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EFFECT OF MAGNETIC FIELD AND ENVIRONMENTAL PH ON THE ADSORPTION EFFICIENCY OF CELLS OF THE GENUS *LACTOBACILLUS*

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Subject. Adsorption of probiotic cultures *Lactobacillus acidophilus*, namely strains 27 and Narine, and *Lactobacillus plantarum*, on high-dispersed silica. **Purpose.** The purpose of this study was to investigate the influence of a magnetic field with intensities of 0.03 and 0.09 T and the pH of the medium on the efficiency of cell adsorption of *Lactobacillus* probiotic strains. **Method.** Microbiological and biotechnological methods were applied in this research. Hydrogels based on high-dispersed silica "Enterogel" and "Toxin.NET" were used as adsorbents. Neodymium magnets with intensities of 0.03 T and 0.09 T were employed to create the magnetic field. The efficiency of adsorption was evaluated by the cell survival of probiotic cultures after adsorption on hydrogel preparations under the influence of the magnetic field and in the case of pH adjustment by adding acid or alkali. The functional properties of the obtained composite preparations were assessed by the time of clot formation after milk fermentation and its acidity. **Results.** The research results demonstrated that immobilized cells of *Lactobacillus acidophilus* on the hydrogel of methylsilicic acid had enhanced protection not only against harsh conditions of the gastrointestinal tract (low pH and bile influence) but also against magnetic fields with intensities of 0.03 T and 0.09 T, which had an overall negative impact on native (non-immobilized) cells. The functional activity of cells immobilized in this way on the hydrogel based on high-dispersed silica "Enterogel," evaluated by acidity and clot formation time, was only slightly reduced: up to 4,3-11,3%. This confirms the assumption that the assessment of cell survival by the Koch's cup method does not fully correspond to the real state of the cells. It was also determined that conducting adsorption in the pH range below 4 is impractical, and the optimal conditions for *L. plantarum* adsorption were in the pH range of 6 to 7. **Scope of results.** The research findings can be utilized for the development of complex probiotic preparations based on high-dispersed silica, which may be appropriate for preserving the functionality of probiotic cells used for starter cultures or in the production of dietary supplements containing beneficial microorganisms.

Key words: high-dispersed silica, lactobacillus, probiotics, adsorption, magnetic field, pH, viability, milk fermentation.

ВПЛИВ МАГНІТНОГО ПОЛЯ ТА pH СЕРЕДОВИЩА НА ЕФЕКТИВНІСТЬ СОРБЦІЇ КЛІТИН РОДУ *LACTOBACILLUS*

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Предмет. Адсорбція пробіотичних культур *Lactobacillus acidophilus*, зокрема штамів 27 і Narine, і *Lactobacillus plantarum*, на високодисперсному кремнеземі. **Мета.** Метою цього дослідження було вивчення впливу магнітного поля напруженістю 0,03 та 0,09 Тл та pH середовища на ефективність клітинної адсорбції пробіотичних штамів *Lactobacillus*. **Методи.** У цьому дослідженні застосовували мікробіологічні та біотехнологічні методи. Як сорбенти використовували гідрогелі на основі високодисперсного кремнезему «Ентеросгель» та «Токсин.NET». Для створення магнітного поля використовували ніодимові магніти з напруженістю 0,03 Тл і 0,09 Тл. Ефективність адсорбції оцінювали за виживаністю клітин пробіотичних культур після адсорбції на препаратах гідрогелів за впливу магнітного поля та у разі створеного за допомоги додавання кислоти або лугу pH. Функціональні властивості отриманих композитних препаратів оцінювали за терміном отримання згустку після сквашування молока та його кислотністю. **Результати.** Результати дослідження показали, що іммобілізовані клітини *Lactobacillus acidophilus* на гідрогелі метилкремнієвої кислоти мали посилений захист не тільки від жорстких умов шлунково-кишкового тракту (низький pH і вплив жовчі), але й від магнітних полів інтенсивністю 0,03 Тл і 0,09 Тл, які, в цілому, негативно впливали на нативні (не іммобілізовані) клітини. Функціональна активність іммобілізованих таким способом на гідрогелі на основі високодисперсного кремнезему «Ентеросгель» клітин, оцінена за кислотністю та терміном утворення згустків, була лише незначно знижена: до 4,3-11,3%, натомість вміст клітин знизився суттєвіше – у середньому на 20%. Це підтверджує припущення про те, що оцінка виживаності клітин чашиковим методом Коха не повністю відповідає реальному стану клітин. Визначено також, що проведення адсорбції в діапазоні pH нижче 4 є недоцільним, найкращими умовами для адсорбції *L. plantarum* було значення pH в діапазоні від 6 до 7. **Сфера застосування результатів.** Результати досліджень можуть бути використані для розробки комплексних пробіотичних препаратів на основі високодисперсного кремнезему, що може бути доцільним для збереження функціональності пробіотичних клітин, використовуваних для заквашувальних культур або у виробництві БАДів із вмістом корисних мікроорганізмів.

