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METHODOLOGICAL APPROACHES TO OPTIMIZING CULTIVATION CONDITIONS OF THE *LIMOSILACTOBACILLUS FERMENTUM* LF-02 STRAIN

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Subject. *Lactic acid bacteria, particularly the Limosilactobacillus fermentum species, are important microorganisms used in the food industry. Studying and optimizing their cultivation conditions to achieve the maximum yield of viable biomass is a relevant task in biotechnological research.* ***Purpose.*** *To determine the optimal cultivation conditions for the L. fermentum LF-02 strain in order to obtain the highest amount of biomass.* ***Methods.*** *The study used the L. fermentum LF-02 strain, which was cultivated to select optimal parameters on standard MRS medium. The cultivation parameters included incubation time: 8, 10, and 12 hours; inoculum volume (% v/v): 1, 2.5, 5, 7.5, and 10; temperature,°C : 28, 30, 32, 34, 37; and initial pH of the medium ranging from 5.0 to 7.5 with an increment of 0.5. The growth of the strain was determined using the serial dilution plating method. To study the growth dynamics and main physiological-biochemical parameters under the selected optimal conditions, the strain growth was determined by serial dilution plating, pH was measured potentiometrically, the amount of reducing sugars was determined by the ferricyanide method, and the content of free amino nitrogen by the ninhydrin method.* ***Results.*** *The optimal cultivation conditions for L. fermentum LF-02 were determined, promoting the accumulation of the maximum biomass amount (9.20±0.03 CFU/cm^3): incubation time – 10 hours, inoculum volume – 5%, temperature – 34°C, and medium pH – within the range of 6.0–7.0. Changes in cell count, pH, reducing sugars, and free amino nitrogen during cultivation under optimal conditions were analyzed. The specific growth rate of the strain was calculated as 0.410 ± 0.04 h^{-1}, the utilization rate of reducing sugars – 72.07±5.12%, and of free amino nitrogen – 26.71±3.56%.* ***Scope of Results.*** *The obtained results can be used for further optimization of nutrient media and the development of a biotechnology for biomass production of the L. fermentum LF-02 strain.*

Key words: *lactic acid bacteria, cultivation parameters, growth dynamics, Limosilactobacillus fermentum, biotechnology*

МЕТОДОЛОГІЧНІ ПІДХОДИ ДО ПІДБОРУ ОПТИМАЛЬНИХ УМОВ КУЛЬТИВУВАННЯ ШТАМУ *LIMOSILACTOBACILLUS FERMENTUM* LF-02

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Предмет. *Молочнокислі бактерії, зокрема вид Limosilactobacillus fermentum є важливими мікроорганізмами, що використовуються у харчовій промисловості. Вивчення та оптимізація умов їх культивування з метою отримання максимальної кількості життєздатної біомаси є актуальними завданнями біотехнологічних досліджень.* ***Мета.*** *Визначення оптимальних умов культивування штаму L. fermentum LF-02, з метою отримання найвищої кількості біомаси.* ***Методи.*** *У роботі використано штам L. fermentum LF-02, який культивували з метою підбору оптимальних параметрів на стандартному середовищі МРС. Параметрами умов культивування обрано час: 8, 10 і 12 годин; внесення посівного матеріалу, (об/об)%: 1, 2,5, 5, 7,5 та 10; температуру,°С: 28, 30, 32, 34, 37; початкове pH поживного середовища від 5,0 до 7,5 із кроком зміни 0,5. Приріст штаму визначали методом висіву серійних розведень. З метою визначення динаміки росту та основних фізіолого-біохімічних показників за підібраних оптимальних умов культивування визначали ріст штаму методом висіву серійних розведень, pH – потенціометрично, кількість редукуючих цукрів – фериціанідним методом, вміст вільного амінного азоту – нінгідриновим методом.* ***Результати.*** *Визначено оптимальні умови культивування штаму L. fermentum LF-02, які сприяють накопиченню максимальної кількості біомаси (9,20 ± 0,03 КУО/см^3): час інкубації – 10 годин, внесення посівного матеріалу – 5%, температура 34°С, pH поживного середовища – діапазон від 6,0 до 7,0. Проаналізовано зміни кількості клітин, pH, редукуючих цукрів та вільного амінного азоту при культивуванні за оптимальних умов. Розраховано питому швидкість росту штаму 0,410±0,04 год$^{-1}$, відсоток утилізації редукуючих цукрів – 72,07±5,12% та вільного амінного азоту – 26,71±3,56%* ***Сфера застосування результатів.*** *Отримані результати можуть бути використані з метою подальшої оптимізації поживних середовищ та розробці біотехнології отримання біомаси штаму L. fermentum LF-02.*

