EVALUATION OF THE PRIMARY METABOLISM OF MONOCULTURES AND YOGHURT STARTERS WITH THE PARTICIPATION OF UREASE-DEFICIENT *STREPTOCOCCUS THERMOPHILUS* STRAINS

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KEYWORDS

Primary metabolism, kinetics, yoghurt starters, urease-deficient, *Streptococcus thermophilus*, modeling

ABSTRACT

The aim of the present work was to evaluate the influence of the lack of urease activity in some *Streptococcus thermophilus* strains on the primary metabolism during lactic acid fermentation. A comparison of the kinetics of the lactic acid process with the participation of urea-utilizing and urease deficient streptococcal strains was performed. It was found that the lack of urease activity in the streptococcal strains had no significant effect on the primary metabolism of the monocultures or the yoghurt starters.

INTRODUCTION

Streptococcus thermophilus strains are important part of the composition of dairy starters. Through their accelerated metabolism they accumulate lactic acid in the media and quickly reduce the pH to ensure the necessary conditions for the growth of the *Lactobacillus delbrueckii* ssp. *bulgaricus* strains. The proven positive symbiotic effect in the growth of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* strains in the composition of yoghurt starters is known as "protocooperation" (Angelov et al., 2009; Driesses, 1987).

It is well known that among all the lactic acid bacteria only *Streptococcus thermophilus* strains possess high urease activity and the ability to hydrolyze urea from raw milk. As a result of the hydrolysis urea is decomposed to ammonia and CO_2, which enhances the anaerobic conditions of the medium, thereby stimulating the growth of the *Lactobacillus delbrueckii* spp. *bulgaricus* strains from the yoghurt starters. At the same time the accumulation of ammonia results in a lower rate of pH reduction, which elongates the fermentation process (Angelov et al., 2009; Arioli et al., 2007; Ninova-Nikolova, 2016).

The main role of *S.thermophilus* in the process of lactic acid fermentation is to accelerate acid formation and accumulation and pH reduction of the medium by the production of lactic acid. Therefore, the rate of acid formation is an important technological parameter as the extension of the acid formation time has a negative impact on product quality and brings negative economic consequences in the organization of industrial process. The rate of acid formation is a strain-specific metabolic trait that is influenced by different physiological properties such as lactose-galactose metabolism, proteolytic system and urease activity (Ninova-Nikolova, 2016).

Urease activity has a serious negative impact on the quality of dairy products - cheese, yogurt, etc. Therefore, its exclusion from metabolism significantly improves the end-products (Ninova-Nikolova, 2016). Since protocooperation between the two types of bacteria is essential in industrial starters, the lack of urease activity must be assessed in relation to possible changes in the primary metabolism and the accumulation of lactic acid. The application of urease-deficient streptococcal strains, result of spontaneous mutation, has practical application due to restrictions on the use of genetically modified organisms (Ninova-Nikolova, 2016).

The purpose of the present work was to study the kinetics of lactic fermentation using urease-deficient streptococcal strains as monocultures and in the composition of yoghurt starters. Thus some of the most common kinetic models to assess the fermentation process were applied.

MATERIALS AND METHODS

Monocultures and yoghurt starters

Symbiotic yoghurt starters and monocultures of *Streptococcus thermophilus* were stored at -196 °C as part of the collection of LB Bulgaricum PLC, Sofia, Bulgaria. The following *Streptococcus thermophilus* monocultures were used: *Streptococcus thermophilus* Ft_3 - isolated from BY LBB 26-12; *Streptococcus thermophilus* Yt3 - isolated from BY LBB 145-18; *Streptococcus thermophilus* Rzt - isolated from BY LBB Razgrad. After natural selection the following urease-deficient *Streptococcus thermophilus* strains were selected: *Streptococcus thermophilus* Ft3uD3 – urease-deficient version of *Streptococcus thermophilus* Ft_3; *Streptococcus thermophilus* Yt3D3-1 – urease-deficient version of *Streptococcus thermophilus* Yt3; *Streptococcus thermophilus* Rzt4uD2 – urease-deficient version of *Streptococcus thermophilus* Rzt4 (Ninova-Nikolova, 2016).

