MICROBIAL GROWTH OF *LACTOBACILLUS DELBRUECKII* SSP. *BULGARICUS* B1 IN A COMPLEX NUTRIENT MEDIUM (MRS-BROTH)

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KEYWORDS
Probiotics, growth kinetics, modeling, optimization, complex nutrient medium, MRS-broth.

ABSTRACT

The microbial growth of the probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* B1, cultivated in a complex nutrient medium (MRS-broth), was studied in the present work. The complex nutrient medium provides not only the carbon source necessary for the growth of biomass, but also all the additional sources of nitrogen, phosphorus and other components that the biomass needs for its growth. The use of non-structural mathematical dependences determines the optimal conditions (substrate concentration) for the accumulation of biomass or lactic acid, depending on the needs of the specific production.

INTRODUCTION

In recent years, there has been increased interest in the use of lactic acid bacteria of the species *Lactobacillus*, *Enterococcus*, *Pediococcus*, *Streptococcus*, *Lactococcus* and *Leuconostoc* for the development of probiotic and synbiotic preparations (Gibson, 2004). In order for a strain of these species to be classified as probiotic, it must meet a number of requirements, one of which is to allow the conduction of industrial cultivation (Saarela et al., 2002; Kostov et al., 2021).

To evaluate this property it is necessary to apply microbial kinetics, which can be used to assess parameters such as: specific growth rate (maximum and current value), specific rate of product accumulation (maximum and current value), different types of constants. saturation, inhibition, etc.). The combination of these parameters makes it possible to assess the growth of microorganism biomass (in particular lactic acid bacteria biomass), to determine the optimal growth conditions that can ensure the accumulation of biomass and/or metabolic products (Bouguettoucha et al., 2011).

In their classical work Baily and Ollis, 1986, proposed different types of models to describe the microbial growth kinetics. These dependencies are based on the S-shaped nature of microbial growth and are divided into four main groups: non-structural non-segregated models; non-structural segregated models; structural non-segregated models and structural segregated models (Bailey and Ollis, 1986). Nonstructural models view the growth of the microbial population as a whole. When applied, it is assumed that the microbial population grows in the conditions of unlimited food sources, unlimited space and lack of factors related to the vital activity of microorganisms. These models follow from the so-called equation of exponential growth (1) and the well-known Monod dependence (2):

$$\frac{dX}{d\tau} = \mu X \quad (1)$$

$$\mu = \mu_m \frac{S}{K_s + S} \quad (2)$$

where: μ_m - maximum specific growth rate, h^{-1}; X - biomass concentration, g/dm^3; S - substrate concentration, g/dm^3; KS - saturation constant, g/dm^3.

A number of non-structural models have been developed based on the Monod equation and they have been usually named after the researcher who proposed them. Such examples are the Tiessier model, the Andrews and Noack model, the Hinshelwood model, the Aiba model, the Ghose and Tyagi model and others, that try to solve various aspects of microbial growth (substrate inhibition; product inhibition; product and substrate inhibition, etc.) (Bailey and Ollis, 1986; Bouguettoucha et al., 2011; Kostov, 2015; Muloiwa et al, 2020). In the present paper we are going to consider other examples as well (see Materials and methods).

Non-structural models describe only the amount of biomass and/or the amount of metabolites accumulated. Thus, they do not reflect the qualitative characteristics of the cell population and the changes that occur in it during cultivation. These changes can only be described by structural models. They are based on the material balance equations, but in their construction it is necessary to select the key changes taking place in the population. In this model type one works with the concentration of the corresponding variable in a volume unit of biophase, taking into account the cell density, the rate of component formation, the cell mass and more. These models are usually quite complex and include a large number of variables that do not always have a clear and precise biological meaning (Bailey and Ollis, 1986; Kostov, 2015; Shopska et al., 2019).

One of the most well known species of lactic acid bacteria is *Lactobacillus delbrueckii* ssp. *bulgaricus*.

Communications of the ECMS, Volume 36, Issue 1,
Proceedings, ©ECMS Ibrahim A. Hameed, Agus Hasan,
Saleh Abdel-Afou Alaliyat (Editors) 2022
ISBN: 978-3-937436-77-7/978-3-937436-76-0(CD) ISSN 2522-2414

Representatives of this species are included as starter cultures for the production of various types of food, as well as for the production of probiotic preparations (Arena et al., 2015; Maisto et al., 2021; Ivanov etl al., 2021).