Ключові слова: високодисперсний кремнезем, пробіотики, лактобактерії, адсорбція, магнітне поле, pH, виживаність, сквашування молока.

Problem formulation. Probiotic microorganisms refer to live microbial agents that can be administered to individuals or animals to elicit beneficial effects, specifically by restoring or enhancing the composition of the intestinal microflora. In the current context, probiotics hold

significant importance in society as they contribute to the maintenance of a healthy intestinal environment and the restoration of microbial balance. Furthermore, probiotic bacteria aid in the treatment of intestinal infections when used in conjunction with antibiotics. However, upon oral administration, probiotic cells can experience damage from the acidic environment and bile present in the gastrointestinal tract (GIT) [1], as well as from ingested antibiotics [2]. Generally, probiotics are available as dietary supplements that contain potentially beneficial bacteria or yeast. While probiotic-enriched lactic acid products are the most commonly used method of delivering probiotic bacteria to the body, alternatives such as tablets, capsules, or powders are also available.

Probiotic bacteria are typically regarded as lactic acid microorganisms such as *Lactobacillus* and *Bifidobacterium*, but there are also other genera utilized as probiotics, including *Lactococcus*, *Propionibacterium*, *Bacillus*, *Saccharomyces*, and certain strains of *Escherichia coli*. Within these genera, numerous species and strains are known for their probiotic properties. Different bacterial and yeast strains possess distinct probiotic advantages they can confer. However, a common challenge for all probiotics is their survival during processing, storage, transit through the gastrointestinal tract (GIT), and colonization within it, as bacteria need to remain viable in order to be active and provide maximum beneficial effects.

The viability of probiotic bacteria after oral consumption can be significantly compromised during their passage through the gastro-intestinal environment [1], as well as unintentional destruction by antibiotics that are intended to target only infecting pathogens [2]. Therefore, in order to protect probiotics from these adverse conditions, methods have been proposed to shield live cells through the creation of complexes with various materials that are neutral to the organism. Despite the biocompatibility of the encapsulation material with the human body, encapsulation can impact the viability of bacteria, especially if the interaction between matrix molecules and bacterial surface components is strong, such as through coordinate-covalent bonding [3].

One such material that has been proposed is high-dispersed silicon dioxide, which is approved and widely used for the treatment of dysbiosis and other GIT issues. The results of our previous studies conducted in 2021 and 2022 [4,5] indicate that probiotic cells of various species immobilized on such preparations as "Enterosgel," "Silix," and "Toxin.NET" indeed exhibit better tolerance to simulated conditions of the GIT. However, similar to being bound to other matrices, they also partially lose viability after immobilization.

Other studies have demonstrated the positive impact of a magnetic field on the preservation of the cellular morphology of *Saccharomyces cerevisiae* yeast cells after drying a suspension droplet on a "solar silicon" plate. One possible explanation for this phenomenon could be the gettering effect, where positively charged impurities migrate to the surface of silicon, promoting better interaction with yeast cells that have a negative zeta potential on their surface at physiological pH values [6,7].

The relevance of our research corresponds to the scientific studies conducted by Hao Wei et al. (2022) [8], which also focus on the development of a composite probiotic consisting of a probiotic bacterial strain (*B. infantis*) encapsulated within an active layer of mesoporous silicon dioxide nanoparticles. In our study, we utilized high-dispersed silicon dioxide, which adsorbs bacteria onto its branched surface, in contrast to the mesoporous silicon dioxide used in Hao Wei's research, which, conversely, attached and encapsulated the culture.

