/g, while $m_s$ is the biomass maintenance energy coefficient, g/g.h. In many cases, the latter was neglected.

## 3. Results

### 3.1. Isolation and Identification of Lactiplantibacillus plantarum AC 11S

*L. plantarum* AC 11S, was isolated from a sample of white brined cheese, homemade on a small farm, in the village of Arda, Rodopa mountain, Bulgaria, near the border with Turkey. The strain is a part of the laboratory collection of the "Stephan Angeloff" Institute of Microbiology, BAS, Bulgaria.

The strain AC 11S was characterized as Gram-positive, catalase, and oxidase-negative rod-shaped cells' morphology (Figure 1 A), non-motile, non-spore-forming facultative anaerobe. It was initially identified as a mesophilic bacterium presumptive *Lactiplantobacillus plantarum* by carbohydrate fermentation test with 49 carbon sources (using API 50 CHL, bioMérieux, Marcy l'Etoile, France). The accurate species affiliation was achieved by multiplex PCR (Figure 1 B), according to Torriani et al. [17]. With primers, targeting the *rec A* gene, a PCR product 318 bp was obtained corresponding to the species *L. plantarum*. This molecular method was preferred as a discriminative approach for three highly similar species from the group of *Lactiplantibacillus* as reported by Toriani et al. [17] and Georgieva et al. [18].

(**a**)　　　　　　　　　　　　　　　(**b**)
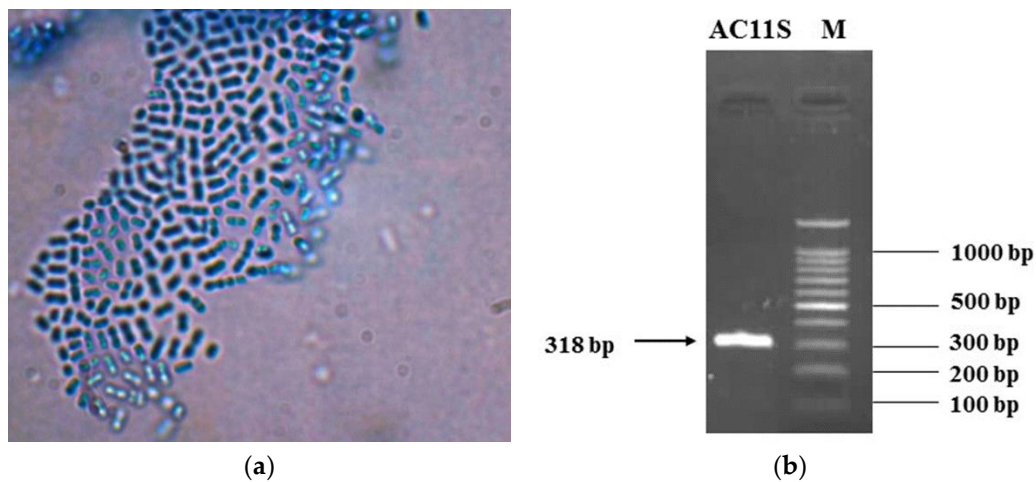
**Figure 1. (a)** Cells morphology of the strain AC 11S (1000x dimension light microscopy Boeco, China microscope); **(b)** Identification of the strain as *L. plantarum*, with Multiplex PCR using primers targeting *recA* gen (according to Torriani et al. [17].

### 3.2. Viability of L. plantarum AC 11S in an Acidic Environment

*L. plantarum* is one of the most often employed as a starter LAB species and/or as an adjunct for various biotechnological processes and food production [28]. So, it may be exposed to various stress conditions, such as temperature, and osmotic stress freeze-drying in fermented food [29]. In addition, probiotics must withstand extremely high acidity levels and, a variety of digestive enzymes throughout the entire gastrointestinal tract [30]. The acid stress is a pivotal issue for microbial survival.

*L. plantarum* AC 11S showed extremely high viability under in vitro passage in simulated stomach juice. After 3-hour exposure to pH 1.5 and pepsin, the growth dynamics is similar to the control. Just a small difference with a longer lag phase may be pointed out (Figure 2). Moreover, the control showed an earlier stationary phase (Figure 2 – red lines)
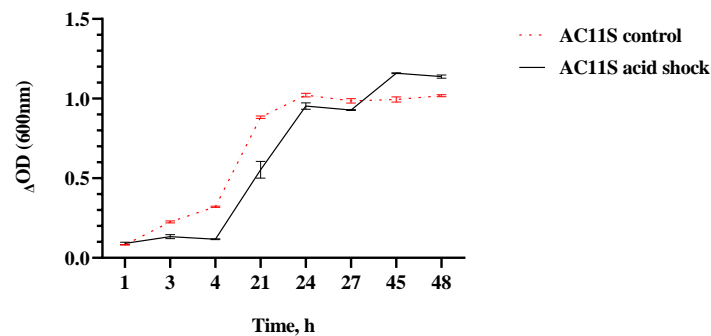


**Figure 2.** Growth curve of *L. plantarum* AC 11S in mMRS broth with lactose (pH 6.5) at 30 °C after an acidic shock in simulated stomach juice.