The ability to accumulate large amounts of biomass in the cultivation of lactic acid bacteria is very important for the production of probiotics. Complex nutrient media are usually used for the cultivation process. One of the most frequently used media for the cultivation of lactic acid media is MRS-broth medium (de Man, Rogosa and Sharpe). MRS-broth medium has been developed primarily for the cultivation of lactobacilli from various sources with the intention of producing a defined medium as a substitute for tomato juice agar. It is used for the cultivation of the whole group of lactic acid bacteria. The medium shows good productivity for nearly all lactic acid bacteria, but the original version is not selective. It was made selective for lactic acid bacteria by lowering the pH to 5.7 and the addition of 0.14% sorbic acid. Some strains from dairy sources show reduced growth rates in MRS. MRS agar is composed of tryptic digest of casein, beef extract, yeast extract, glucose, sorbitan monooleate, di-potassium hydrogen orthophosphate, magnesium sulfate, manganese (II) sulfate, ammonium citrate, sodium acetate, agar, and distilled or deionized water (Corry et al., 2003).

The main metabolite of lactic acid fermentation is lactic acid. It is known that its increasing concentration during fermentation has an inhibitory effect on the growth of the microbial population. The sensitivity to the accumulating lactic acid is strain-specific (Bouguettoucha et al., 2011; Gordeev et al., 2017).

The aim of the present work was to study the growth characteristics of the probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* when cultivated in a complex nutrient medium such as MRS-broth. The strain has demonstrated a number of probiotic characteristics and had been isolated from homemade yogurt (Goranov et al, 2015; Teneva et al., 2015). As already commented, in some cases, strains isolated from dairy products show reduced growth in MRS-broth medium. Six non-structural mathematical models (see Materials and methods) based on the Monod equation were used to model the microbial growth. The obtained data were used to determine the optimal concentrations of the complex food source in order to improve the accumulation of biomass or lactic acid.

MATERIALS AND METHODS

Microorganisms and cultivation conditions

The study was conducted with *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 isolated from home made yougurt (Goranov et al., 2015; Teneva et al., 2015).

The strain was cultivated in MRS-broth, produced by Merck, with the following qualitative and quantitative composition (g/dm^3): peptone from casein - 10.0; meat extract - 8.0; yeast extract - 4.0; D(+)-glucose - 20.0; dipotassium hydrogen phosphate - 2.0; Tween® 80 - 1.0; di-ammonium hydrogen citrate - 2.0; sodium acetate - 5.0; magnesium sulfate - 0.2; manganese sulfate – 0.05.

The cultivation was performed in a bioreactor with mechanical stirring, shown in Fig.1.The apparatus has a geometric volume of 2 dm^3 and a working volume of 1.5 dm^3 and is equipped with a Sartorius A2 control device, which includes all the measuring instruments for the fermentation process: temperature, pH, dissolved oxygen, etc. The fermentation process was carried out at a stirring speed of 150 rpm at 37±1°C.

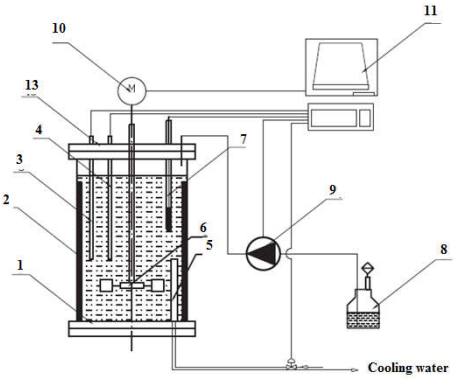

Figure 1: Laboratory Bioreactor
1 - vessel with a geometric volume of 2 dm^3; 2 - baffles; 3 - temperature electrode (thermometer); 4 - cooling/heating device (water jacket); 5 - an additional cooling/heating device; 6 - turbine stirrer; 7 - pH/Eh electrode; 8 - fermentation medium/inoculum/pH adjustment medium; 9 - peristaltic pump; 10 - stirrer drive; 11 – Sartorius A2 control device;

Methods of analysis and nutrient medium for analysis

- Determination of titratable acidity (ISO/TS 11869:2012);
- Determination of number of viable lactobacilli cells (ISO 7889:2005).
- Nutrient media (ISO 7889:2005)
  - MRS-broth;
  - MRS-agar;
  - Saline solution.