Ключові слова: *молочнокислі бактерії, параметри культивування, динаміка росту, Limosilactobacillus fermentum, біотехнологія*

Problem statement. Lactic acid bacteria (LAB) are industrially valuable microorganisms used in various fields of industry [1, 2]. The overall demand for LAB biomass drives research focused on providing optimal cultivation conditions for strains to increase the yield of viable biomass [3], lactic acid synthesis [4], and production of specific antimicrobial metabolites [5].

The vast majority of industrially significant LAB strains have complex physiological and metabolic characteristics, which require careful selection of specific cultivation parameters. Currently, much research attention is devoted to the optimization of nutrient media for LAB, particularly the selection of carbon and nitrogen sources and growth factors, due to the high adaptive potential of these microorganisms to different substrates [3, 6]. Studies have shown that the choice of carbon source significantly affects the growth, biomass yield, and metabolic activity of strains, as observed for *Limosilactobacillus fermentum* cultivated in glucose-maltose media [7]. Despite this, the influence of such parameters as temperature, cultivation duration, aeration level, or medium pH on biomass and metabolite production has been studied much less frequently [4, 8].

For many LAB, the optimal growth temperature is close to 37°C. At the same time, literature reports studies investigating the effects of broader or shifted temperature ranges on the growth and metabolism of *Lactobacillus plantarum*, including 22–42°C [9], 30–38°C [4], and 30–45°C [1]. The cultivation duration for many LAB strains is often defined by standard intervals, for example, 12-24 hours for *L. reuteri* [10], 15-24 hours for *L. plantarum* [11], or 8-14 hours for *L. acidophilus*, *L. fermentum*, and *L. mucosae* [8]. Another critical factor determining LAB growth and viability is the pH of the medium. For most lactic acid bacteria, the optimal pH lies within the range of 6.0-6.5 [6]. However, studies have also examined *L. plantarum* growth at pH 5–7 [12], 4.5–8.5 [13], and 5.5–8.0 [9]. An equally important factor influencing the growth and metabolic activity of lactic acid bacteria is the amount of inoculum introduced. Reported studies show a variety of inoculum ranges, including 1–10% for *L. plantarum* [14], 1–8% for *L. plantarum* [9, 12], as well as 1–10% for *L. rhamnosus* and *L. paracasei* [9].

Thus, numerous studies on various LAB representatives confirm the significant influence of temperature, cultivation duration, pH, and inoculum level on growth and metabolic activity. At the same time, most of these studies focus on *L. plantarum*, *L. reuteri*, *L. acidophilus*, and several other

species, whereas data on *L. fermentum* remain scarce and fragmentary. The limited number of publications devoted to the comprehensive optimization of cultivation conditions for *L. fermentum* strains highlights the need for studies that take into account not only the composition of the medium but also the physical cultivation parameters.

The aim of this study was to determine the optimal cultivation conditions for the *L. fermentum* LF-02 strain in order to obtain the highest biomass yield.