### 3.3. Influence of pH and Temperature

Fermentation productivity is strongly influenced by the medium pH value and temperature. Although LAB can grow in a broad range of temperatures and pH levels, their growth rate and population density are affected by these factors [12].

With the aim to determine optimum conditions for *L. plantarum* AC11S two series of experiments were carried out. In the first one, the initial medium pH was varied from 4.5 to 8.5 at 30 °C., and in the second set of experiments, conducted at pH 6.5, the temperature was changed from 24 to 40 °C. 100 mL LA broth was inoculated with 10% seeding culture and fermentation was carried out at appropriate temperature and pH value under anaerobic and static conditions for 24 hours.

The results of these experiments are presented in Figure 3. As can be seen from the figure the optimum conditions for lactic acid production are pH = 6.5 and temperature of 30 °C. The influence of pH is more pronounced, especially on the cell concentration.
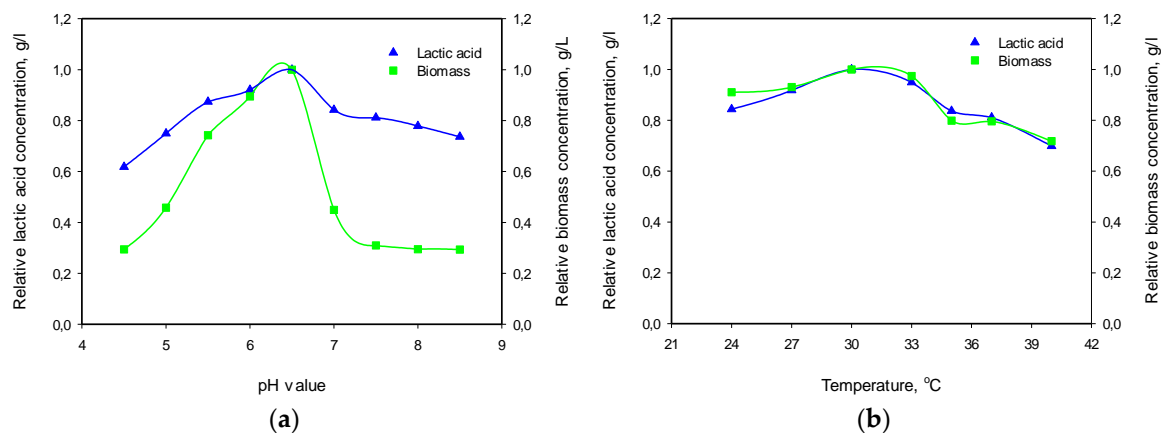


**Figure 3. (a)** Influence of initial pH value on biomass growth and lactic acid production at 30 °C; **(b)** Influence of temperature on biomass growth and lactic acid production at initial pH 6.5.

*L. plantarum* is a mesophilic bacterium, capable of growing at temperatures between 10 and 40 °C. In support of our findings, other researchers also cultivated *L. plantarum* at 30 °C as an optimal temperature [31–35], but there are enough investigations in which the strain was cultivated at 37 °C - see for example [5,6,9].

*L. plantarum* possesses high acidity tolerance and can grow in pH values between 4 and 7. It is generally accepted that lactic acid production is growth growth-associated process. Anyway, during the fermentation, due to the lactic acid accumulation, the pH value of the broth decreases to about 3.0-3.5 in pH uncontrolled mode [36,37]. Many authors assumed that both dissociated and undissociated forms of the acid could exhibit an inhibition effect on cell growth, as the undissociated form is the stronger inhibitor. It is logical because at low pH (below pKa) the acid is mainly in undissociated form. As pointed out by Peetermans et al. [38] other factors besides pKa, like volatility, and lipophilicity of the acid, as well as medium pH or acid concentration influence the microbial growth inhibition in the presence of weak acids. Mercier et al. [39] investigated lactic acid fermentation with glucose as substrate in the pH range of 5.4 – 7.8. Based on experimental results the authors suggest a pH value between 6.0 and 6.5 as optimal for maximal yields for biomass and lactic acid production. W. Fu and A.P. Mathews [9] studied the lactic acid production from lactose with *L. plantarum* in the pH range of 4.0-7.0 and found optimal values for cell growth and acid production between 5.0 and 6.0. Yetiman et al. [4] have characterized a new *L. plantarum* strain isolated from *shalgam* – a traditional fermented beverage. The authors investigated the cell growth at different pH values (2, 3, 4, 5, and 7) at two temperatures – 30 and 37 °C. Maximum cell density was achieved at pH = 7.0 for both temperatures, but the lag phase was shorter, and the specific growth rate was higher at 37 °C. The same behavior was observed in the presence of different concentrations of bile salts. It is worth mentioning that despite a longer lag phase and lower specific growth rate the final cell densities were higher at 30 °C, especially in the presence of bile salts.