Among the commonly used materials for immobilization, silica gels are known for their high chemical and biological stability. Microbial cells, plant cells, and pancreatic islets have been successfully incorporated into materials derived from silica gels [9-11].

The interaction between bacteria and hydrogel containing high-dispersed silicon dioxide can occur through various mechanisms. One such mechanism is electrostatic interaction, where charged groups on the surface of bacteria attract charged groups on the surface of silicon dioxide, facilitating the adhesion of bacteria to the silicon dioxide surface.

Additionally, other interaction mechanisms exist. For example, certain bacteria can produce exopolysaccharides that serve as a "glue" to anchor bacteria onto the silicon dioxide surface. Some bacteria can also produce fibrin (a protein material) that can attach to the silicon dioxide surface and serve as a site for bacterial attachment.

Overall, the interaction between bacteria and high-dispersed silicon dioxide can be complex and depends on several factors, such as the surface properties of silicon dioxide, the composition and properties of bacteria, as well as the conditions of interaction (e.g., temperature and pH of the environment).

The primary concern of this research is to investigate the influence of a magnetic field and pH of the environment on the efficiency of *Lactobacillus* probiotic cell adsorption.

To achieve the set objective, the following tasks were performed:

- Investigate the functionality of immobilized *Lactobacillus* probiotic cells on silicon dioxide-based enterosorbents in terms of their ability to ferment milk.

- Examine the influence of a constant magnetic field with intensities of 0.03 and 0.09 T during adsorption on the viability of probiotic cells in a composite drug based on high-dispersed silicon dioxide.

- Study the impact of pH during adsorption on the viability of probiotic cells in a composite drug based on the sorbent "Enterosgel."

Materials and methods.

Biological Agents. For this study, the following biological agents were used: *Lactobacillus acidophilus* strains 27 and Narine; *Lactobacillus plantarum* 3206. These strains were provided by the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine. Monoculture biomass was utilized at concentrations ranging from 10^8 to 10^{10} CFU/g.

Lactobacillus acidophilus are facultative anaerobes. The fermentation type is homofermentative, producing DL-lactic acid. The optimum growth temperature is within the range of 35–38°C. They grow over initial pH values ranging from 5.0 to 7.0, with an optimum pH range of 5.5–6.0.

L. plantarum is a heterofermentative bacterium that metabolizes glucose and fructose, producing lactic acid, lactate, acetate, ethanol, and CO₂. They are also capable of amino acid metabolism and amino acid synthesis. *L. plantarum* can grow over a wide range of temperatures, from 15 to 40 °C. The optimal growth temperature is approximately 30–37 °C. The culture can grow over a wide range of pH values, from 4.0 to 8.5, with an optimal pH range of around 6.0–7.0 [12].

Materials and Reagents.

The *magnetic field* was generated by neodymium magnets with intensities of 0.03 T and 0.09 T.

Adsorbents for Immobilization. Hydrogels of highly dispersed silica were used as sorbents:

1. "Enterosgel", PJSC "EOF "KREOMA-PHARM", Ukraine (active substance: methylsilicic acid; auxiliary substances: sodium saccharin dihydrate, sodium cyclamate, purified water) (Fig. 1).

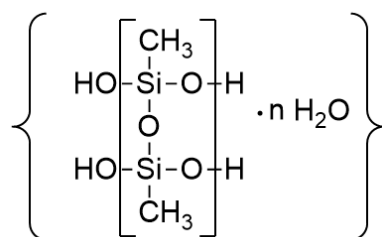


Fig. 1. Structural formula of polymethylsiloxane polyhydrate

2. "Toxin.NET" with inulin, Ilan Farm LLC, Ukraine (active substance: silicon dioxide; auxiliary substances: citric acid, sodium benzoate, potassium sorbate, glycerin, carboxymethyl cellulose, purified water, "banana" flavoring).

Reagents: 40% NaOH, 1M HCl, 2.5% fat milk, gentian violet, phenolphthalein, GA medium (hydrolyzed agar), physiological saline solution, distilled water.