The yoghurt starters used were: Starter LBB BY 26-12; Starter b26 + Ft3uD3; Starter LBB BY 145-18; Starter BY 145-Yt3uD3-1; Starter LBB BY RAZGRAD; Starter

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BY Rzb2 + Rzt4uD2. Each yoghurt starter consisted of a *Lactobacillus delbrueckii* ssp. *bulgaricus* strain and a mutant urease-deficient *Streptococcus thermophilus* strain (Ninova-Nikolova, 2016).

Media

Milk based fermentation media by "LB Bulgaricum" PLC were used in the present work.

Bioreactor, culture conditions and sample analysis

Cultivation was performed in a bioreactor with a working volume of 2 dm^3, equipped with a system for monitoring and control of the fermentation process "BIOFLO". The system is equipped with circuits for automatically maintaining the pH. The adjustment of pH was carried out with 2 M NaOH at continuous stirring. The anaerobic conditions of the fermentation were guaranteed by its conduction under inert gas – nitrogen medium. The temperature of the culture medium was maintained automatically through control actions, generated by the control device.

The fermentation process was carried out at temperatures of 39 °C and 43 °C, pH 5.90 and 6.20 at a stirring speed of 150 rpm. After loading the medium and its sterilization at 120 °C for 20 min and subsequent cooling down to the fermentation temperature, it was inoculated with 5% culture of the studied *Streptococcus thermophilus* strain or yoghurt starter. Milk samples were taken by sterile sampling system every 30 minutes since the beginning of the process. They were analyzed to determine the concentration of viable cells and the titratable acidity. The changes in the acidity of the medium were monitored by the amount of the NaOH used for the neutralization of the lactic acid accumulated in the apparatus.

The total number of viable cells of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* in cfu/cm^3 was determined by the pour plate method and the spread plate method in synthetic MRS medium and M_{17} medium, according to the methodology described in ISO 7889: 2005 (ISO 7889: 2005).

The amount of lactic acid was calculated based of the amount of 2M NaOH used for the neutralization (ISO/TS 11869:2012).

Mathematical models and determination of the kinetic characteristics

The kinetics of the lactic acid fermentation process were examined by the system of differential equations (Birol et al., 1998; Kostov et al., 2012):

$$\begin{aligned} \frac{dX}{dt} &= f(X,S,P) \\ \frac{dP}{dt} &= f(X,S,P) \\ \frac{dS}{dt} &= f(X,S,P) \end{aligned} \qquad (1)$$

The used kinetic equations (Birol et al., 1998; Kostov et al., 2012) through which the system (1) acquires a certain type are presented in Table 2.

Table 2

Mathematical models for description of the kinetics of the fermentation process

№	Model	dX/dt	dP/dt	dS/dt
1	**Monod**	$\mu_{\max}\left(\frac{S}{K_{sx}+S}\right)$	$q_{P\max}\left(\frac{S}{K_{sp}+S}\right)X$	$-\frac{1}{Y_{x/s}}\frac{dX}{dt}-\frac{1}{Y_{p/s}}\frac{dP}{dt}$
2	**Tiessier**	$\mu_{\max}\left(1-\exp\left(\frac{S}{K_{sx}}\right)\right)X$	$q_{P\max}\left(1-\exp\left(\frac{S}{K_{sP}}\right)\right)X$	
3	**Hinshelwood**	$\mu_{\max}\left(\frac{S}{K_{sx}+S}\right)(1-K_{PX}P)X$	$q_{P\max}\left(\frac{S}{K_{sp}+S}\right)(1-K_{PP}P)X$	
4	**Aiba**	$\mu_{\max}\left(\frac{S}{K_{sx}+S}\right)\exp(-K_{PX}P)X$	$q_{P\max}\left(\frac{S}{K_{sp}+S}\right)\exp(-K_{PP}P)X$	

Parametric identification of the models was carried out in MATLAB environment (Kostov et al., 2012; Mitev and Popova, 1995; Popova 1997). The sum of squared errors of the model output data:

$$F(r)=\left(X(k_1,...,k_n)-X^e\right)^2+\left(S(k_1...,k_n)-S^e\right)^2+\left(P(k_1,...,k_n)-P^e\right)^2 \qquad (2)$$

was minimized. For that purpose the function "fmincon" was applied.