Materials and methods. The study used the *L. fermentum* LF-02 strain isolated from oat kvass. Liquid MRS medium was used for cultivation according to [15]. The strain was pre-cultivated for 16 hours at a temperature of 37±1°C. The main cultivation parameters in this series of experiments were defined as follows: time – 8, 10, and 12 hours; inoculum volume (% v/v) – 1, 2.5, 5, 7.5, and 10; temperature (°C) – 28, 30, 32, 34, and 37; initial pH of the nutrient medium – from 5.0 to 7.5 with an increment of 0.5. Growth parameters of the strain were assessed by plating serial dilutions on solid MRS medium.

After determining the optimal cultivation parameters, the changes in the main growth indicators were evaluated: biomass increase – by plating serial dilutions, pH – potentiometrically, reducing sugars – by the ferricyanide method according to [7, 16], and free amino nitrogen – by the ninhydrin method according to [17].

The specific growth rate (μ, год$^{-1}$) was calculated based on data obtained for the exponential growth phase of the strain between 2 and 6 hours using the formula [18]:

$$\mu = \frac{ln(N_6 - N_2)}{t_6 - t_2},$$

where N_6 is the number of CFU/cm^3 after 6 hours of cultivation, N_2 is the number of CFU/cm^3 after 2 hours of cultivation, and t_2 and t_6 represent the cultivation times of 2 and 6 hours, respectively.

The experiments were conducted in triplicate. The obtained results are presented as mean values ± standard deviation. To determine the statistical significance of differences between means, one-way analysis of variance (ANOVA) was applied, and Duncan's test was used for mean comparison at a significance level of $p < 0.05$. Mean values marked with different letters indicate statistically significant differences, while identical letter markings indicate no statistically significant difference between groups.

Results and discussion. To determine the optimal cultivation time for the maximum biomass accumulation by the strain, cultivation was carried out at a temperature of 37°C with an inoculum volume of 10% (v/v), which is standard for the cultivation of most LAB. The results are presented in Figure 1.

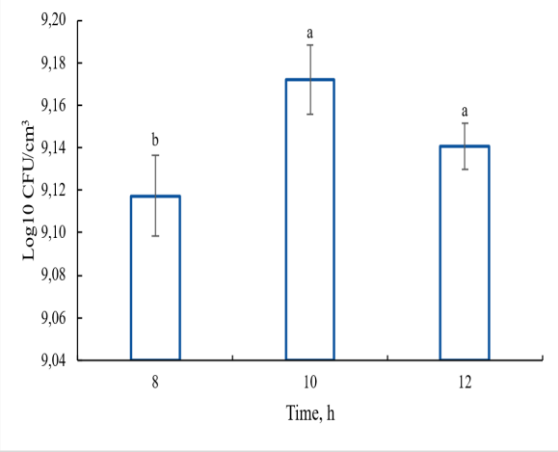

Fig. 1. Effect of cultivation duration on biomass accumulation of *L. fermentum* LF-02

The highest biomass accumulation was observed after 10 and 12 hours of cultivation (9.17 ± 0.02 CFU/cm^3 and 9.14±0.01 CFU/cm^3, respectively). The obtained values showed no statistically significant difference when compared with each other. A comparatively lower value was observed after 8 hours of cultivation, indicating insufficient time to achieve the maximum cell count. The absence of statistically significant differences between 10 and 12 hours of cultivation indicates that the culture had entered the stationary growth phase. Therefore, 10 hours was selected as the optimal cultivation time, ensuring the highest biomass accumulation within a shorter period.

According to the literature, for most LAB

such as *L. fermentum*, *L. reuteri*, *L. rhamnosus*, *L. acidophilus*, and *L. plantarum*, the exponential growth phase lasts on average 10–12 hours, followed by a transition to the stationary phase, which is consistent with the obtained results [14, 19, 20].

The effect of inoculum volume on biomass accumulation by the strain was analyzed at a temperature of 37°C and a cultivation duration of 10 hours. The results are presented in Figure 2.