### 3.4. Influence of Initial Substrate Concentration

In order to elucidate the influence of substrate concentration on cell growth and lactic acid production a set of experiments was carried out at 30 °C and initial pH 6.5 with lactose monohydrate concentrations 11, 22, 33, 44, and 55 g/L without pH control during fermentation. The results obtained are presented in Figure 4. From the figure, it is clearly seen that while at 11 g/L substrate concentration, the conversion is almost complete, and close to the theoretical. The degree of conversion decreased with increasing the substrate concentration, probably due to the inhibition of

high substrate concentration and/or product accumulation. It is worth mentioning that during the fermentation period (about 50 h) cell death was not observed.
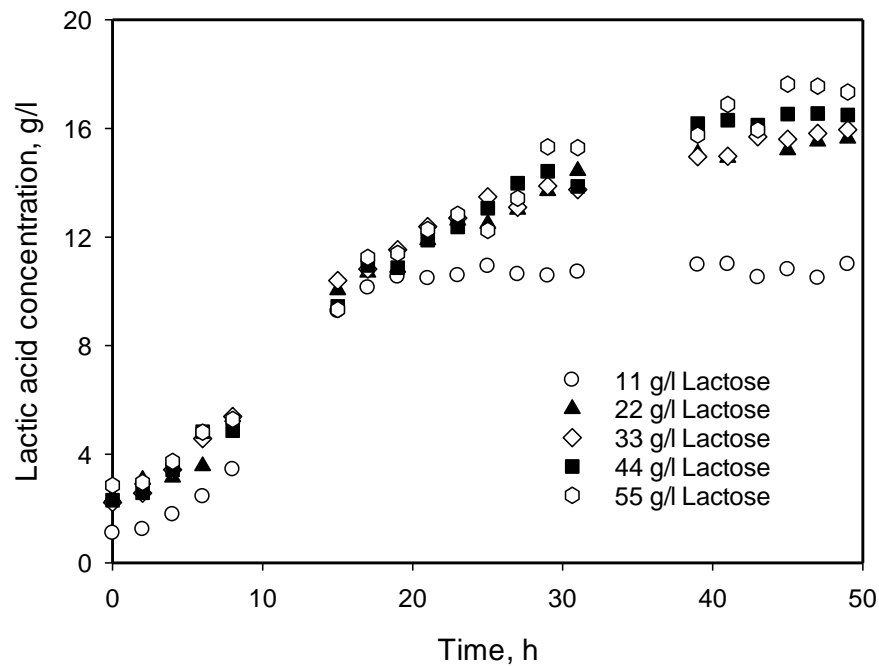


**Figure 4.** Influence of initial substrate concentration on the lactic acid production at 30 °C and initial pH value 6.5 without pH control.

### 3.5. Modeling of Cell Growth, Substrate Consumption, and Product Accumulation

As can be seen in Figure 5 (growth, substrate, and product for 11 g/L) no cell death was observed during the process. The same hold and for others initial substrate concentrations.
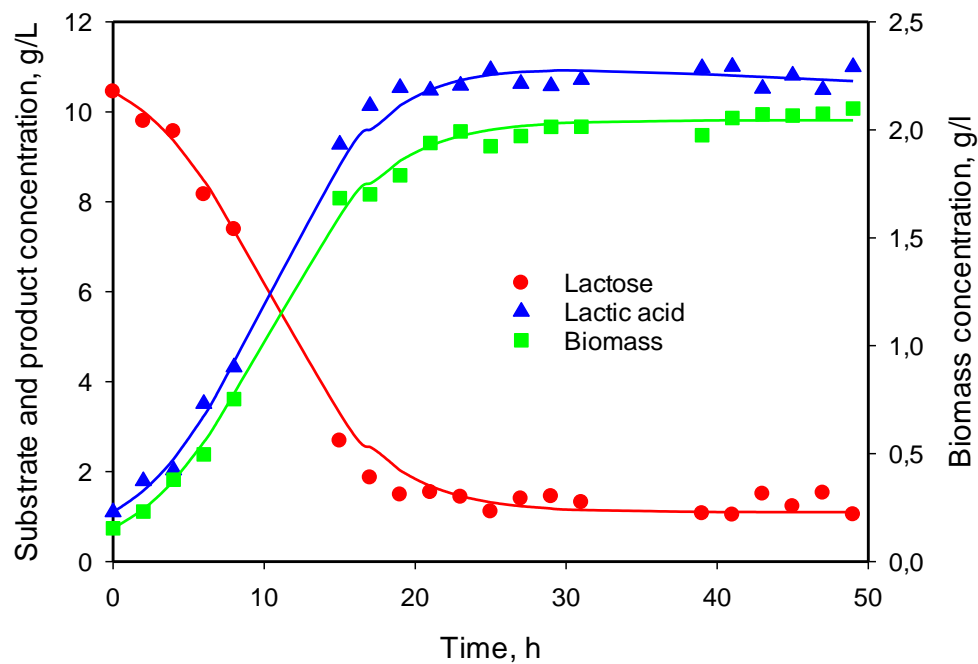


**Figure 5.** Time course of substrate consumption, biomass growth, and lactic acid production from 11 g/L lactose monohydrate at 30 °C and initial pH value 6.5 without pH control.