Equipment and Laboratory Glassware: binocular microscope (Fischer Bioblock); adventurer electronic balances from OHAUS; thermostat; CERTOMAT® SII shaker; burette; tweezers; measuring pipettes with a volume of 1 cm³; cover slips and microscope slides; sterile glass test tubes; alcohol burner; sterile glass bottles with a volume of 200 cm³; glass Petri dishes; tube racks; conical glass flasks of various volumes.

Proportional calculations for preparing complex preparations based on high-dispersion silicon dioxide. When preparing complex preparations based on high-dispersion silicon dioxide, different amounts of biomass and pharmaceutical preparations were used to achieve a specific concentration of biomass in the final mixture. The calculation aimed to create a weight of biomass for the analysis of survival with 0.1 g of biomass.

For studies, calculations were made to have 10% of biomass at a concentration of 1010 CFU/g in the mixture with hydrogel.

Samples were created for different values of the magnetic field strength to compare the survival rates in the "Enterogel" preparation, "Toxin.NET," and the control point – pure biomass.

Investigation of the magnetic field impact on the adsorption of probiotic cultures onto high-dispersion silicon dioxide preparations. Adsorption was conducted at a temperature of 28°C. Hydrogel preparations with biomass were uniformly mixed for 5 minutes on the surface of the magnets, except for the control sample (Fig. 2). The procedure was carried out under aseptic conditions. Subsequently, the mixture was left undisturbed for 30 minutes. A predetermined amount of the obtained preparation was taken for survival analysis, ensuring an equal amount of biomass in all samples.

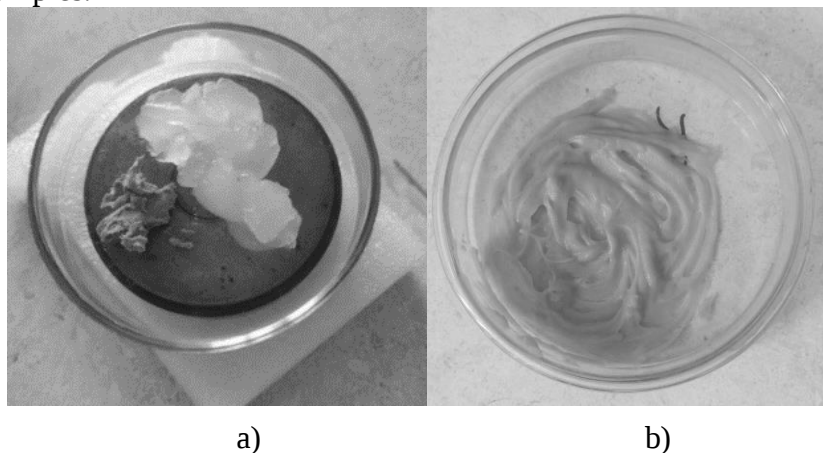


Fig. 2. Appearance of complex preparation: a) components prior to sorption on the magnet (on the right – "Enterogel" preparation, on the left – biomass);
b) components after the adsorption on the magnet

Study of the Viability of Probiotic Microorganisms. To investigate the survival of probiotic microorganisms, a culture was prepared by inoculating the microorganisms onto Petri dishes containing hydrolyzed agar at dilutions ranging from the third to the tenth in a physiological saline solution. To determine the viability of the microorganisms, colony-forming units (CFUs) were counted after incubation in a thermostat at a temperature of 37°C for a duration of 3 days.

Investigation of the functional properties of immobilized probiotic cultures through milk fermentation. To study the functional characteristics of immobilized probiotic cultures, the following experiments were conducted: 0.1 g of biomass was added to 100 cm³ of 2.5% milk. The mixture was thoroughly mixed and incubated in a thermostatic chamber at 37 °C. To evaluate the results of the experiments, the time of clot formation (in hours) and acidity were determined.

Determining the acidity of the clot formed by the immobilized culture of probiotic microorganisms was carried out by the traditional method and was measured in Turner degrees.