The output is vector of "fmincon" are model parameters $k=[k_1,k_2,...,k_n]$, where $k_1,k_2,...,k_n$ are constants.

The overall differential equations s0ystem the function "ode45" was used.

RESULTS AND DISCUSSION

The results of the identification of kinetic models are summarized in Table 3 to Table 6. On Figures 1 and 2 are presented comparisons of the models for some of monocultures and starter cultures.

Monod-based kinetics

The results of the parameters identification of Monod's model are presented in Table 3. Data showed that monocultures grew with a good fermentation rate that varies within a relatively narrow range between 0,040 and 0,510 h^{-1}. Only one of the variants was characterized by a higher maximum specific growth rate, but the average rate of the process was comparable to that of the other variants, a fact that will be explained later.

The accumulation of cells of the urease-deficient strains was not affected by the lack of urease in their metabolism. Similar values of the specific growth rate were established in all variants. Consequently, the

absence of the gene responsible for the utilization of urea from the composition of milk did not affect the growth of the cells.

It was typical for streptococcal monocultures that in terms of the accumulation of viable cells they had high affinity to the substrate. The saturation constant K_{SX} for all variants was 0. Only in variant Yt3 no affinity was observed which lowered the maximum specific growth rate and it was within the already commented range between 0,040 and 0,510 h^{-1}.

The accumulation of lactic acid by the streptococcal monocultures proceeded with high specific rate of lactic acid production and with high affinity between the substrate and the culture. The rate varied between 0,365 and 0,513 g/(g.h), which led to higher economic coefficient that ensured that the substrate was transformed mainly to lactic acid.

All models of the Mono-based kinetics described experimental data with high accuracy, the average error between them and the data was between 4 and 60.

Data showed that the exclusion of the gene responsible for the utilization of urea from milk did not change the primary metabolism of any monoculture. Therefore urease-deficient strains can be successfully included in the composition of yoghurt starters.

Table 3

Kinetic parameters of the growth of monocultures and yoghurt starters in the model of Monod

	μ_{max}	K_{SX}	q_{pmax}	K_{SP}	$Y_{X/S}$	$Y_{P/S}$	Error*10
Monocultures							
Ft3 (pH 5,9/ t 43°C)	0,040	0,000	0,444	0,000	0,376	1,185	5,62
Ft3uD3 (pH 5,9/ t 43°C)	0,048	0,000	0,468	0,000	0,268	1,436	3,00
Yt3 (pH 6,2/ t 43°C)	0,057	0,000	0,513	0,001	0,263	1,542	1,87
Yt3uD3-1 (pH 6,2/ t 43°C)	0,049	0,000	0,500	0,041	1,000	1,022	1,22
Yt3 (pH 6,2/ t 39°C)	0,121	10,000	0,372	0,000	0,713	0,882	3,21
Yt3uD3-1 (pH 6,2/ t 39°C)	0,043	0,000	0,365	0,000	0,237	1,697	4,09
Rzt4 (pH 6,0/ t 43 °C)	0,044	0,000	0,463	0,000	0,224	1,540	4,58
Rzt4uD2 (pH 6,0/ t 43°C)	0,043	0,000	0,474	0,000	0,465	1,140	3,96
Yoghurt starters							
LBB BY 26-12 (medium 1)	0,047	0,000	0,334	0,000	0,300	1,221	2,60
LBB BY 26-12 (medium 2)	0,043	0,000	0,284	0,123	0,350	1,801	1,42
b26+Ft3uD3 (medium 1)	0,077	10,000	0,329	0,000	1,000	0,852	0,51
b26+Ft3uD3 (medium 2)	0,050	0,000	0,233	0,000	0,239	3,636	0,35
LBB BY 145-18 (medium 1)	0,041	3,244	0,256	0,000	0,079	10,000	0,53
LBB BY 145-18 (medium 2)	0,083	1,258	0,244	0,083	0,269	10,000	0,29
BY 145-Yt3uD3-1 (medium 1)	0,035	0,000	0,227	0,000	0,193	1,048	0,68
BY 145-Yt3uD3-1 (medium 2)	0,064	1,496	0,232	0,000	0,214	10,000	0,49
LBB BY RAZGRAD (medium 1)	0,049	0,000	0,349	0,012	0,118	2,853	0,49
LBB BY RAZGRAD (medium 2)	0,083	2,985	0,311	0,564	0,196	10,000	0,4
BY Rzb2+Rzt4uD2 (medium 1)	0,039	0,000	0,289	0,000	0,198	2,255	0,82
BY Rzb2+Rzt4uD2 (medium 2)	0,054	1,095	0,234	0,000	1,000	0,983	0,31

