

Determination of the kinetic parameters of batch fermentation of *Lactobacillus paracasei* RN5 with probiotic potential

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Determination of the kinetic parameters of batch fermentation of *Lactobacillus paracasei* RN5 with probiotic potential. One of the requirements for probiotic strains is to allow the carrying out of industrial processes, including industrial cultivation with accumulation of a high concentration of viable cells. In culturing of *Lactobacillus paracasei* RN5 with probiotic properties in a bioreactor with constant agitation and under static conditions high concentration of viable cells (10^{14} cfu/cm³) was achieved. The mathematical models showed that in the bioreactor better conditions for the development of the lactobacillus strain were created.

Key words: *Lactobacillus*, probiotic, batch fermentation, mathematical model

INTRODUCTION

Probiotics are defined as live microorganisms that confer beneficial effects to the health of the host when administered in adequate amounts [2; 8]. Not all lactobacilli can be included in the composition of probiotics and probiotic foods, but only those which have certain properties [6], one of which is to enable the conduction of industrial processes - industrial cultivation, aimed at obtaining of concentrates with high content of viable cells; immobilization and lyophilization, and to maintain their activity during the storage of the lyophilisates.

Providing optimal conditions for the growth of the microbial cells that would ensure the accumulation of a high concentration of viable cells is of particular importance in the culturing process. According to Pirt, 1975 [5], in batch fermentation of lactic acid bacteria each microbial species increases its biomass at a rate which is a function of the chemical and physical conditions of the medium. The composition of the culture medium is of particular importance. Proper implementation of the fermentation process in respect not only to the culture conditions, but also to the change of the morphology of the microbial cells provides the obtaining of preparations that contain a high concentration of viable cells. Culturing in a bioreactor allows better development of the microbial cells and also provides an opportunity for culturing of mixed cultures. Conditions for obtaining standardized starters with homogeneous properties and biochemical activity are created [3].

Knowledge of the kinetic parameters of the microbial population is necessary for the study, implementation and management of cultivation processes, for their scaling from laboratory to industrial scale. Furthermore, knowledge of the kinetic features of development of the population allows the selection of the most appropriate probiotic strain, namely the one that has the highest maximum specific growth rate and the maximum specific rate of acidformation.

The purpose of the present study was to investigate the possibilities for batch fermentation in a bioreactor with continuous agitation and under static conditions of *Lactobacillus paracasei* RN5 with probiotic properties and to determine the kinetic parameters of the processes.

MATERIALS AND METHODS

Microorganisms

The studies in the present work were carried out with *Lactobacillus paracasei* RN5 with probiotic properties, isolated from spontaneously fermented sourdough.

Media

1. LAPTg10 - broth. Composition (g/dm³): peptone - 15; yeast extract - 10; Tryptone - 10; glucose - 10. pH was adjusted to 6.6 - 6.8, and Tween 80 was added - 1cm³/dm³. Sterilization - 20 minutes at 121°C.

2. LAPTg10 - agar. Composition (g/dm³): Medium LAPTg10 - broth +2% agar. Sterilization - 20 minutes at 121°C.

Batch fermentation in a bioreactor with constant agitation and under static conditions.

The batch fermentation was carried out in LAPTg10-broth without pH adjustment. The medium in the bioreactor was inoculated with 5% (v/v) inoculum from a fresh 24-hour culture of *Lactobacillus paracasei* RN5. Fermentation was carried out at 37±1°C, 150 rpm, without aeration. The duration of fermentation was 24 hours, with periodic sampling of the culture fluid for the determination of the number of viable cells of *Lactobacillus paracasei* RN5 (cfu/cm³) and the titratable acidity. Laboratory bioreactor with a volume of 2 dm³, working volume of 1,5 dm³, provided with a control unit "Sartorius A2", which included a control loop for agitation rate, temperature, pH and etc. was used.

In parallel with the held batch fermentation in a bioreactor with constant agitation, fermentation under static conditions (in an incubator) at 37±1°C was carried out as well.