Therefore, in the eq. 1 the second term was omitted as well as the last term in eq. 4 and the set of differential equations describing the process becomes:

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

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}}\frac{dX}{dt} - \frac{1}{Y_{P/S}}\frac{dP}{dt} \tag{9}$$

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X$$

with initial conditions t = 0, X = $X_0$, S = $S_0$, P = $P_0$.

    With the aim of finding the best expression for specific biomass growth rate, all kinetic models listed in Table 1, as well as the modified logistic and Gompertz equations were used.

    An algorithm for simultaneously solving the model equations describing the fermentation process at different initial substrate concentrations was developed.

    Using this algorithm, own experimental data were used to identify the model parameters by least square function minimization using MATLAB 2013A software.

    For this purpose, the experimental data were processed by minimization procedure of the target function Q, being the sum of the squares of the differences between the measured biomass, substrate, and lactic acid concentrations and the model values:

$$Q\left(\mu_{max}, k_i, X_{max}, \Upsilon_{X/S} \ \& \ \beta, Y_{P/S}\right) =$$

$$\frac{1}{N}\sum_{j=1}^{N}\left(X_j - X_j^{exp}\right)^2 + \frac{1}{N}\sum_{j=1}^{N}\left(S_j - S_j^{exp}\right)^2 + \frac{1}{N}\sum_{j=1}^{N}\left(P_j - P_j^{exp}\right)^2 \to min \tag{10}$$

where $i$ is the number of constants in each equation for $\mu$, and $j$ is the number of experimental points.

    The attempt to solve the model with the data for all 5 substrate concentrations wasn't very successful, the discrepancies between model and experimental data were large and the Q function value was too big.

    Analyzing the data for product accumulation in Figure 4 it can be concluded that while at 11 g/L substrate concentration, the conversion is almost complete, while increasing the substrate concentration from 22 to 55 g/L leads to strong inhibition of the process and the conversion drops up to about 30%. It was decided to solve the model for 11 g/L separately, using an expression for $\mu$ without any additional terms for substrate and product inhibition. Four equations were used – the Monod equation, Verhulst equation, modified logistic, and modified Gompertz equations.

    The results are presented in Figure 6 and the values of the model's parameters are given in Table 2. All four equations described very well the biomass growth as Gompertz and Verhulst's equations gave the best fit. Solving together the system for biomass growth, substrate consumption, and product formation, however, the results were a little bit different – the best fit was obtained with the Verhulst equation, while the value of the Q function was higher in case of the Gompertz equation and the discrepancies between model and experimental data are high for substrate and product. The obtained values for maximum cell concentration (*Xmax* and *A*) are very close, as well as some other model parameters. A very short lag phase was observed and modified Gompertz and logistic models predict - 3-4 h. From the values of the parameters $\alpha$ and $\beta$ it is evident that the production of lactic acid with *L. plantarum* AC 11S is related to biomass growth and practically there is no lactic acid production during the stationary phase.