Modeling of microbial growth and identification of parameters in kinetic models

The following system of differential equations was used to model the kinetics of microbial growth:

$$\begin{cases} \dfrac{dX}{d\tau} = \mu(\tau)X(\tau) \\ \dfrac{dP}{d\tau} = q(\tau)X(\tau) \\ \dfrac{dS}{d\tau} = -\dfrac{1}{Y_{X/S}}\dfrac{dX}{\tau} - \dfrac{1}{Y_{P/S}}\dfrac{dP}{d\tau} \end{cases} \quad (3)$$

where: X – biomass concentration, g/dm^3; P – lactic acid concentration, g/dm^3; S – substrate concentration, g/dm^3; $Y_{P/S}$, $Y_{X/S}$ – yield coefficients; μ – specific growth rate, h^{-1}; q – specific lactic acid accumulation rate, g/(g.h).

The following dependences were used to model the biomass specific growth rate and the specific rate of lactic acid accumulation (Bailey and Ollis, 1986; Bouguettoucha et al., 2011; Kostov, 2015; Muloiwa et al, 2020):

- Monod model

$$\mu = \mu_{\max}\frac{S}{K_{SX}+S} \qquad q = q_{p\max}\frac{S}{K_{SP}+S} \tag{4}$$

- Haldane model

$$\mu = \mu_{\max}\frac{S}{K_{SX}+S+\frac{S^2}{K_{Xi}}} \qquad q = q_{p\max}\frac{S}{K_{SP}+S+\frac{S^2}{K_{Pi}}} \tag{5}$$

- Aiba model

$$\mu = \mu_{\max}\frac{S}{K_{SX}+S}\exp(-K_{PX}P) \qquad q = q_{p\max}\frac{S}{K_{SP}+S}\exp(-K_{PP}P) \tag{6}$$

- Haldane-Aiba model

$$\mu = \mu_{\max}\frac{S}{K_{SX}+S+\frac{S^2}{K_{Xi}}}\exp(-K_{PX}P) \qquad q = q_{p\max}\frac{S}{K_{SP}+S+\frac{S^2}{K_{Pi}}}\exp(-K_{PP}P) \tag{7}$$

- Haldane model for product inhibition

$$\mu = \mu_{\max}\frac{S}{K_{SX}+S+\frac{S^2}{K_{Xi}}}\left(1+\frac{P}{K_{PX}}\right) \qquad q = q_{p\max}\frac{S}{K_{SP}+S+\frac{S^2}{K_{Pi}}}\left(1+\frac{P}{K_{PP}}\right) \tag{8}$$

- Haldane-Jerusalimski model

$$\mu = \mu_{\max}\frac{S}{K_{SX}+S+\frac{S^2}{K_{Xi}}}\left(\frac{K_{PX}}{P+K_{PX}}\right) \qquad q = q_{p\max}\frac{S}{K_{SP}+S+\frac{S^2}{K_{Pi}}}\left(\frac{K_{PP}}{P+K_{PP}}\right) \tag{9}$$

where: μ_{max} – maximum specific growth rate, h^{-1}; q_{pmax} – maximum specific rate of lactic acid formation, h^{-1}; K_{sx} и K_{sp} – Monod constants for saturation of biomass and product by substrate, g/dm^3; K_{xi} и K_{pi} – substrate inhibition constants for biomass and product, g/dm^3; K_{px} и K_{pp} – product inhibition constants for biomass and product, g/dm^3.

The parameters in the kinetic equations are calculated by solving the system of differential equations using the Runge-Kuta method of the 4th row, by minimizing the sum of the squares of the difference between the experimental and model data. The software used was Microsoft Excel 2013 (Choi et al., 2014).

RESULTS AND DISCUSSION

The dynamics of the studied fermentation process in the complex nutrient medium MRS-broth is presented in Fig. 2.