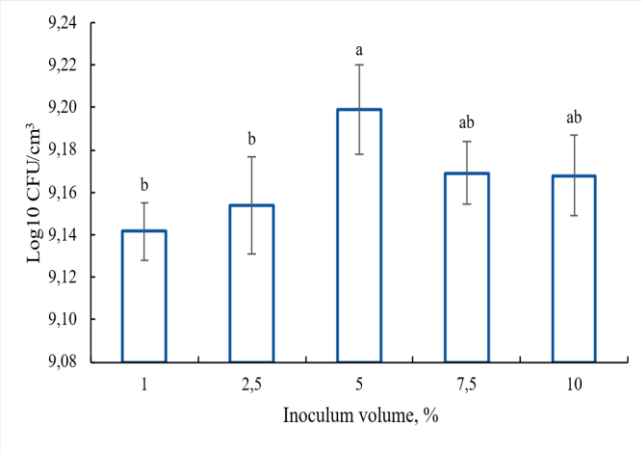

Fig. 2. Effect of inoculum volume on biomass accumulation of *L. fermentum* LF-02

According to the obtained results, the lowest cell growth values were observed with 1% and 2.5% inoculum volumes, with no statistically significant difference between these variants. The maximum biomass increase was recorded with a 5% inoculum (9.20±0.02 CFU/cm^3), while further increasing the inoculum volume to 7.5% and 10% did not result in a significant rise in biomass compared to 5%. Thus, for further experiments, an inoculum volume of 5% was selected as optimal.

In the study on optimizing the cultivation conditions of *L. plantarum* AS-14, the highest biomass yield was obtained with a 5% inoculum volume [21]. For *L. brevis* RK03, an increase in biomass growth was observed with increasing inoculum volume [22]. The study [23] identified 3% as the optimal inoculum volume for *L. fermentum* BLN3. Similarly, in [24], the authors reported maximum biomass accumulation by *L. plantarum* when using a 3% inoculum. For the *L. plantarum* p6 strain, the optimal inoculum volume was determined to be 2%, providing the highest growth performance compared to 4% and 6% [12]. The obtained results are partially consistent with the findings presented in this study.

To investigate the effect of different temperatures on the growth of the strain, a cultivation time of 10 hours and an inoculum volume of 5% were selected. The results are shown in Figure 3.

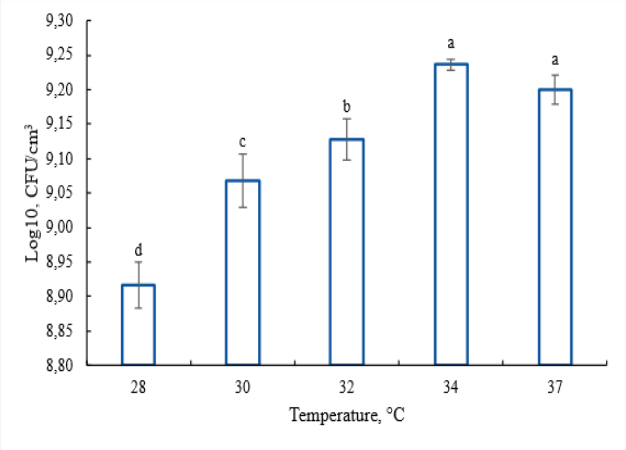

Fig. 3. Effect of temperature on biomass accumulation of *L. fermentum* LF-02

It was found that lower temperatures (28°C, 30°C , and 32°C) were unfavorable for biomass accumulation by the strain, with the lowest value recorded at 28°C (8.92±0.03 CFU/cm^3). Statistically significant differences were observed for each tested temperature, except for 34°C and 37°C, which showed the highest values of 9.24 ± 0.01 CFU/cm^3 and 9.20±0.02 CFU/cm^3, respectively. Considering the obtained results and the economic feasibility of using a lower cultivation temperature, 34°C was selected as the optimal temperature and applied in further experiments.