**Table 2.** Model parameters determined by solving the system 9 for 11 g/L initial substrate concentrations.

| Model | $\mu_{max}$ | $K_S$ | $X_{max}$ | $\lambda$ | A | $Y_{X/S}$ | $Y_{P/S}$ | $\alpha$ | $\beta$ | Q |
|---|---|---|---|---|---|---|---|---|---|---|
| Monod | 0.420 | 5.511 | | | | 0.175 | 4.016 | 5.965 | 0.0197 | 1.019 |
| Verhulst | 0.253 | | 2.045 | | | 0.202 | 6.394 | 5.396 | 0.0077 | 0.352 |
| Gompertz | 0.048 | | | 2.959 | 1.925 | 0.205 | 5.986 | 6.086 | 0.0042 | 3.846 |
| Logistic | 0.138 | | | 4.0705 | 1.875 | 0.229 | 2.011 | 4.591 | 0.0 | 1.949 |
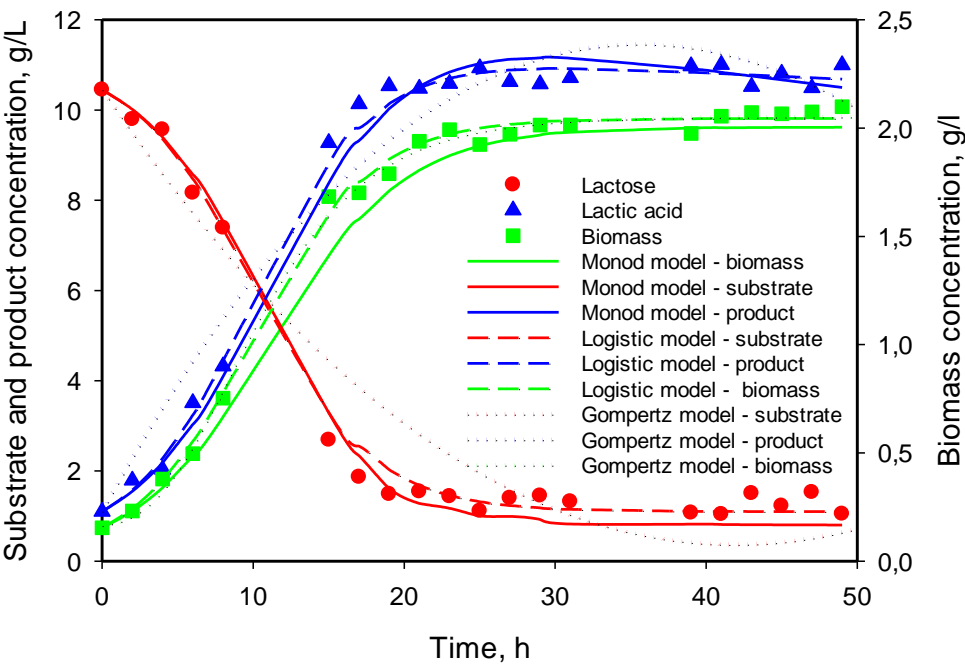
**Figure 6.** Experimental and model results for microbial growth, substrate consumption, and lactic acid production from 11 g/L initial substrate concentration.

For the rest of the experimental data (from 22 to 55 g/L initial substrate concentration) the mathematical model was solved with all equations that include different types of inhibition in the expressions for specific growth rate $\mu$ listed in Table 1. Calculated values of model parameters from simultaneous solution for all 4 initial substrate concentrations are listed in Table 3 and presented in Figure 7.

**Table 3.** Model parameters determined by solving the system 9 for 22 - 55 g/L initial substrate concentrations.

| Model No | $\mu_{max}$ | $K_S$ | $K_P$ | $K_i$ | $X_{max}$ | $P_{max}$ | $n_1$ | $n_2$ | $Y_{X/S}$ | $Y_{P/S}$ | $\alpha$ | $\beta$ | Q |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.179 | | | | 1.754 | | | | 0.121 | 3.650 | 8.337 | 0.0156 | 0.991 |
| | 0.322 | | | | 3.673 | | 2.060 | | 0.154 | 3.346 | 7.015 | 0.0014 | 0.348 |
| 2 | 0.257 | | | | 2.268 | 19.389 | | | 0.419 | 3.803 | 7.263 | 0.0069 | 0.494 |
| | 0.302 | | | | 3.013 | 17.087 | 2.248 | 1.767 | 0.151 | 2.951 | 6.948 | 0.0029 | 0.305 |
| 3 | 0.365 | 3.464 | 0.247 | | | | | | 0.155 | 1.855 | 6.784 | 0.005 | 0.463 |
| 4 | 0.168 | 0.550 | | 0.077 | | | | | 0.144 | 5.269 | 6.232 | 0.0549 | 4.099 |
| 5 | 0.113 | 17.306 | 2.506 | 6.406 | | | | | 0.011 | 58.27 | 29.07 | 1.2367 | 4.71 |
| 6 | 0.185 | 4.293 | 1.052 | | | | | | 0.114 | 1.231 | 7.287 | 0.0231 | 3.287 |
| 7 | 0.233 | 4.847 | 0.064 | | | | | | 0.158 | 3.262 | 6.524 | 0.0028 | 1.348 |