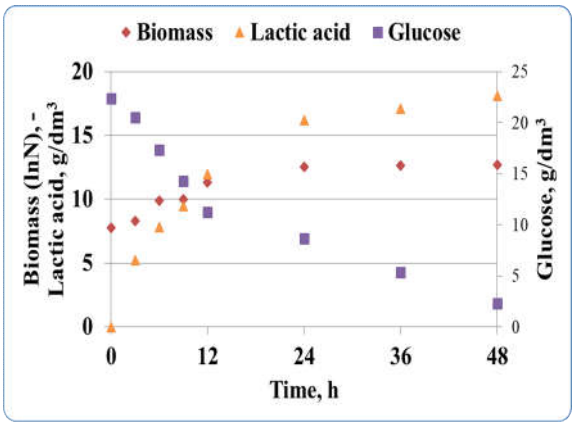

Figure 2: Dynamics of Lactic Acid Fermentation in Cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth in Bioreactor with Stirring

The data show that the fermentation process developed according to the trends for this process type. The duration of the lag phase was about 3 hours, after which the culture entered the exponential growth phase. The exponential growth phase lasted about 24 hours, and at the end of this phase a high number of viable cells was reached - 12.57 logarithmic units. In the next 24 hours, the culture was in the stationary phase, and it retained the high number of viable cells. In this phase, the substrate continued to be utilized at a high rate and lactic acid was constantly accumulating. At the end of the process, the lactic acid concentration reached 18.09 g/dm^3 and the substrate concentration decreased to 2.3 g/dm^3. The unutilized substrate was due to the influence of the product and the substrate inhibition processes, which should be taken into account. This means that in order to optimize the process, opportunities for the complete utilization of the substrate should be sought, which is achieved by optimizing the concentration of the substrate. It is known that the process of cultivation of lactic acid bacteria is substrate and product dependent (inhibited) (Bouguettoucha et al., 2011; Kostov, 2015).
The first two models we are going to discuss at are the classic Monod model and the Haldane model. The data for the kinetic parameters and the errors of the models are shown in Table 1, and the convergence of the models to the experimental data is given in Fig. 3 and Fig. 4.

Table 1: Kinetic Constants in the Different Models Used to Describe the Microbial Growth Kinetics of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1

	Monod	Haldane	Aiba	Haldane-Aiba	Haldane for product inhibition	Haldane-Jerusalimski
μ_{max}, h^{-1}	0.072	0.038	0.576	0.049	0.113	0.011
K_{SX}, g/dm^3	43.05	32.51	213.78	47.59	65.69	17.55
K_{Xi}, g/dm^3	-	85.45	-	156.67	163.23	283.82
q_{pmax}, h^{-1}	0.065	0.3982	0.266	0.063	0.256	0.065
K_{SP}, g/dm^3	11.13	132.36	29.71	0.005	29.12	41.39
K_{SPi}, g/dm^3	-	441.11		180.72	96.60	169.39
K_{PX}, g/dm^3	-	-	0.16	0.249	22.38	70.09
K_{PP}, g/dm^3	-	-	0.11	0.138	18.53	3.36
$1/Y_{x/s}$	0.3416	0.3055	0.6181	0.3318	0.3190	0.6680
$1/Y_{p/s}$	12	0.9820	2.2487	1.9694	0.0400	0.0400
$Y_{x/s}$	2.9277	3.2733	1.6176	3.0139	3.1348	1.4970
$Y_{p/s}$	0.0833	1.0183	0.4021	0.5078	25	25
R^2 (biomass)	0.7895	0.8473	0.8860	0.8712	0.9378	0.871
Error (biomass)	0.84	0.69	0.33	0.27	0.38	0.48
R^2 (product)	0.9507	0.9720	0.9763	0.983	0.9814	0.8895
Error (product)	2.63	2.42	1.42	1.38	1.42	1.80
R^2 (substrate)	0.9194	0.9361	0.9946	0.9907	0.9943	0.9697
Error (substrate)	2.75	2.25	1.27	1.23	1.27	1.12
S_{opt} (biomass), g/dm^3	-	52.71	-	86.35	103.55	70.58
S_{opt} (product)), g/dm^3	-	241.63	-	0.96	53.04	82.37

The data from Fig. 3 and Fig. 4, as well as those in Table 1, show that the Monod model and the Haldane model agree very well with the experimental data.