A similar study of the kinetics of the primary metabolism with inclusion of urease-deficient strains in the composition of several yoghurt starters was performed. Due to the specific requirements for the obtaining of the yoghurt starters the cultivation was conducted in two different media.

Data in Table 3 showed that the symbiotic yoghurt starter strains of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* grew with maximum specific growth rate in the range of 0,036-0,083 h^{-1}, but also at higher specific rates of accumulation of lactic acid - 0,284 - 0,349 g/(g.h). The affinity of the strains to the substrate in the medium was high with slight deviations from this summarization concerning only few strains. The inclusion of urease-deficient strains in the composition of yoghurt starters did not alter the primary metabolism; on the contrary, it accelerated the process and milk coagulated 30 min earlier in variants with urease deficient *Streptococcus thermophilus* strain than in the variants in which urea was utilized. This was due to the previously mentioned fact that the accumulation of ammonia leads to the neutralization of the pH, hence, to slower coagulation.

An interesting fact is that the exclusion of sodium acetate from the composition of the medium caused lack of affinity of the yoghurt starter to the substrate, which was probably due to adversely influencing the utilization of the carbon source and the need for microorganisms to accumulate additional substances which would allow them to utilize lactose from milk.

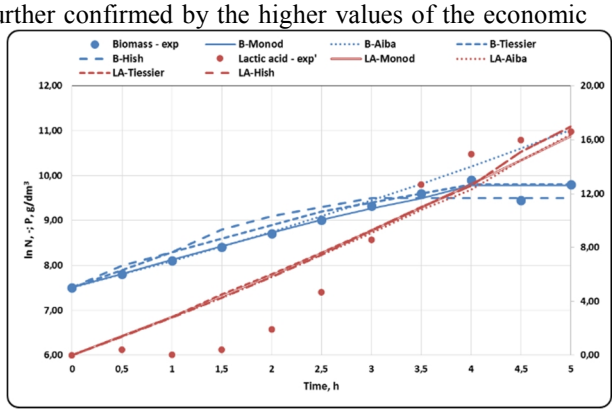
The main amounts of substrate were utilized and converted to lactic acid, which was confirmed by the higher values of the coefficients $Y_{P/S}$ compared with $Y_{X/S}$ (Table 3).

Aiba based kinetics

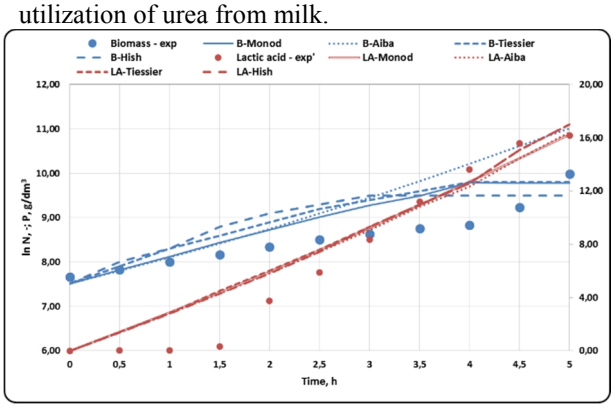
A common characteristics of many biological processes is that the accumulation of the metabolic products may be associated with the inhibition of growth. The model of Aiba assumes exponential growth inhibition as a result of

the accumulated lactic acid in the medium. Data from the parameters identification of the model of Aiba are presented in Table 4.