One can see that the best fit (lowest Q value) was obtained with the model proposed by Altıok [20]. The models of Verhulst [19], and Aiba [21] also very well described the growth and production kinetics.
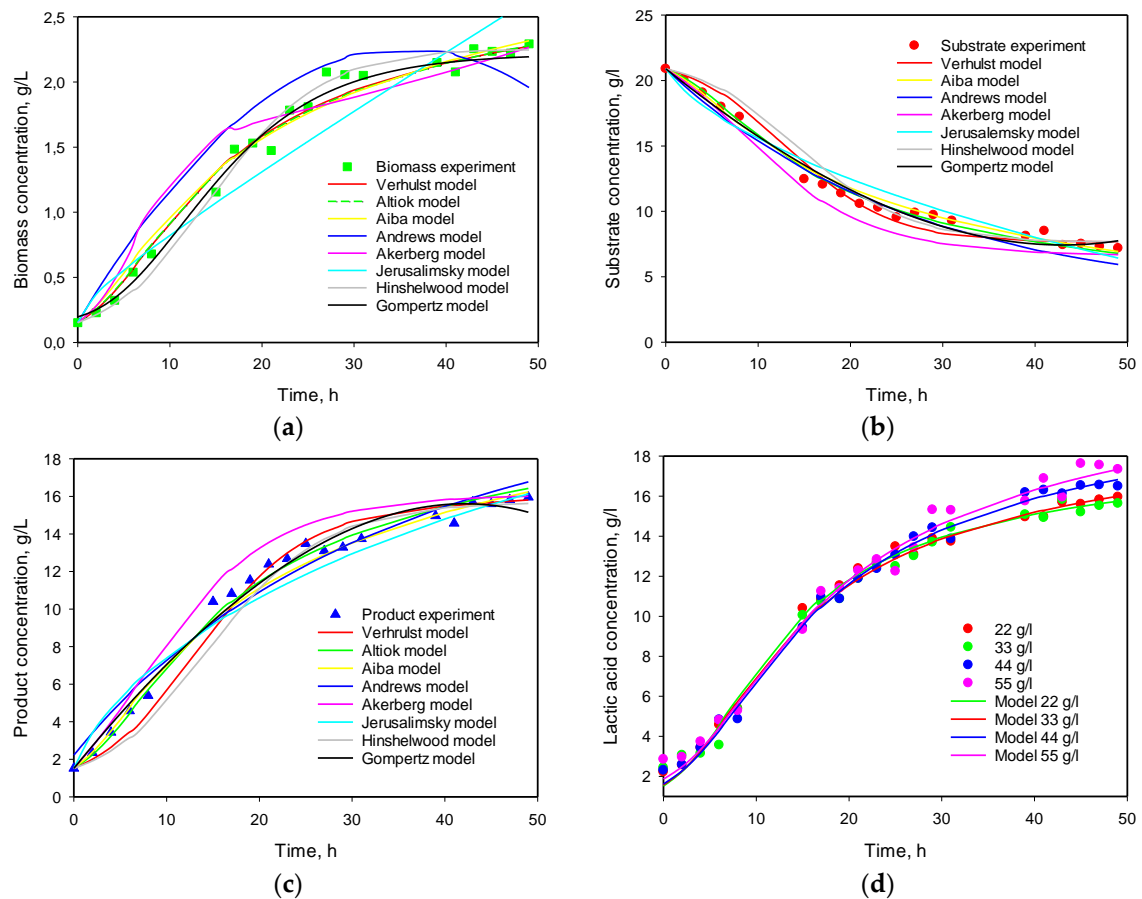
**Figure 7. (a)** Comparison of various model data for biomass growth – 22 g/l initial substrate concentration; **(b)** Comparison of various model data for substrate consumption – 22 g/l initial substrate concentration; **(c)** Comparison of various model data for product formation – 22 g/l initial substrate concentration; **(d)** Comparison of experimental data and Altıok model predictions for lactic acid production – 22-55 g/l initial substrate concentration.

None of both models including substrate inhibition produce good agreement with experimental data. The same observation was made also by Altıok et al. [20]. Åkerberg et al. [23] also reported that the substrate inhibition was very small compared to the product one.

The values of the yield coefficients $Y_{X/S}$ and $Y_{P/S}$ are of the same magnitude except those obtained by the Monod-Jerusalimsky model. The ratio between values of the parameters $\alpha$ and $\beta$ is also high which confirms that product formation is growth-related in this concentration range.

Analyzing the literature data, it is obvious that kinetics constant values are not only strain and substrate specific but also depend on other factors like temperature, pH, media composition, etc. Some of the published data for values of maximal growth rate µ are summarized in Table 4.

**Table 4.** Values of the maximal growth rate determined by other authors.

| Microorganism | Substrate | Growth model | $\mu_{max}$ (h⁻¹) | Reference |
|---|---|---|---|---|
| *L. amylophilus* | Glucose 20 g/L | $\mu = \mu_{max}\left(1 - X/X_{max}\right)$ | 0.32 (pH 6.5) | [39] |
| *L. casei* | Lactose 20 g/L | $\mu = \mu_{max}\left(1 - X/X_{max}\right)$ | 0.511 | [40] |
| *L. plantarum* | Glucose 100 g/L | $\mu = \mu_{max}\left(1 - X/X_{max}\right)$ | 0.64 | [41] |
| *L. lactis* | Glucose 20 g/L | $\mu = \left(\mu_{max}S\left(K_P + P\right)\right)/\left(\left(K_S + S\right)P\right)$ | 0.66 (pH 6.5) | [13] |
| *L. acidophilus* | Glucose 10 g/L | Four parameter Gompertz model | 0.35 (pH 6.5, 30 °C) | [42] |