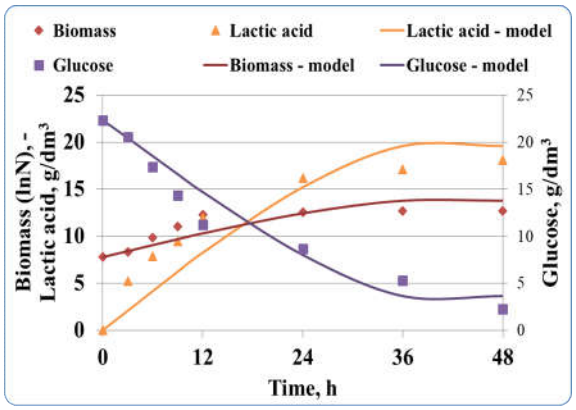

Figure 3: Kinetics of Lactic Acid Fermentation in Cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth Described with the Monod Model

The correlation coefficients range from 0.7895 to 0.9720. The values of the calculated errors vary in the range of 0.69 to 2.25. The Monod model gives almost twice the maximum specific growth rate (0.072 h^{-1}) compared to the Haldane model - 0.038 h^{-1}, due to the fact that the Haldane model takes into account the substrate inhibition of the lactic acid fermentation process. In both models there is an increased saturation constant of the substrate - 43.05 g/dm^3 and 32.51 g/dm^3, respectively, (in this case the substrate is equated to the concentration of the carbon source - glucose 20 g/dm^3), which confirms the observation that glucose is the substrate limiting the fermentation process. The two models also give different values with respect to the maximum specific lactic acid accumulation rate. The fact that the Haldane model gives about 6 times higher rate of acid formation (0.3982 h^{-1}) than the Monod model (0.065 h^{-1}) is very interesting. This also leads to significant differences in the saturation constants by product - 11.13 g/dm^3 for the Monod model and 132.36 g/dm^3 for the Haldane model. This difference shows that according to the Haldane model the rate of acid formation depends to a greater extent on the concentration of the limiting substrate.

It is interesting to determine theoretically at what substrate concentrations the culture will undergo substrate inhibition. Information on this is given by the substrate inhibition constants for biomass and product in the Haldane model - K_{Xi} and K_{SPi}, respectively. From the data presented in Table 1 it can be seen that the inhibitory effect of the substrate on cell proliferation and growth will begin to be observed at K_{Xi} = 85.45 g/dm^3 and K_{SPi} = 441.11 g/dm^3, which is 8.545% and 44.11% substrate (glucose) in the nutrient medium, respectively. K_{Xi} is close to the experimental results of various authors who found that at concentrations of the substrate (glucose) in the nutrient medium higher than 10%, its inhibitory effect on the specific growth rate of lactic acid bacteria becomes noticeable.

However, the K_{SPi} calculated by the Haldane model has an abnormally high value, which deviates greatly from the K_{Xi}. In this case, the value of K_{SPi} is real from mathematical point of view, but not from biological point of view, because such high glucose concentration in the medium will not allow the growth and accumulation of high numbers of viable cells that actively produce lactic acid. It should be noted that the

inhibition may be due not only to the carbon source, but also to some of the complex components in the nutrient medium. However, this is difficult to account for with non-structural models that are usually used to describe the fermentation process.

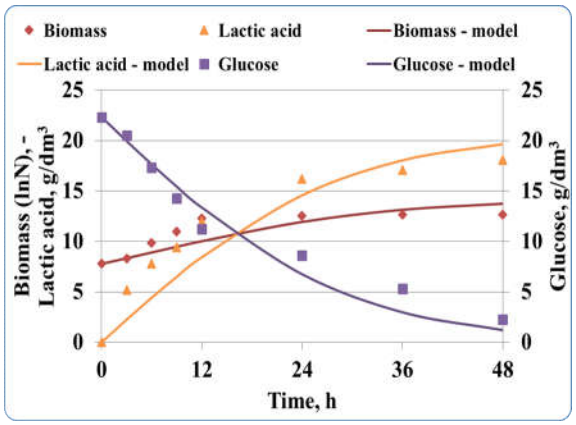

Figure 4: Kinetics of Lactic Acid Fermentation in Cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth Described with the Haldane Model