Identification of the model parameters

To describe the kinetics of the process the model of the logistic curve for the biomass and the product (expressed as titratable acidity) was applied:

$$\frac{dX}{dt} = [\mu_m - \beta X]X \quad X = \frac{X_H e^{\mu_m (t - \tau_{lag})}}{1 - \frac{X_H}{X_{kp}} (1 - e^{\mu_m (t - \tau_{lag})})} \quad (1)$$

$$\frac{dP}{dt} = q_{pm} \left(1 - \frac{P}{P_{kp}}\right) P \quad P = \frac{P_H e^{q_{pm} (t - \tau_{lag})}}{1 - \frac{P_H}{P_{kp}} (1 - e^{q_{pm} (t - \tau_{lag})})} \quad (2)$$

Wherein: μ_m - maximum specific growth rate, h⁻¹; X_H and X_{kp} - initial and final concentration of viable cells, cfu/cm³; q_{pm} - maximum specific rate of acidformation, °T/(cfu.cm³.h); P_H and P_{kp} - initial and final titratable acidity; τ_{lag} - length of the lag phase, h; τ - time, h.

RESULTS AND DISCUSSION

Lactobacillus paracasei RN5 was cultured in a laboratory bioreactor with continuous agitation and under static conditions in LAPTg10-broth medium. For 24 hours of culturing in a bioreactor the concentration of viable cells reached 1x10¹⁴cfu/cm³ at titratable acidity of 127,77°T (Fig. 1) and redox potential +87,70 mV.

Under static conditions for the same time the number of living cells was 5.10¹³cfu/cm³ at titratable acidity of 134,33°T (Fig. 2). The redox potential (Eh) increased from the beginning of the process and followed the course of the growth curve, and at the 24th hour it was +90 mV.

A comparison of experimental data with model data for the dynamic and static culturing of *Lactobacillus paracasei* RN5 is shown on Fig. 3. The mathematical models reflected the experimental data with sufficient accuracy. The cells of the strain were not sensitive to the mechanical action of the stirrer. They were supplied with the necessary quantity of oxygen, being microaerophiles, by the surface aeration, provided by the stirrer.

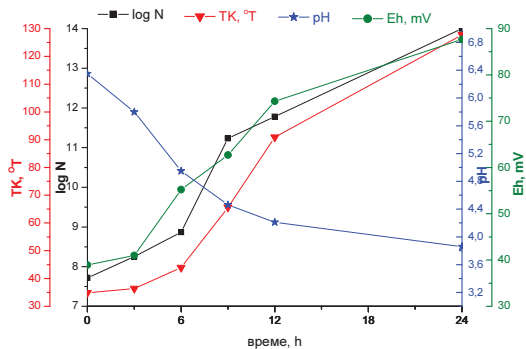


Fig. 1. Batch fermentation of *Lactobacillus paracasei* RN5 in LAPTg10-broth medium in a bioreactor with constant agitation

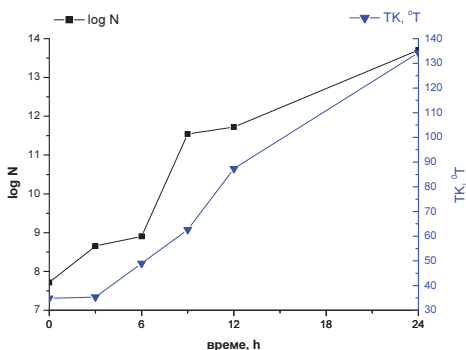
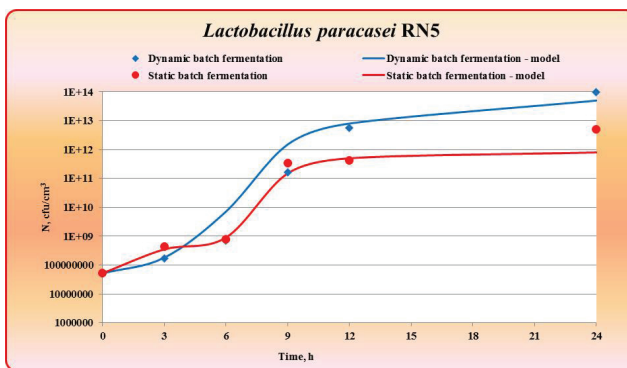


Fig. 2. Static batch fermentation of *Lactobacillus paracasei* RN5 in LAPTg10-broth medium



| Strain | Dynamic batch fermentation | | | Static batch fermentation | | |
|--------|----------------------------|-------------------------|-----------------|---------------------------|------------------------|-----------------|
| | μ, h^{-1} | $\beta, cm^3/(cfu.h)$ | τ_{lag}, h | μ, h^{-1} | $\beta, cm^3/(cfu.h)$ | τ_{lag}, h |
| RN5 | 0,756 | 0.108×10^{-15} | 3 | 0,26 | 0.87×10^{-13} | 6 |

