

MODELING OF OXYGEN EFFECT ON KINETICS OF BATCH FERMENTATION PROCESS FOR YOGURT STARTER CULTURE FORMATION

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ABSTRACT

The aim of this work is to describe the kinetics of yogurt starter culture production by *S.thermophilus* 13a and *Lb. bulgaricus* 2-11 and to quantitatively analyse the effect of different dissolved oxygen concentration in the milk on the process trend. Five different mathematical models for description of process kinetics are tested and the best one is selected. The increase of initial concentration of dissolved oxygen leads to a proportional decrease in the specific growth rate of the population and of the rates of lactose consumption and lactic acid production. On the basis of this investigations, two zones of initial dissolved oxygen concentration are defined. In each of these zones, the associated pair of microorganisms have different behaviour.

INTRODUCTION

The interaction between *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in a yogurt starter culture is characterized with an ecological indication named proto-cooperation [Driessen 1987, Simova et al. 1999]. The proto-cooperation is a basis for the symbiotic relationship build up between two species and collaborative metabolism with positive effect on fermented milk. The symbiotic co-existence of the

selected strains *S.thermophilus* and *Lb. bulgaricus* determines the typicality and strict individuality of yogurt. These two microorganisms that mutually provide for their development modify milk composition and as a result a product with specific nutritional profile is obtained. Furthermore, the microorganisms produce the specific aroma-defining compounds that in combination with other metabolites lead to specific aroma of the target product. The creation of a stable symbiotic association between a *S.thermophilus* and *Lb. bulgaricus* is really hard and time consuming process sometimes with no result [Driessen 1987, Driessen et al 1982a, Tamime and Robinson 2003].

Contributions of each of two micro organisms for such an interaction are investigated and some basic elements of mutual growth of lactobacillus and streptococcus are defined [Driessen 1987, Radke-Michell and Sandine 1986, Driessen et al. 1982a, Driessen et al. 1982b, Amoroso and Nanca de Nandra 1990, Farrow and Collins, 1984; Louaileche et al. 1993, Marshall, 1987, Radke-Michell and Sandine 1984, Tammam et al. 2000, Thunell and Sandine 1985, Tinson et al. 1982, Zourari et al. 1992]. The allegation that carbon dioxide is the unique element of proto-cooperation [Driessen et al. 1982b] has no scientific explanation by now. There is no proof that CO₂ takes part in *Lb. bulgaricus* metabolism too.

In answer to the question which is the stimulating factor for *Lb. bulgaricus* in the case of low urea activity of its partner in the associate pair, a detailed investigation of the specific role of milk dissolved oxygen in thermophilic

streptococcus and lactobacillus metabolism is carried out [Simova et al. 1999, Simova and Beshkova 2007, Simova 2007]. It is proven that the oxygen-tolerance of *S.thermophilus* 13a is the main growth factor for both investigated species in the starter culture as well as the main factor for proto-cooperation between them. *S.thermophilus* 13a assimilates oxygen in milk very quickly and in this way assures the anaerobic conditions required by *Lb. bulgaricus* 2-11. The Lactobacillus is actively cultivated and stimulates the metabolism of the mixed culture [Simova et al. 1999, Simova and Beshkova 2007, Angelov et al. 2002, Simova 2002, Simova 2007]. The main purpose of this work is to describe the kinetics of a yogurt starter culture consisting of *S.thermophilus* 13a and *Lb. bulgaricus* 2-11, using the investigation methods mentioned above, and to quantitatively analyse the effect of different oxygen concentrations on the starter culture formation.

MICROORGANISMS AND CULTIVATION CONDITIONS

Natural strains *S.thermophilus* 13a and *Lb. bulgaricus* 2-11 are isolated from home-made yogurts manufactured in Rodopite – mountain region in Bulgaria. A new highly effective symbiotic starter culture with a high degree of proto-cooperation between strains and high technological characteristics for the production of original Bulgarian yogurt is developed from *S.thermophilus* 13a and *Lb. bulgaricus* 2-11 [Simova et al. 1999, Simova and Beshkova 2007, Angelov et al. 2002, Simova 2002, Simova 2007].