It is also interesting to determine the metabolic (trophic) coefficients - $1/Y_{X/S}$ and $1/Y_{P/S}$, showing the consumption of substrate for biomass growth and for product synthesis. Since they are the reciprocal values of the respective economic coefficients, the latter can also be determined. From the data presented in Table 1 it is evident that both models show higher substrate consumption for biomass formation – the trophic coefficients being 0.3416 and 0.3055, respectively, while the economic coefficients being 2.9277 and 3.2733, respectively, and a smaller part of the substrate goes to lactic acid synthesis ($1/Y_{P/S}$ - 12 and 0.9820, respectively, and $Y_{P/S}$ - 0.083 and 1.0183, respectively).
The Haldane mathematical model has an advantage over the Monod model - one can determine theoretically what the optimal substrate concentration will be at the optimal (maximum) specific growth rate:

$$\mu = \mu_{\max}^{opt} \Rightarrow S_{opt} = \sqrt{K_{SX} K_{SXi}} \qquad (10)$$

Similarly, the optimal substrate concentration at which the rate of lactic acid synthesis will be optimal (maximum value) can be determined:

$$q_p = q_{p\max}^{opt} \Rightarrow S_{opt} = \sqrt{K_{SP} K_{SPi}} \qquad (11)$$

Then, according to the Haldane model, S_{opt} for the growth and reproduction of the strain will be 52.71 g/dm^3, which is 5.271% glucose in the nutrient medium. This value is also close to experimentally determined values by other authors (Bouguettoucha et al., 2011). Here, too, it should be noted that the concept of substrate should be considered as a balanced set of components that are necessary for cell growth. In this case, the result obtained is very close to the total amount of components in the used complex nutrient medium. For the acid formation rate S_{opt} is 241.63 g/dm^3.

According to these data obtained from the Haldane model, it can be concluded that for *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 the substrate concentration should be in the range from 52.71 g/dm^3 to 241.63 g/dm^3, and if the substrate concentration is above 241.63 g/dm^3 there will be complete inhibition of both the growth of the strain and its biosynthetic ability.
Lactic acid fermentation is also a product-inhibited process (Bouguettoucha et al., 2011;), which is why the growth kinetics and lactic acid biosynthesis of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 with the Aiba model have been modeled. The results are shown in Fig.5, and the parameters of the model are presented in Table. 1.

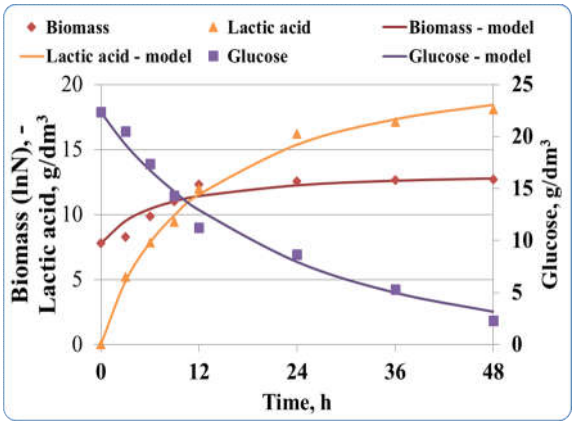

Figure 5: Kinetics of Lactic Acid Fermentation in Cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth Described with the Aiba Model

The Aiba model is characterized by high correlation values ranging from 0.8860 to 0.9946 and low identification errors. It gives relatively high rates of the specific growth rate - 0.576 h^{-1} and 0.266 h^{-1}, but also confirms product inhibition. This is evidenced by the relatively close values of the constants K_{PX} and K_{SP} - 0.16 g/dm^3 and 0.11 g/dm^3. This in turn confirms that the cultivation process of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 must be carried out with neutralization of the lactic acid produced in order to achieve high maximum growth rate of the culture and high concentration of active cells. Low values of the product inhibition constants can also be considered as an indirect indicator of the sensitivity (resistance) of the strain to the acidic pH of the stomach, which means that this strain is good to be used in encapsulated form, as a probiotic strain. The presence of product inhibition increases the saturation constant value for biomass (213.78 g/dm^3). The Aiba model again shows that most of the substrate is used for biomass formation. The values of the trophic coefficients - $1/Y_{X/S}$=0.6182 and $1/Y_{P/S}$=2.2487, and therefore the values of the economic coefficients $Y_{X/S}$=1.6176 and $Y_{P/S}$=0.4021 serve as a proof of this conclusion.
The data described so far show that lactic acid fermentation is both a substrate- and a product-inhibited process, which should be taken into account in its

modeling. Combined models such as the Haldane-Aiba model (equation 7), the Haldane model for product inhibition (equation 8) and the Haldane-Jerusalimski model (equation 9) can be used for this purpose. The results of the modeling of the cultivation process of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 are reflected in Fig. 6 to Fig. 8, as well as in Table 1.