Similarly to Monod's model, the monocultures grew primarily by accumulating lactic acid, which was proven by the high values of the specific rate q_{pmax} and the relatively high values of μ_{max}. This observation was further confirmed by the higher values of the economic coefficient, which were associated with the accumulation of product obtained from one unit of substrate. The model parameters did not show substrate or product inhibition; the error in describing the test results was minimized. Data from Aiba's model showed that the primary metabolism of streptococcal monocultures was not affected by the exclusion of the gene responsible for the utilization of urea from milk.



а) monoculture of Yt3 (pH 6,2/ t 43°C)



б) monoculture of Yt3uD3-1 (pH 6,2/ t 43°C)

Fig.1. Comparison of kinetic models for cultivation of monoculture of Yt3 and Yt3uD3-1

Table 4

Kinetic parameters of the growth of monocultures and yoghurt starters in the model of Aiba

	μ_{max}	K_{SX}	q_{pmax}	K_{SP}	$Y_{X/S}$	$Y_{P/S}$	Kpx	Kpp	Error*10
Monocultures									
Ft3 (pH 5,9/ t 43°C)	0,066	0,000	0,424	0,000	0,999	1,078	0,000	0,000	14,20
Ft3uD3 (pH 5,9/ t 43°C)	0,073	0,000	0,447	0,000	0,962	1,101	0,000	0,000	11,50
Yt3 (pH 6,2/ t 43°C)	0,108	5,342	0,496	0,001	1,000	1,074	0,000	0,000	5,90
Yt3uD3-1 (pH 6,2/ t 43°C)	0,069	1,468	0,489	0,055	1,000	1,048	0,000	0,000	4,80
Yt3 (pH 6,2/ t 39°C)	0,077	0,000	0,359	0,000	1,000	1,143	0,000	0,000	10,29
Yt3uD3-1 (pH 6,2/ t 39°C)	0,058	0,000	0,355	0,000	0,959	1,084	0,000	0,000	6,20
Rzt4 (pH 6,0/ t 43 °C)	0,083	0,000	0,441	0,000	0,969	1,245	0,000	0,000	17,00
Rzt4uD2 (pH 6,0/ t 43°C)	0,075	0,000	0,449	0,000	0,986	1,209	0,000	0,000	16,40
Yoghurt starters									
LBB BY 26-12 (medium 1)	0,065	0,000	0,323	0,000	0,991	1,033	0,000	0,000	6,90
LBB BY 26-12 (medium 2)	1,425	0,000	0,293	0,428	0,104	0,730	6,231	0,000	2,42
b26+Ft3uD3 (medium 1)	0,061	3,665	0,327	0,000	0,128	10,000	0,000	0,000	3,10
b26+Ft3uD3 (medium 2)	1,500	0,167	0,225	0,000	0,108	0,700	7,051	0,000	11,80
LBB BY 145-18 (medium 1)	0,817	0,000	0,250	0,000	0,257	0,774	4,448	0,000	2,87
LBB BY 145-18 (medium 2)	0,085	0,789	0,243	0,010	1,000	1,120	0,000	0,000	0,59
BY 145-Yt3uD3-1 (medium 1)	0,051	0,000	0,224	0,000	0,107	10,000	0,071	0,000	4,41
BY 145-Yt3uD3-1 (medium 2)	0,056	0,210	0,236	0,001	0,702	1,173	0,000	0,000	1,21
LBB BY RAZGRAD (medium 1)	0,871	0,000	0,336	0,000	0,470	0,767	2,470	0,000	3,50
LBB BY RAZGRAD (medium 2)	1,354	0,483	0,281	0,002	1,000	0,953	5,863	0,000	1,71
BY Rzb2+Rzt4uD2 (medium 1)	0,052	0,000	0,283	0,000	0,990	1,058	0,000	0,000	3,38
BY Rzb2+Rzt4uD2 (medium 2)	0,049	0,000	0,234	0,000	0,994	1,031	0,000	0,000	0,88