Investigation of the adsorption efficiency on high-dispersion silicon dioxide preparations "Enterosgel" and "Toxin.NET" at different pH values. The study on the influence of pH on the adsorption of probiotic cells on high-dispersion silicon dioxide preparations was conducted using the culture fluid of *L. plantarum*. Acid or alkali was added to 40 cm³ of the culture fluid to create the desired pH environment, followed by the addition of 1.23±0.2 g of the "Enterosgel" preparation. The mixture was then incubated for 10-40 minutes with continuous stirring, after which 1 cm³ was quickly sampled into a test tube containing sterile physiological saline solution. From this, a series of dilutions were prepared for sowing on hydrolyzed agar to determine the number of colony-forming units using Petri dishes.

Results and discussion. *Investigation of the Influence of Magnetic Field on the Viability and Functional Activity of Complex Probiotic Preparations.* The conducted studies (Table 1) revealed that the magnetic field with intensities of 0.03 T and 0.09 T generally suppresses the functional properties of the probiotic culture, as determined by the maximum acidity of fermented milk, which ranged from 73% to 75% (rows 1-3). In the case of immobilization on the Toxin.NET (TN) preparation, the maximum acidity decreased less dramatically (rows 7-9). The control and immobilized samples on Toxin.NET were digested after 4-5 hours, but the acidity was measured not immediately after clot formation, but the next day, after placing the samples in a refrigerator at 10 °C overnight.

Table 1

The effect of magnetic field on the fermentability of milk by native and immobilized cultures of *Lactobacillus acidophilus*, Narine

№	Experiment Conditions	Acidity, °T (after 6 hours)	Maximum Acidity, °T
1	Biomass (Control)	200±10	260±13
2	Biomass 0.03 T	-*	190±9.5
3	Biomass 0.09 T	-*	195±9.8
4	Biomass + Enterosgel	200±10	-**
5	Biomass + Enterosgel 0.03 T	187±9.3	-**
6	Biomass + Enterosgel 0.09 T	192±9.6	-**
7	Biomass + Toxin.NET	162±8.1	250±12.5
8	Biomass + Toxin.NET 0.03 T	128±6.4	230±11.5
9	Biomass + Toxin.NET 0.09 T	156±7.8	250±12.5

* The milk failed to undergo proper fermentation for 6 hours, consequently yielding no result

**Not investigated

In the case of adsorption on the Enterosgel (EG) preparation, compared to adsorption on TN, the negative impact of the magnetic field was significantly reduced: the acidity of the formed coagulum after 16 hours (rows 5-6) reached approximately 95% of the acidity of the product fermented with the native preparation (row 1). Unfortunately, the data for the acidity of the coagulum for the enterosorbent with inulin (TN) were found to be unacceptable (rows 7).

In other words, immobilized cells were found to be better protected not only from the influence of extreme gastrointestinal conditions (low pH and action [4,5]) but also from external physical factors, such as electromagnetic fields (rows 2-3 and 8-9). Thus, immobilization of microorganisms on high-dispersion silica is a favorable factor for their viability and synthesis of metabolic products in industrial biotechnology, which is consistent with the widely accepted theory.

It is worth noting that under the influence of a magnetic field with an intensity of 0.09 T, the indicators are better than under the influence of 0.03 T. It can be assumed that the magnetic field positively affects key enzymes involved in the fermentation process, which is also in line with previous research [4,5], for example, on the positive role of the magnetic field in increasing the rate of the enzymatic reaction of pyruvate to lactate conversion by lactate dehydrogenase.

Immobilization has almost no negative impact on the functionality of probiotics, which is confirmed by our previous research [4,5] without the influence of a magnetic field. Indeed, the fermentation time (clot formation) and acidity decreased insignificantly (Table 2). It should be noted that the clot formation time under the influence of a magnetic field with an intensity of 0.09 T was shorter than under the influence of 0.03 T, and the acidity was higher, which confirms previous data.

Table 2

Influence of magnetic field on the functional properties of the complex preparation of *Lactobacillus acidophilus* culture, strain 27 on EG

№	Experiment conditions	Time of milk fermentation, hours	Acidity (at the time of clot formation), °T
1	Biomass (Control)	4.0±0.02	106±4
2	Biomass + Enterosgel	4.2±0.02	104±3
3	Biomass + Enterosgel 0.03 T	4.4±0.02	94±3
4	Biomass + Enterosgel 0.09 T	4.25±0.02	97±3

It should also be noted that the milk fermented with the probiotic preparation based on Toxin.NET acquired an unpleasant bitterness. This may be a consequence of the presence of flavor additives in the preparation, rather than its main properties. Survival assessment of the adsorbed probiotic cultures under the influence of magnetic fields with intensities of 0.03 and 0.09 T was also conducted (Table 3).