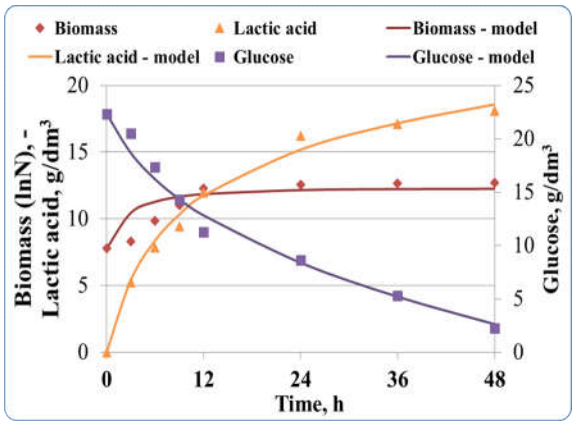

Figure 6: Kinetics of Lactic Acid Fermentation in Cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth Described with the Haldane-Aiba Model

The data in Table. 1 show that all three models, including product and substrate inhibition, describe experimental data with high accuracy. The correlation coefficients vary in the range of 0.87 to 0.9943, the errors - in the range of 0.27 to 1.8 for the individual indicators.

The data in Table 1 show that the Haldane-Aiba model (Equation 7) and the Haldane-Jerusalimski model (Equation 9) predict lower biomass specific growth rate of 0.049 h^{-1} and 0.011 h^{-1}, respectively. The Haldane model for product inhibition (Equation 8) predicts growth rate of 0.113 h^{-1}. This difference is due to the approach used by the three models to describe the processes of product and substrate inhibition. The Haldane-Jerusalimski model determines lower value of the saturation constant - 17.55 g/dm^3 compared to the Haldane-Aiba model - 47.59 g/dm^3 and the Haldane model for product inhibition - 65.69 g/dm^3. Despite this difference, the results are within the range expected in the cultivation of lactic acid bacteria. The data in Table 1 show that the three models predict growth inhibition by the substrate at concentrations above 15.67%, i.e. well above the current value of glucose in the MRS-broth medium. The models show that complete inhibition of growth by the substrate will occur only at glucose concentrations in the medium above 28.38%, and such values are not typical for nutrient media designed for the cultivation of lactic acid bacteria.

Data on the specific rate of lactic acid formation are of great interest in the enlisted models. The Haldane-Aiba model and the Haldane-Jerusalimski model give quite low values of q_{pmax} - 0.063 h^{-1} - 0.065 h^{-1}, which means that the MRS-broth medium is designed to allow enhanced synthesis of biomass at the expense of lactic acid production. The Haldane model for product inhibition predicts more intense process of lactic acid biosynthesis, as evidenced by the significantly higher maximum specific rate of acid formation (Table 1).

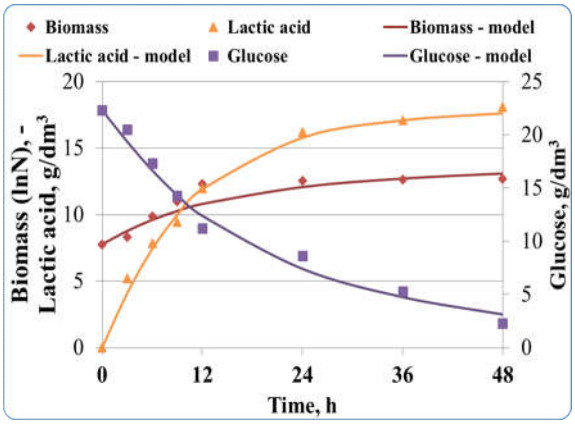

Figure 7: Kinetics of Lactic Acid Fermentation in Cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth Described with the Haldane model for Product Inhibition

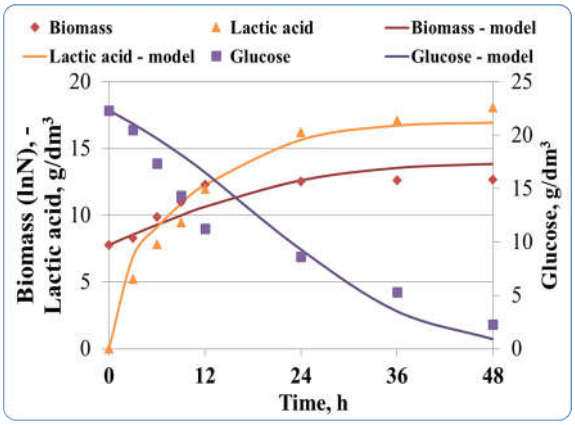

Figure 8: Kinetics of Lactic Acid Fermentation in Cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth Described with the Haldane-Jerusalimski Model