The inoculum of monocultures and associations is prepared in the following way: after microbial and biochemical indexes control the whole cow milk is sterilized at 121°C for 15 minutes, then the milk is cooled to 43°C and is inoculated with 2% of the corresponding culture.

Batch cultivations of starter culture *S.thermophilus* 13a + *Lb. bulgaricus* 2-11 are carried out in bioreactor MBR AG Ltd. (Switzerland) with a geometric volume of 2 dm³ and control device IMCS – 2000.

The laboratory bioreactor MBR AG Ltd. is shown in Figure 1. The bioreactor is equipped with a six-blade turbine stirrer and four repulse devices. There are two orifices on the lid: one for feeding and the other for the installation of heat exchangers, sensors for temperature, pH and dissolved oxygen. The installation includes sensors and mechanisms for monitoring and control of the main physico-chemical process variables – pH, dissolved oxygen concentration and stirrer speed.

The feed (sterilized and restored dry milk containing 12% of dry material) and inoculates are inserted in the apparatus using a peristaltic pump that is indicated with number 10 in Figure 1.

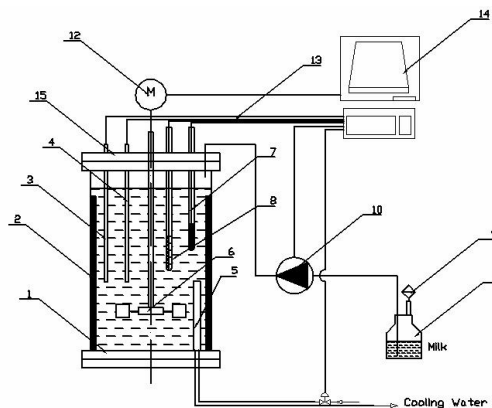


Figure 1: Laboratory bioreactor MBR AG Ltd scheme
 1 - apparatus with geometric volume 2 dm³; 2 - four repulse devices; 3 – thermo-strength Pt100; 4 – heater; 5 - heat exchanger for cold water; 6 – stirrer; 7 – pH electrode; 8 – dissolved oxygen electrode; 9 – filter; 10 – peristaltic pump; 11 – flask with sterilized milk; 12 – motor; 13 - control links; 14 – control device.

Dissolved oxygen concentration could be set using the oxygen control scheme that includes a steam-sterilized electrode of ‘Clark’ Ingold type. The control system is calibrated in distilled water. The measurements are taken and expressed in percentage of saturation.

The partial pressure of the milk-dissolved carbon dioxide is measured by an extra outline of steam-sterilized potentiometric CO₂-electrode Ingold and CO₂ amplifier 525, Ingold (Switzerland). The system is calibrated using the method proposed by Spinnler [Spinnler et al. 1987].

Prior to fermentation the milk is maintained at the temperature of 43°C for coagulation. The coagulants are kept refrigerated at 4°C.

During the fermentation, the following biochemical state variables are measured off-line: concentration of lactobacillus plus streptococci (CFU ml⁻¹), concentration of substrate lactose (g l⁻¹) and the concentration of lactic acid (g l⁻¹). Samples are taken at constant time intervals of 0.5 hour. Each experiment is carried out till the total acidity of the medium reaches 80⁰T then the process is stopped. The number of viable cells of lactic acid bacteria is measured in CFU (cm³)⁻¹ according to the IDF Standard 117B, 1977. The lactic acid and lactose are measured by enzyme-based methods (UV test Boehringer Mannheim, GmbH Biochemica).