| Organism | Substrate | Equation | Value | Ref |
|---|---|---|---|---|
| | | | 0.43 (pH 6.5, 37 °C) | |
| L. helveticus | Whey ultrafiltrate powder | $\mu = \mu_{max} 1/\left(1 + ce^{dt}/\left(\mu^m - c\right)\right)$ | 0.56 | [43] |
| | | | 0.993 | |
| L. paracasei | Rice flour | $\mu = \mu_{max}\left(S/\left(S + K_S X\right)\right)$ $\mu = \mu_{\max}\left(1 - X/X_{\max}\right)$ $\mu = \mu_{max} S/\left(K_S + S\right)$ | 0.619 | [44] |
| | | | 0.811 | |
| L. plantarum | Sucrose 20 g/L | $\mu = \mu_{max}\left(1 - \left(X/X_{max}\right)^n\right).$ | 0.0545 | [45] |
| L.plantarum | Hydrolysed wheat flour | $\mu = \mu_{max}\left(S/\left(K_S + S + S^2/K_i\right)\right)\left(1 - K_P P\right)^n$ | 0.403 | [23] |
| L. plantarum | Lactose 40 g/L | $\mu = \mu_{max} S/\left(K_S + S\right)$ | 0.364 (pH 6.0) | [9] |
| L. plantarum | Dairy waste water | $\mu = \mu_{max}\left(1 - X/X_{max}\right)$ | 0.35 | [6] |
| L. casei | Waste potato starch | $\mu = \mu_{max} S/\left(K_S + S\right)$ | 0.115 | [46] |
| L. lactis | Glucose | $\mu = \mu_{max}\left(1 - X/X_{max}\right)$ | 0.687 | [47] |
| L. amylovorus | Glucose Sucrose Starch | $\mu = \left(\mu_{max} S/\left(K_S + S\right)\right)\left(K_I/\left(K_I + P\right)\right)$ | 0.58 0.32 0.61 | [48] |
| L. delbrueckii | Glucose 10 g/L | Logistic equation | 0.031 | [49] |
| | | | 0.55 | |
| L. delbrueckii | Glucose | $\mu = \mu_{max}/\left(1 + S/K_I\right)$ $\mu = \mu_{max} e^{-S/K_I}$ $\mu = \mu_{max}\left(1 - S/S_m\right)^n$ | 0.59 | [50] |
| | | | 0.58 | |
| L. casei | Whey lactose | $\mu = \mu_{max}\left(S/\left(K_S + S\right)\right)\left(1 - X/X_{max}\right)$ | 0.265 | [20] |
| L. delbrueckii | Potato starch | $\mu = \mu_{max} e^{-K_P P}\left(K_S + S + S^2/K_i\right)$ | 0.372(pH 5.5) | [51]a |
| L. helveticus | Whey lactose | $\mu = \mu_{max}\left(1 - X/X_{max}\right)$ | 0.64 | [52] |
| L. helveticus | Lactose 50 g/L | $\mu = \left(\mu_{max} S/\left(K_S + S\right)\right) e^{-\left(S/K_I\right)^{n1}} e^{-\left(P/K_P\right)^{n2}}$ | 0.25 | [53] |
| L. casei | Glucose 50 g/L | $\mu = \mu_{max}\left(K_P/\left(K_P + P\right)\right)\left(S/\left(K_S + S\right)\right)\left(1 - P/P_c\right)$ | 0.45 | [54] |
| L. plantarum | Irish brown seaweeds L. digitata L. saccharina | Modified Gompertz equation | 0.4 0.29 | [55] |
| L. plantarum | Glucose- 20 g/L Vegetable juice – 3 g/L RS | Modified Gompertz equation | 0.6 0.45 | [56] |
| L. plantarum | Glucose- 20 g/L Vegetable juice – 3 g/L RS | Logistic equation | 0.62 0.53 | [56] |

Because other authors have solved models separately for each initial substrate concentration, similar calculations were made with the proposed model (eq. 9). Some of the calculated values are

presented in Table 5. Experimental values of $\alpha$ were calculated from experimental data for biomass and product according to the equation 11:

$$\alpha = \frac{\left(P - P_0\right)}{\left(X - X_0\right)} \tag{11}$$

**Table 5.** Experimental and calculated values of $\alpha$ and $\mu_{max}$.

| Initial substrate concentration, g/L | $\alpha$ exp | $\alpha$ calc | $\mu_{max, calc}$ |
|---|---|---|---|
| 11 | 5.091 | 6.850 | 0.249 |
| 22 | 6.748 | 6.782 | 0.334 |
| 33 | 6.959 | 6.768 | 0.343 |
| 44 | 6.947 | 7.197 | 0.311 |
| 55 | 7.651 | 7.195 | 0.309 |
| Mean value | 6.679 | 6.958 | 0.309 |