However, the three models give different degrees of influence of lactic acid formation on cell growth. The Haldane-Aiba model shows relatively strong product inhibition due to low K_{PX} and K_{PP} values of 0.249 g/dm^3 and 0.138 g/dm^3, respectively. The Haldane model for product inhibition gives higher K_{PX} and K_{PP} values - 22.38 g/dm^3 and 18.53 g/dm^3, which according to this model means that the process is not product inhibited so strongly. The value of the product inhibition constant for the biomass given by the Haldane-Jerusalimski model is abnormally high. From a mathematical point of view, this value is correct and brings the values calculated by the model closer to the experimental ones, but its biological meaning is doubtful, as this is too high a concentration of lactic acid at which the specific growth rate would be half the maximum value. For K_{PP} this model gives a biologically realistic value - 3.36 g/dm^3.

The three models, including product and substrate inhibition, also allow the determination of the optimal

substrate concentrations to provide maximum (optimal) specific growth rate or acid formation (Table 1). The data presented in the table show that the Haldane-Aiba model and the Haldane-Jerusalimski model give close values of the optimal glucose concentration - 86.35 g/dm^3 and 70.58 g/dm^3, respectively. Once again, one must recall that the models determine the optimal concentration of the balanced set of components, rather than just the concentration of the carbon source in the medium. The Haldane model for product inhibition rather sets the substrate concentration limit (103.55 g/dm^3) at which inhibition of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 growth will begin.

Similar conclusions can be made for S_{opt} for lactic acid synthesis. This time, however, the Haldane model for product inhibition and the Haldane-Jerusalimski model predict substrate concentrations that are characteristic of lactic acid bacteria. Only the Haldane-Aiba model showed an abnormally low value for S_{opt} - 0.96 g/dm^3.

The results obtained (Fig. 2 to Fig. 8 and Table 1) do not support the statement cited in the introduction that the MRS-broth medium may not be suitable for the cultivation of strains originating from dairy products. In the cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 in MRS-broth data show that the strain grew at relatively high growth rates and lower rates of lactic acid accumulation. The effects of substrate and product inhibition are normal for this type of fermentation, which allows relatively higher concentrations of viable cells to accumulate at the end of fermentation.

CONCLUSION

An important requirement for selection of strains to be included in the production of probiotic foods and preparations is the ability of the selected strains to be cultivated in industrial conditions and to accumulate high concentrations of viable cells. In the present work, the cultivation of *Lactobacillus delbrueckii* ssp. *bulgaricus* B1, isolated from home-made yoghurt, cultivated in a complex culture medium (MRS-broth) was studied. Complex media provide a balanced set of components - carbon, nitrogen and phosphorus source, micro- and macroelements. These media are usually designed to provide optimal growth, but in some cases are unsuitable for certain strains. The obtained results show that the selected probiotic strain *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 grew at relatively high specific growth rates and accumulated moderate amounts of lactic acid, determined by moderate specific acid formation rates. The data show that the strain may be sensitive to lactic acid, which is why pH adjustment and neutralization of lactic acid accumulated in the medium can be applied in industrial cultivation. This will ensure complete absorption of the substrate by the cells and the accumulation of maximum cell numbers in the medium.

The data obtained show that, although of dairy origin, *Lactobacillus delbrueckii* ssp. *bulgaricus* B1 can be cultivated in the complex nutrient medium MRS-broth.

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ACKNOWLEDGEMENTS

This work were supported by the Bulgarian Ministry of Education and Science under the National Research Programme "Healthy Foods for a Strong Bio-Economy and Quality of Life" approved by DCM № 577/17.08.2018 and by the project "*Strengthening the research excellence and innovation capacity of University of Food Technologies - Plovdiv, through the sustainable development of tailor-made food systems with programmable properties*", part of the European Scientific Networks National Programme funded by the Ministry of Education and Science of the Republic of Bulgaria (agreement № Д01-288/07.10.2020).

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