Using the model of Aiba to describe the kinetics of the growth of symbiotic yoghurt starters showed other trends in comparison to those obtained by Monod's model. The maximum specific growth rate for some variants reached values between 1,35 and 1,50 h^{-1}, but at the same time substrate inhibition in these variants was observed. Thus, the overall growth rate would not be different from the other variants. Substrate inhibition was observed with both types of media, which was most likely due to the excess of some components of the medium. There was no product inhibition of the process of accumulation of lactic acid - the inhibition constant K_{pp} equaled 0 for all variants.

The inclusion of urease-deficient *Streptococcus thermophilus* strain in the composition of the yoghurt starters did not alter the primary metabolism of the yoghurt starter. Data from Aiba's model showed slight

increase in the specific growth rate of the symbiotic culture.

Hinshelwood based kinetics

Hinshelwood's model assumes a non-linear growth inhibition and the accumulation of a product which unlike the exponential one, is not as strong. Data from the identification of the parameters are presented in Table 5. Data from Hinshelwood's model, in accordance with the other two tested models, showed that monocultures were growing with maximum specific growth rate at maximum affinity to the substrate. The main amount of the substrate was utilized for the accumulation of lactic acid, the rate of this process being 8-10 times higher than that for the accumulation of biomass. This was confirmed by the values of the economic coefficient $Y_{P/S}$. The metabolism of the cells was not affected by the lack of urease in the mutants.

There were significant differences in studying the yoghurt starters in comparison with the conclusions drawn from the previous models. Minor to moderate lack of affinity to the substrate was typical for some of them. It might be provoked by the observed substrate inhibition of the growth of the symbiotic culture. Again, there was no product inhibition.

The error of the model as compared to the experimental data was minimal. The addition of the urease-deficient strain in the composition of the yoghurt starter did not change substantially the primary metabolism.

Table 5

Kinetic parameters of the growth of monocultures and yoghurt starters according to the model of Hinshelwood

	μ_{max}	K_{SX}	q_{pmax}	K_{SP}	$Y_{X/S}$	$Y_{P/S}$	Kpx	Kpp	Error*10
Monocultures									
Ft3 (pH 5,9/ t 43°C)	0,065	0,000	0,423	0,000	0,153	13,977	0,000	0,000	11,400
Ft3uD3 (pH 5,9/ t 43°C)	0,073	0,000	0,447	0,000	0,161	16,188	0,000	0,000	11,500
Yt3 (pH 6,2/ t 43°C)	0,075	0,158	0,495	0,000	1,144	1,046	0,000	0,000	5,850
Yt3uD3-1 (pH 6,2/ t 43°C)	0,063	0,388	0,487	0,003	0,189	2,344	0,000	0,000	4,810
Yt3 (pH 6,2/ t 39°C)	0,178	16,197	0,352	0,000	100,000	0,919	0,000	0,000	10,150
Yt3uD3-1 (pH 6,2/ t 39°C)	0,058	0,000	0,355	0,000	7,525	0,934	0,000	0,000	6,180
Rzt4 (pH 6,0/ t 43 °C)	0,076	0,000	0,438	0,000	0,214	3,759	0,000	0,000	15,210
Rzt4uD2 (pH 6,0/ t 43°C)	0,073	0,000	0,450	0,000	0,469	1,380	0,000	0,000	14,520
Yoghurt starters									
LBB BY 26-12 (medium 1)	0,064	0,000	0,324	0,000	0,205	3,154	0,000	0,000	7,310
LBB BY 26-12 (medium 2)	0,096	6,145	0,279	0,101	100,000	0,999	0,000	0,000	2,860
b26+Ft3uD3 (medium 1)	0,091	0,000	0,321	0,000	1,763	0,873	0,100	0,000	3,150
b26+Ft3uD3 (medium 2)	0,059	0,300	0,231	0,000	27,928	0,853	0,000	0,000	1,650
LBB BY 145-18 (medium 1)	0,192	50,000	0,253	0,000	0,089	5,511	0,024	0,000	2,800
LBB BY 145-18 (medium 2)	0,499	37,470	0,239	0,016	4,216	0,894	0,000	0,000	0,632
BY 145-Yt3uD3-1 (medium 1)	0,049	0,000	0,224	0,000	0,101	100,000	0,051	0,000	4,450
BY 145-Yt3uD3-1 (medium 2)	0,470	47,102	0,231	0,001	2,025	0,969	0,000	0,001	1,280
LBB BY RAZGRAD (medium 1)	0,070	0,948	0,343	0,000	0,103	100,000	0,028	0,000	2,280
LBB BY RAZGRAD (medium 2)	0,060	0,006	0,290	0,035	0,324	1,707	0,029	0,000	1,260
BY Rzb2+Rzt4uD2 (medium 1)	0,052	0,000	0,283	0,000	1,515	0,991	0,000	0,000	3,810
BY Rzb2+Rzt4uD2 (medium 2)	0,053	0,419	0,233	0,000	2,809	0,905	0,000	0,000	0,880