Fig. 3. Comparison of the dynamics of the fermentation process of *Lactobacillus paracasei* RN5 under dynamic and static conditions using the model of the logistic curve

One of the most important characteristics of the microbial population is the maximum specific growth rate. A mandatory requirement for probiotic strains is to have high maximum specific growth rate. The maximum specific growth rate ($\mu_{\max} = 0.756 \text{ h}^{-1}$) was higher under dynamic conditions in comparison with the values of the same parameter under static conditions - $\mu_{\max} = 0.261 \text{ h}^{-1}$. The coefficient of internal population competition in the dynamic culturing ($\beta = 0.108 \times 10^{-15} \text{ cfu}/(\text{cm}^3 \cdot \text{h})$) was two orders of magnitude lower than that in the static culture ($\beta = 0.87 \times 10^{-13} \text{ cfu}/(\text{cm}^3 \cdot \text{h})$). This confirmed that the conditions for growth and development in the bioreactor were significantly better than these of the static culture. All this reflected on the length of the lag phase. It was three hours shorter under dynamic conditions of cultivation than under static conditions (Fig. 3).

Another important feature of the culture is the specific rate of acidformation. The produced lactic and other organic acids by the lactobacilli resulted in a rapid acidification of the medium, which suppressed the growth of pathogenic and putrefactive microorganisms. Therefore, the maximum specific rate of acidformation was calculated using the model of the logistic curve (2) [1, 4]. The results are presented on Fig. 4 and Fig. 5

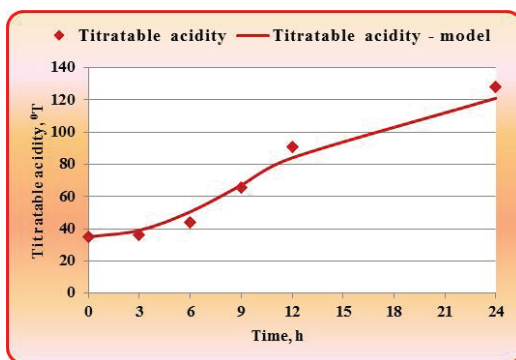


Fig. 4. Comparison of experimental data with model data for the dynamic cultivation of *Lactobacillus paracasei* RN5, $q_{pm} = 0,183 \text{ }^\circ\text{T}/(\text{cfu} \cdot \text{cm}^3 \cdot \text{h})$

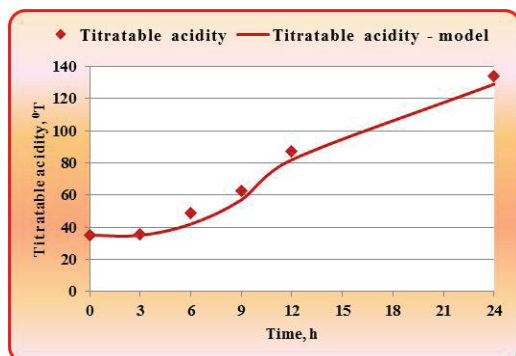


Fig. 5. Comparison of experimental data with model data for the static cultivation of *Lactobacillus paracasei* RN5, $q_{pm} = 0,25 \text{ }^\circ\text{T}/(\text{cfu} \cdot \text{cm}^3 \cdot \text{h})$

The selected model described well the kinetics of acidformation expressed as titratable acidity. In contrast to the specific growth rate, the specific rate of acidformation was higher in static culture (titratable acidity 134°T), while the acidity reached 128°T at the

end of the process in the dynamic cultivation. The higher titratable acidity was due to the fact that lactic acid was accumulated in large quantities under anaerobic conditions. These studies confirmed the results, reported by Schiraldi, 2003 [7], that biomass was accumulated at a lower acidity for a shorter period of time under microaerophilic conditions, while acid was accumulated under anaerobic conditions.

CONCLUSION

The dynamics of the growth of *Lactobacillus paracasei* RN5 with probiotic potential in a bioreactor with constant agitation and under static conditions was determined. The made mathematical models showed that the growth conditions in the bioreactor were better and for 24 hours preparations with a high concentration of viable cells (10^{14} cfu/cm³) at lower value of the titratable acidity were obtained.

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This paper has been reviewed