Both mean values are very close to the value predicted by the model solving all data for different substrate concentrations simultaneously.
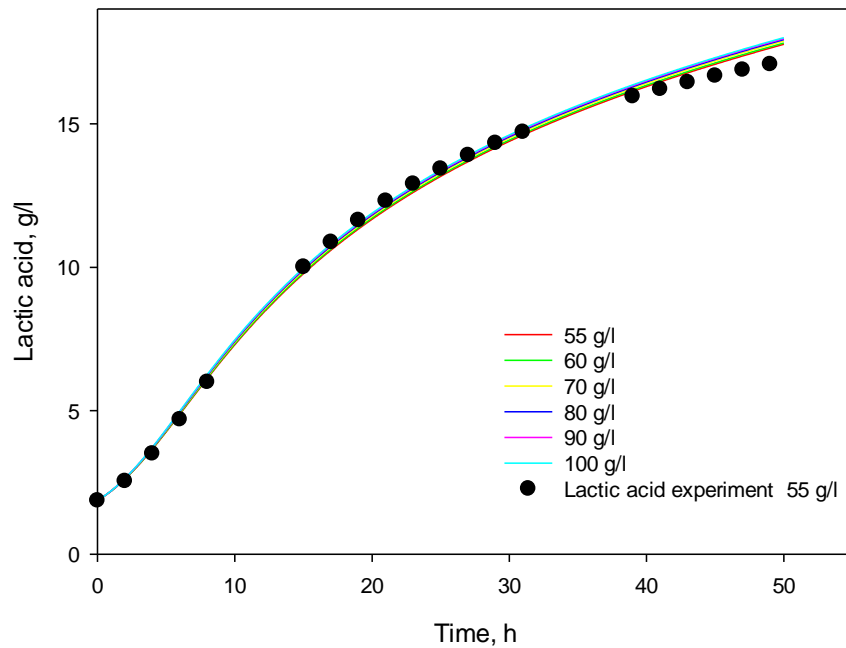
*3.6. Model Predictions*

One of the advantages of the proper mathematical models of a fermentation process is its ability to predict the system behavior in various conditions. With determined model parameters, some calculations were made in view to predict the starting point of the inhibition and lactic acid production at high substrate concentrations. The model was solved for 6 initial substrate concentrations between 11 and 22 g/L. The model predictions are presented in Figure 8. From the inner picture, one can see that the inhibition starts between 15 and 16 g/L.
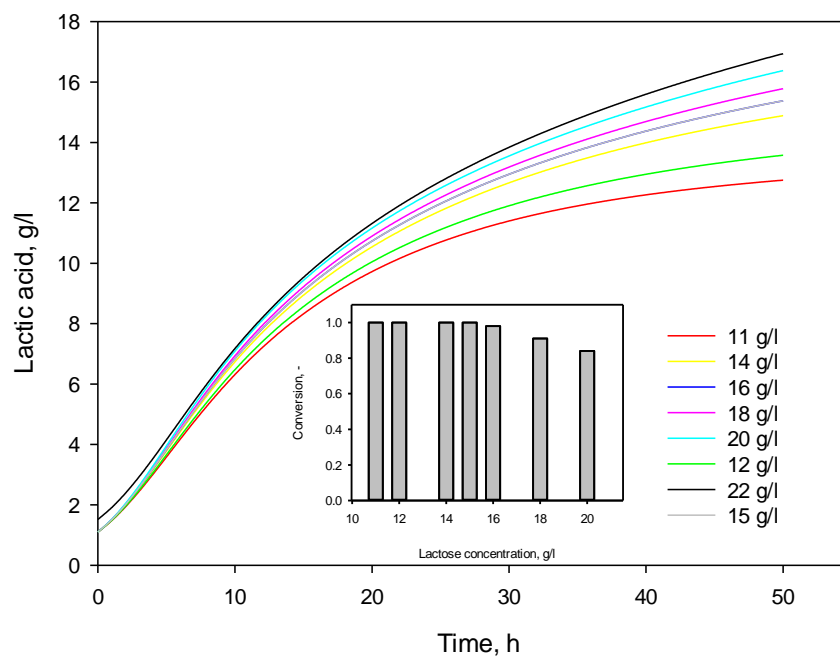


**Figure 8.** Model predictions for initial substrate concentrations in the range of 11-22 g/L.

The model was also solved for higher initial substrate concentrations – from 60 to 100 g/l and the results are presented in Figure 9. The model predicted a very low increase in product concentration – from 17.07 g/L at 55 g/L to 18.00 at 100 g/L. These values are close to the $P_{max}$ value determined according to model 3 (see Table 3).

**Figure 9.** Model predictions for initial substrate concentrations in the range of 55-100 g/L.

## 4. Conclusions

Lactic acid fermentation on lactose by means of *Lactiplantibacillus plantarum* strain AC 11S was investigated in batch mode without pH control. The optimum pH and temperature were determined to be 6.5 and 30 ºC. The strain is extremely acid-resistant and easily recovers from an acid shock. A mathematical model was developed including equations describing biomass growth, substrate consumption, and product formations. When solving the model only for bacterial growth, the modified Gompertz equation gave the best fit for all initial substrate concentrations in the investigated range. Including this equation in the system with equations for substrate depletion and product accumulation, however, didn't produce a good fit with experimental data. The best fit was achieved with a modified Verhulst equation with an added product inhibition term. The results obtained are a good base for further investigation of the strain capabilities in pH-controlled fermentation at fed-batch and continuous mode.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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