KINETIC'S MODELS

In general, the dynamics of a batch fermentation process is described by the material balance equations as follows:

$$\begin{cases} \frac{dX}{d\tau} = \mu X \\ \frac{dP}{d\tau} = q_p X \\ \frac{dS}{d\tau} = -q_s X \end{cases} \quad (1)$$

Where X is the viable cells' concentration, P is the product's (lactic acid), S is the substrate's (lactose), μ , q_s and q_p are the specific growth rates of population growth, substrate consumption and metabolite production, respectively. The dependences of the specific kinetic rates on the process state variables determine the model structure [Bastin and Dochain 1990]. For the kinetics of *S.thermophilus* 13a + *L. bulgaricus* 2-11 growth, substrate consumption and product formation the following models are proposed [Biryukov 2004, Pirt 1975]:

Model 1:

$$\mu = \mu_{\max} \frac{S}{k_s + S} \quad (2a)$$

$$q_s = \frac{\mu}{Y} + m \quad (2b)$$

$$q_p = a + b\mu \quad (2c)$$

Model 2:

$$\mu = \mu_{\max} \frac{S}{k_s + S + k_i S^2} \quad (3a)$$

$$q_s = \frac{\mu}{Y} + m \quad (3b)$$

$$q_p = a + b\mu \quad (3c)$$

Model 3:

$$\mu = \mu_{\max} \frac{S}{k_s + S} \cdot \frac{k_p}{k_p + P} \quad (4a)$$

$$q_s = \frac{\mu}{Y} + m \quad (4b)$$

$$q_p = a + b\mu \quad (4c)$$

Model 4:

$$\mu = \mu_{\max} \frac{S}{k_s + S + P/k} \quad (5a)$$

$$q_s = \frac{\mu}{Y} + m \quad (5b)$$

$$q_p = a + b\mu \quad (5c)$$

Model 5:

$$\mu = \mu_{\max} \frac{k_p}{k_p + P} \quad (6a)$$

$$q_s = \frac{\mu}{Y} + m \quad (6b)$$

$$q_p = a + b\mu \quad (6c)$$

It can be observed that, the main difference between the five models appears in relation to the specific growth rates (a). The models for specific substrate (b) and product formation (c) rates are the same for all models. Therefore, the identification task is reduced to the identification of the most suitable relation to describe the specific growth rate, as well as the model parameters using the experimental data. The process data base is designed using generalized values from three independent experiments. The data is used for testing all models (2-6). The system of differential equations (1) is analytically solved using models (2) to (6) in a sequence. The model parameters are iteratively modified until the pre-set criteria – based on the square error between experimental and model data - are satisfied.

RESULTS AND DISCUSSION

The choice of kinetic dependences is made taking into account the expert's knowledge of the investigated process. At the beginning, the classical Monod law (2a) was tested. However, it is known from the literature that the growth kinetics of this process does not always follow this relationship [Biryukov 2004, Pirt 1975]. Therefore, in the work, models are preferred which account for growth inhibition by the substrate and/or product. The Haldane's growth kinetics (3a) was then tested as well. The results showed a reasonable dependence of cell's growth on the substrate and the product concentrations. Then the blocks of growth investigated next (4a and 5a) include the influence of substrate and product inhibition on microbial growth. It is well known that the lactic acid process is inhibited by the product concentration [Pirt 1975]. For this reason, the model of Jerusaliwski and Enganbervediev (6a) was also tested. The model describes the inhibitor effect of product concentration only. The tested models have simple structures, but they describe with good accuracy the investigated lactic acid process taking into account the experiments with different initial concentrations of milk-dissolved oxygen.

Specific lactose consumption rate (2b-6b) and product accumulation rate (2c-6c) are expressed by relations where those parameters are functions of the specific growth rate. For specific rate of product accumulation (2c-6c), the Luedeking-Piret [Pirt 1975] law is proposed to be tested. According to this law, a part of the product is continuously accumulated independent of microbial growth while the remaining part is proportional to the growth [Biryukov 2004]. Furthermore, the test of this relation is reasonable because it is specifically derived for lactic acid fermentations [Biryukov 2004, Pirt 1975]. For the lactose consumption rate (2b-6b), a relation proposed by Pirt [Pirt 1975] is recommended. This relation describes substrate consumption as a function of

specific growth rate and remains constant for microbe population viability. One part of the substrate is used for irreplaceable products of the metabolism, which have to be taken into account in the model [Biryukov 2004].