It was determined that the survival rate was slightly lower compared to the survival rate of preparations obtained without the influence of magnetic fields: the colony-forming units (CFU) count had the same order as the adsorbed preparation. This fact, along with the results of milk fermentation experiments, confirms the previous assumption regarding a methodological flaw in this technique: adsorbed cells do not have physical access to the nutrient medium of hydrolyzed agar, thus hindering colony growth.

Table 3

Survival of probiotic strains immobilized on high-dispersion silicon dioxide preparations under the influence of a magnetic field

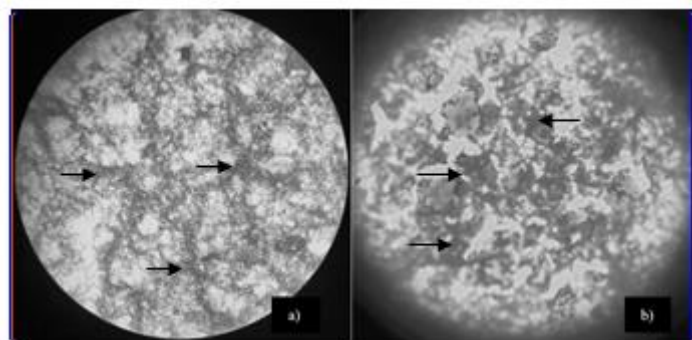
<i>Lactobacillus acidophilus</i> , 27		<i>Lactobacillus acidophilus</i> , Narine	
Experiment conditions	Colony-forming units lg CFU/cm ³	Experiment conditions	Colony-forming units, lg CFU/cm ³
Biomass (Control)	10.43±0.05	Biomass (Control)	8.0±0.07
Biomass + Enterosgel	8.84±0.05	Biomass + Enterosgel	6.0±0.04
Biomass + Enterosgel 0.03 T	8.3±0.04	Biomass + Enterosgel 0.03 T	6.0±0.05
Biomass + Enterosgel 0.09 T	8±0.04	Biomass + Enterosgel 0.09 T	6.0±0.05
Biomass + Toxin.NET	8.8±0.05	Biomass + Toxin.NET	6.0±0.05
Biomass + Toxin.NET 0.03 T	8.3±0.04	Biomass + Toxin.NET 0.03 T	-
Biomass + Toxin.NET 0.09 T	7.7±0.04	Biomass + Toxin.NET 0.09 T	6.0±0.03

The statistical analysis of the obtained data also revealed that the survival and functional activity results of the culture were not correlated. The Pearson correlation coefficient was calculated as 0.39, and the coefficient of determination was 0.15, indicating that there is no dependence between the values.

Under the experimental conditions of this study, local accumulation of bacterial cells around high-dispersion silicon dioxide was confirmed and documented (Fig. 3).

It is mentioned in the literature that live cells do not grow in the direction parallel to the layer of silicon dioxide (silica) unlike organic polymers. This is likely due to the strength of the

material, which hinders cell proliferation. Cell growth occurs vertically towards the silica layer through cell outflow from the surface. In the experimental conditions, colony formation was not observed in the silica gel fragments. Air bubbles trapped in the ash, which appeared after mixing, were quickly captured by rapid gel formation. These open spaces were often filled with growing bacteria, creating dark spots inside the transparent silica gel [13]. Based on the results of our study, we can assume that bacteria also do not grow pervasively into the depths of the sorbent, but rather form cultures in the gaps between the drug particles, consuming a nutrient medium that is not present in the sorbent.



**Fig. 3. Microphotographs of immobilized cells
(arrows show the clusters of adsorbed cells on the sorbents):**

a) *L. acidophilus*, Narine on "Enterosgel"; b) *L. acidophilus*, Narine on "Toxin.NET"

Thus, the highly dispersed silica containing the adsorbed cells probably prevents their access to the nutrient medium in the Petri dishes. Using a similar mechanism, enterosorbents protect probiotic bacteria from the harmful effects of gastric juice and bile, and also shield them from the influence of a magnetic field. Therefore, the development of complex probiotic preparations based on high-dispersion silicon dioxide is a promising and relevant area of research.