The optimization of the cultivation medium and conditions for *L. plantarum* AS-14 conducted in [21] indicated that the optimal temperature of 40°C was more effective for achieving higher biomass yields compared to 35°C. However, in [24], 35°C was identified as the optimal temperature. The highest biomass yield for *L. plantarum* 200655 was obtained at 30°C, compared to 32°C and 35°C [25]. In study [5], it was reported that temperatures of 32°C, 37°C, and 40°C had no significant effect on the growth of *L. fermentum* SA715, whereas lowering the temperature to 25°C did not favor biomass accumulation. Similar results were obtained for *L. fermentum* BLN3, with the optimal temperature ranging from 33°C to 37°C [23]. These findings are, to some extent, consistent with the data obtained in this study.

The determination of the optimal initial pH value of the cultivation medium was carried out by incubating the culture for 10 hours with a 5% inoculum at a temperature of 34°C, as shown in Figure 4.

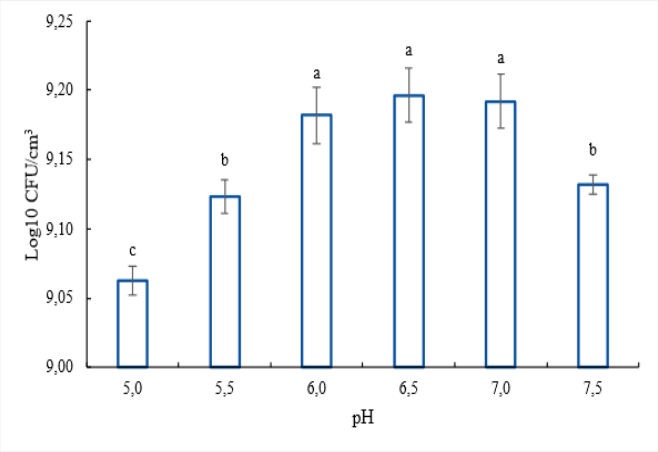

Fig. 4. Effect of initial medium pH on biomass accumulation by *L. fermentum* LF-02

A negative effect was observed at more acidic initial pH values of 5.0 and 5.5, as well as at the alkaline value of 7.5. The pH range from 6.0 to 7.0 was found to be the most favorable for biomass accumulation by the strain. Therefore, it can be concluded that the optimal pH range for biomass accumulation lies between 6.0 and 7.0. For further studies, the initial pH of 6.5 was selected, corresponding to the standard pH of MRS medium.

The study [26] reported an optimal initial pH value of 6.28 for the cultivation medium when optimizing biomass accumulation conditions for *Lactobacillus rhamnosus* LS-8. For *L. plantarum*, the study [24] identified an initial pH of 6.5 as the most favorable for biomass accumulation, although a pH of 7.0 promoted faster biomass production compared to 6.5. Investigation of the pH effect on biomass accumulation by *L. plantarum* 200655 indicated an optimal range between 6.5 and 7.5 [25]. In the case of *L. fermentum* IMDO 130101, the highest relative growth rate was observed at medium pH values from 5.5 to 6.5, which partially corresponds with our results [27]. The study [23] reported an optimal pH value of 6 for the cultivation of *L. fermentum*.

To investigate the dynamics of growth, acid formation, and the consumption of key nutrients in the medium, changes in the concentrations of carbohydrates and free amino nitrogen were analyzed during the 10-hour cultivation of the strain, with a 5% inoculum, at 34°C, and an initial medium pH of 6.5. The results are shown in Figure 5.