Tiessier based kinetics

Tiessier's models are modified Monod's models, but they suggest exponential reduction of the specific rates due to substrate inhibition. The results of the parameters identification are presented in Table 6.

The results of this model were highly contradictory. On one hand, the model describes satisfactorily part of the experimental data. With the other part of the data an error was observed, especially when describing the amount of the utilized substrate. On the other hand, the model showed that part of the monocultures had no affinity to the substrate, but, quite unexpectedly, a trend associated with urease deficit was not observed. On the contrary, some of the strains having urease activity, showed no affinity to the substrate. The explanation might be sought in the cultivation conditions. All monocultures grew at low specific growth rate, while those which had high values of the maximum specific growth rate showed no affinity to the substrate.

All monocultures showed high specific rate of accumulation of lactic acid. Data indicated that the substrate was utilized mainly for the production and accumulation of lactic acid.

As with the other models there was no effect on the primary metabolism in urease-deficient monocultures and yoghurt starters.

The description of the lactic acid process in the symbiotic yoghurt starter also showed interesting tendencies. For example, for almost all yoghurt starters medium 2 proved to be unsuitable for the conduction of the fermentation process. In this medium there was a significant lack of affinity to the substrate, wherein the overall growth rate decreased. The model of Tiessier gave conflicting

information about the metabolism in the yoghurt starters but it could be concluded that the primary metabolism was not affected.

Tiessier's model showed that changes related to the inclusion of urease-deficient streptococcal strains in the composition of the yoghurt starters can be observed, but this would be a subject of further investigation.

CONCLUSION

It was found that the primary metabolism of monocultures of urease-deficient streptococci and yoghurt starters with the inclusion of urease-deficient strains of *Streptococcus thermophilus* was not affected by the lack of urease activity. All tested models showed that the lactic acid fermentation process was characterized by good growth rates and very high values of the specific rates of accumulation of lactic acid. The process of lactic acid fermentation was not associated with substrate or product inhibition, except for few variants, but the inhibition in these variants was weak.

The results obtained with Tiessier's model, which showed lack of affinity of some monocultures and yoghurt starters to the culture medium, are interesting and give the possible ground for future research.