Table 1 Optimal criteria for searching of the best model to fit experimental data

DO _{int} , [%]	Model 1	Model 2	Model 3	Model 4	Model 5
5	16,97	16,92	9,98	10,17	10,28
10	14,76	14,76	7,96	8,22	8,16
15	11,09	11,07	7,63	7,64	7,60
20	14,98	14,88	8,42	8,64	8,57
40	12,73	12,60	7,41	7,48	7,45
90	32,30	32,34	14,41	13,33	14,31

The results concerning all the tested models are summarized in the Table 1. It can be observed that, the best results are given by Model 3. The deviations between the experimental data and the calculated values are 1.4 and 3.4 times lower than for other models. The models that include the inhibitor effect of product on bacterial growth (Models 3-5), give a better description of lactic acid production.

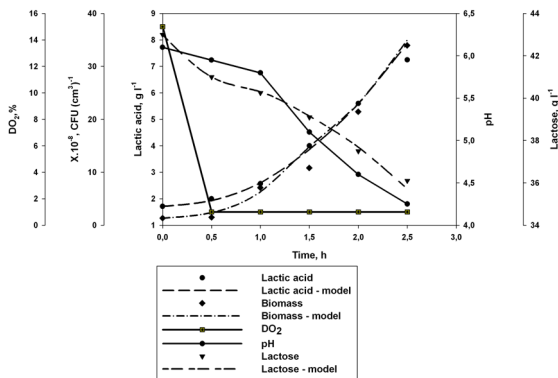


Figure 2: Kinetics of lactic acid process with 15% initial dissolved oxygen concentration in milk

Table 2 Process Model 3 parameters values

DO _{in} , [%]	μ [h ⁻¹]	K_s [g l ⁻¹]	K_p [g l ⁻¹]	a [g g ⁻¹]	b [g g ⁻¹]	m [g l ⁻¹ h ⁻¹]
5	8	6,02	0,85	0,376	1,46	6,10 ⁻⁶
10	8	9,24	0,85	0,999	1,21	1,6,10 ⁻⁵
15	7,60	8,70	0,78	1,223	1,12	1,2,10 ⁻⁵
20	3	5	5	1	1,40	4,2,10 ⁻⁶
40	8	8,61	0,91	0,417	1,40	4,2,10 ⁻⁶
90	8	5,84	0,71	1,359	1,40	4,2,10 ⁻⁶
		9,96	0,67	1,2,10 ⁻⁵	2,63	1,9,10 ⁻⁵
		1	4		2	

The quadratic error criteria for Model 3 shows the best values for dissolved oxygen concentrations between 5% and 20%. This result is very useful for industrial

practice. Hence, the relationships in Model 3 are chosen to be the most suitable ones to describe the process kinetics of lactic acid.

Figure 2 shows the simulation results in comparison with experimental data for the selected model. The experimental points are represented as circles and the model (1) output with kinetic dependences (4a) – (4c) represented as lines.

The multiplicative kinetic equation (4a) describes the relation between the specific growth rate of lactobacillus, substrate (lactose) concentration and the target product (lactic acid) concentration in the bioreactor. This model could be used for simulation of the process in the cases where some parameters and/or initial conditions are changed. The kinetic equation for the substrate consumption rate (4b) describes the relationship between the specific substrate consumption rate and the lactobacillus growth rate, where Y is a constant coefficient and μ is a maintenance coefficient for microorganism's viability. The kinetic equation of metabolite formation (4c) describes the relationship between the specific metabolite production rate and the specific growth rate.

The quantitative assessment of the oxygen's effect on the fermentation process can be based on the values obtained for the process parameters. Table 2 and Figure 3 indicate that the inhibitory constant K_p shows a trend of inverse proportionality to the changes of milk-dissolved oxygen concentration. The growth-inhibiting effect, in the case of increasing substrate, starts at lower concentrations of the inhibiting component – lactic acid. This confirms the experimental observations i.e. that 40% of milk-dissolved oxygen is the critical concentration: at higher concentrations prolonged cultivation time deteriorates the lactic acid process to the extent of full milk coagulation.

High oxygen concentrations in milk (40%-90%) lead to an augmentation trend for the value of coefficient b in Eq. 4c (see Figure 4) which describes the product accumulation in the medium. This means that cell culture becomes slower in the stationary phase.