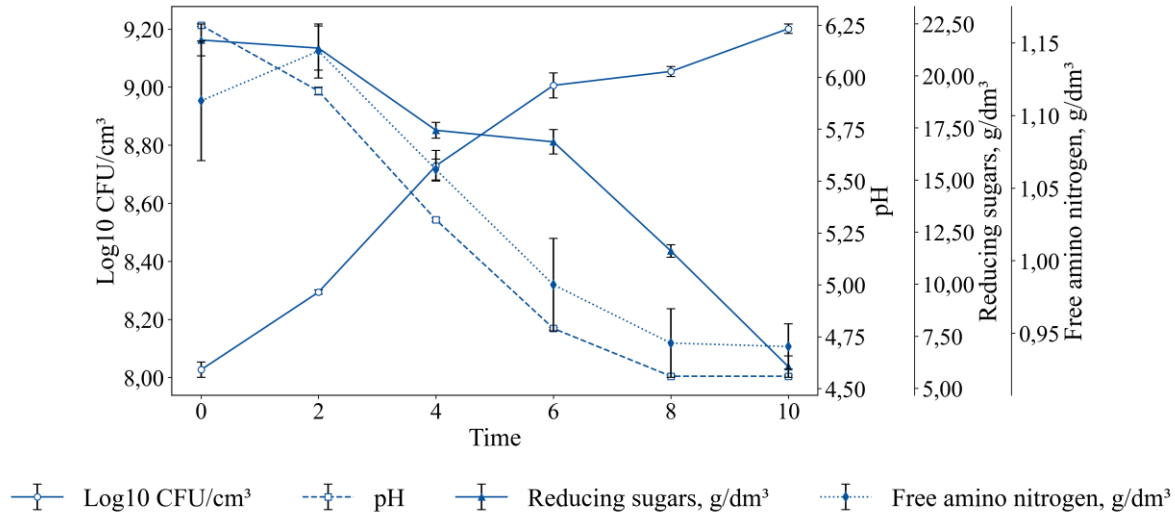

Fig. 5. Dynamics of growth, acid formation, and consumption of major medium nutrients by the strain

The main increase in cell number was observed during the first 8 hours of cultivation, accompanied by a decrease in pH to 4.56±0.01. After 10 hours, a further increase in CFU/cm^3 to 9.20±0.03 was detected, consistent with previously obtained data; the specific growth rate was 0.410 ± 0.04 h^{-1}. The pH value did not change after 8 hours compared to the previous measurement, which may indicate maximal acid accumulation by the strain. Changes in the concentrations of reducing sugars and free amino nitrogen showed their gradual depletion relative to the initial level:

sugar utilization reached 72.07±5.12%, while free amino nitrogen utilization was 26.71±3.56%. Thus, it can be concluded that during the logarithmic growth phase, the strain consumed carbon sources more actively than nitrogen sources.

Similar results regarding growth dynamics were observed during the cultivation of the *L. fermentum* IMDO 130101 strain. The culture entered the stationary growth phase within the first 10 hours of cultivation in a medium containing 1% glucose, reaching 9.3–9.4 CFU/cm^3. Complete depletion of 10 g/dm^3 of glucose was recorded after 10 hours of incubation, which corresponds to the findings obtained in the present study [27]. The study [5] demonstrated comparable results, with the stationary phase observed at the 10th hour of cultivation based on optical density and Log10 CFU/cm^3 data; however, the specific growth rate was 0.72 h^{-1}, which may be attributed to the use of a different carbon source – sucrose.

Conclusions. The optimal parameters for cultivating *Limosilactobacillus fermentum* LF-02 on the commonly used MRS medium were determined. The highest biomass yield was observed after 10 hours of cultivation with a 5% inoculum at a temperature of 34°C. The optimal initial pH range of the cultivation medium for strain growth was found to be between 6.0 and 7.0, with no statistically significant differences between these values. The dynamics of growth and changes in key physiological and biochemical parameters were analyzed under the selected optimal cultivation conditions. The maximum cell concentration reached 9.20±0.03 CFU/cm^3, accompanied by a decrease in pH to 4.56 ± 0.01.

The obtained results indicate the possibility of achieving high growth performance of the strain under the selected cultivation conditions, allowing for the maximum biomass yield and more efficient utilization of energy and material resources.

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