Table 6

Kinetic parameters of the growth of monocultures and yoghurt starters according to the model of Tiessier

	μ_{max}	K_{SX}	q_{pmax}	K_{SP}	$Y_{X/S}$	$Y_{P/S}$	Error*10
Monocultures							
6Ft3 (pH 5,9/ t 43°C)	0,055	1,608	0,432	0,094	6,000	0,940	11,600
Ft3uD3 (pH 5,9/ t 43°C)	0,063	1,945	0,455	0,076	6,000	0,946	11,700
Yt3 (pH 6,2/ t 43°C)	0,061	2,266	0,500	0,269	100,000	0,933	6,110
Yt3uD3-1 (pH 6,2/ t 43°C)	0,688	156,419	0,489	0,952	100,000	0,930	4,810
Yt3 (pH 6,2/ t 39°C)	0,107	11,585	0,358	0,014	95,693	0,919	10,300
Yt3uD3-1 (pH 6,2/ t 39°C)	0,051	0,000	0,360	0,007	0,955	1,060	6,310
Rzt4 (pH 6,0/ t 43 °C)	0,714	147,222	0,442	0,137	98,697	1,006	11,800
Rzt4uD2 (pH 6,0/ t 43°C)	0,201	202,500	0,455	0,269	98,697	1,000	12,100
Yoghurt starters							
LBB BY 26-12 (medium 1)	0,056	0,335	0,329	0,092	0,649	0,979	7,410
LBB BY 26-12 (medium 2)	1,813	250,000	0,278	0,884	66,830	0,998	2,800
b26+Ft3uD3 (medium 1)	0,270	75,560	0,326	0,019	0,685	0,974	3,180
b26+Ft3uD3 (medium 2)	1,867	226,463	0,229	0,001	217,022	0,850	1,720
LBB BY 145-18 (medium 1)	0,692	250,000	0,254	0,805	0,083	7,090	2,380
LBB BY 145-18 (medium 2)	1,494	131,465	0,242	0,657	29,876	0,865	0,630
BY 145-Yt3uD3-1 (medium 1)	0,373	122,830	0,224	0,027	0,185	1,163	4,480
BY 145-Yt3uD3-1 (medium 2)	0,058	1,934	0,237	0,363	39,202	0,862	1,200
LBB BY РАЗГРАД (medium 1)	0,475	100,872	0,342	0,001	0,229	1,110	3,510
LBB BY РАЗГРАД (medium 2)	2,377	250,000	0,290	0,661	69,911	0,867	1,220
BY Rzb2+Rzt4uD2 (medium 1)	0,039	1,035	0,000	249,643	13,802	5,802	1,170
BY Rzb2+Rzt4uD2 (medium 2)	1,209	227,701	3,995	0,086	16,415	37,806	1,120

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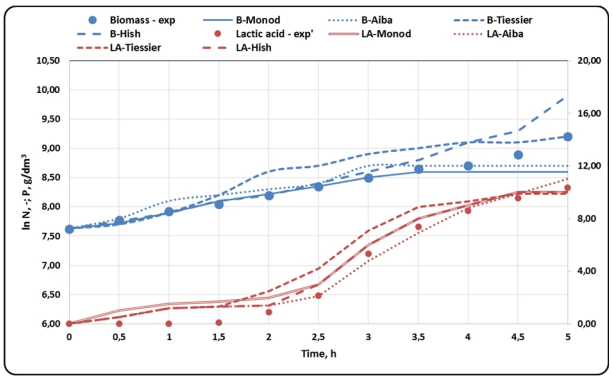
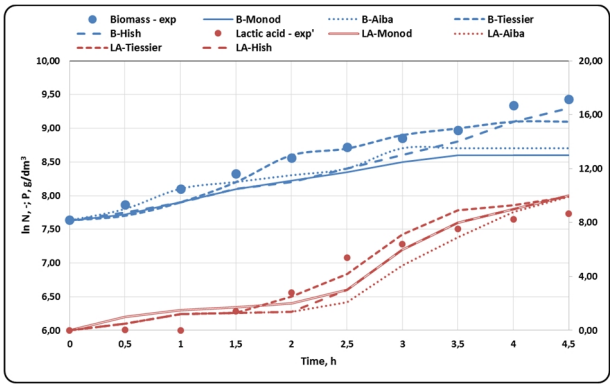


а) medium A



б) medium B

Fig.2. Comparison of kinetic models for cultivation of starter cultures with contribution of Yt3uD3-1

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