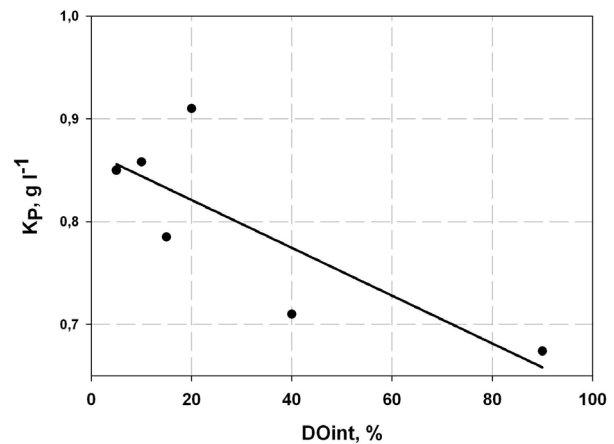


Figure 3: Effect of initial milk-dissolved oxygen concentration on inhibiting constant K_p

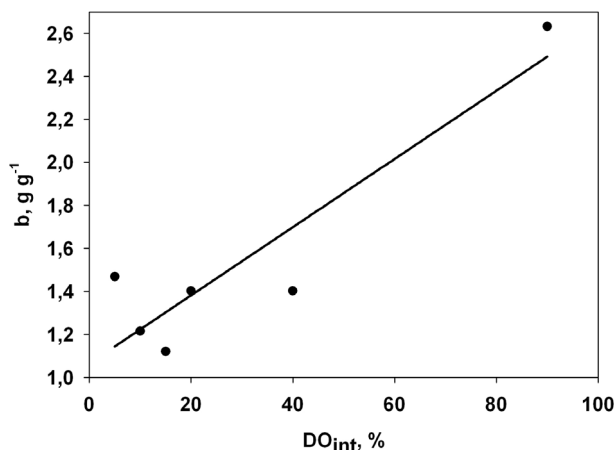


Figure 4: Influence of initial milk-dissolved oxygen concentration on model coefficient b

In the case of high dissolved oxygen concentration, a decrease in viable cells titer is observed for the same fermentation time. A smaller number of cells results in slower oxygen consumption by streptococci and as a result in a delay of total process rate.

CONCLUSIONS

This work has analyzed the kinetics of yogurt starter culture production by *S.thermophilus* 13a and *Lb. bulgaricus* 2-11 focusing on the quantitative effects of different milk-dissolved oxygen concentrations on the process. When the initial concentration of milk-dissolved oxygen is within the 5% - 30% interval, negative consequences on thermophilic streptococcus and lactobacillus growth are not observed. The values of the kinetic parameters of mixed cultures are characterized by an extreme value that could be observed between 1.5 and 2 hour of fermentation.

The effect of dissolved oxygen $DO_{int} = 40\%$ is more notable on *Lb. bulgaricus* 2-11 (on starter culture respectively): its concentration decreases 3 times at the end of the specified interval. A decrease in lactobacillus concentration is mainly observed with no effect on the lactic acid process. The desired interaction between *S.thermophilus* 13a and *Lb. bulgaricus* 2-11 (3:1) can be obtained at $DO_{int} < 40\%$. In the same zone, a maximum ratio of both species becomes larger than it is technologically desirable.

More significant is the effect of $DO_{int} = (60 - 90)\%$ on the lactic acid process and on the status of both populations. A delay of *Lb. bulgaricus* 2-11 growth is detected because of the inability of *S.thermophilus* 13a to consume that high an oxygen concentration in milk and to establish the anaerobic conditions for the growth of *Lb. bulgaricus* 2-11. This results in a delay of *Lb. bulgaricus* 2-11 growth and in a prolongation of the milk coagulation time. The increase of initial concentration of milk-dissolved oxygen from 5% to 90% leads to a proportional decrease in the specific growth rate of *S.thermophilus* 13a + *Lb. bulgaricus* 2-11 and of

the rates of lactose consumption and lactic acid production.

On the basis of this investigation, two zones of DO_{int} in milk are defined whose limit is 40% of DO saturation. In each of these zones, the associated pair of lactic acid bacteria of starter culture show different behaviour.

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