Investigation of the Impact of pH Environment on the Efficiency of Adsorption of Probiotic Microorganisms. To evaluate the adsorption efficiency of the "Enterosgel" and "Toxin.NET" preparations containing high-dispersion silicon dioxide, a study was conducted to examine the immobilization dependence on pH level, following the methodology described in Section 2. The results of the microorganism survival experiment are presented in Fig. 4.

According to the provided diagram, the culture cells were unable to grow at extremely acidic pH values, which likely indicates their loss of viability and adsorption capacity prior to the addition of the sorbent. Even in the 4th dilution, no colony growth was observed. Thus, conducting adsorption at pH values below 4 is impractical as the cells quickly perish or lose functionality before immobilization occurs.

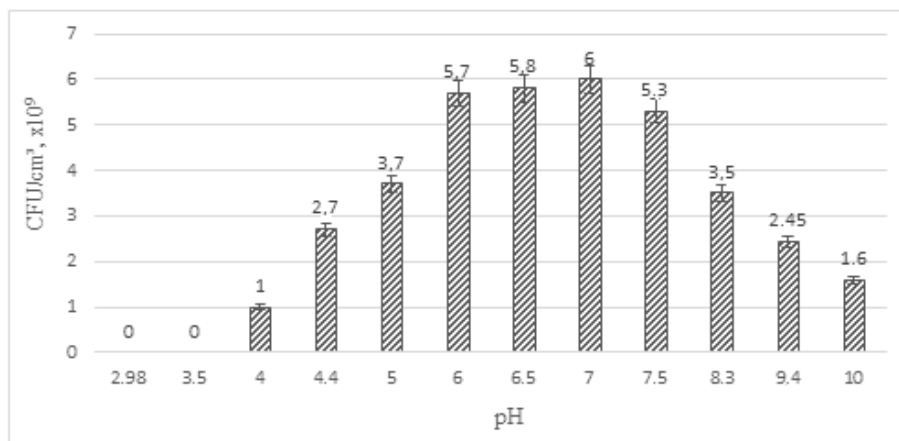


Fig. 4. Survival of *L. plantarum* probiotic culture sorbed on the "Enterosgel" preparation at different pH levels (sorption duration 30-40 min)

In the pH range of 7.5 to 10, survival decreased less intensively, but at high pH values (9-10), the preparation became darker and acquired an unpleasant odor. Based on the survival results, the optimal conditions for adsorption (for *L. plantarum*) are pH values in the range of 6 to 7.

Interestingly, under unfavorable pH conditions, increasing the duration of mixing from 10 to 40-50 min had a positive effect on survival (Table 4).

Table 4

Survival rate of *L. plantarum* probiotic culture sorbed on the "Enterosgel" preparation under unfavorable pH conditions with varying adsorption durations

Experiment conditions		Cell concentration in the sample, CFU/cm ³	
pH	Mass of EG, g.	Mixing duration 10 min	Mixing duration 30-40 min
2.98	1.22	No growth	No growth
3.5	1.23	No growth	No growth
4.0	1.23	$1.0 \cdot 10^5$	$(0.9-1.1) \cdot 10^9$
8.0	1.26	$0.8 \cdot 10^9$	-*
8.28	1.22	-*	$3.5 \cdot 10^9$

* Not investigated

The positive impact of sorption in cases of detrimental pH levels is visually demonstrated. Specifically, at a pH value of 4 during slow stirring ($t=20^\circ\text{C}$) for 10 minutes, the survival rate of the culture in the complex preparation was four orders of magnitude lower compared to the case of a longer adsorption process.

Thus, under unfavorable acidic pH values, the cells did not die immediately but rather lost viability or the ability to adhere and develop in a solid agarized environment. If the contact with the sorbent lasted longer, the cells adhered better to the sorbent, which protected them from adverse conditions, allowing for subsequent colony development under restored physiological conditions. The same conclusion can be drawn from the adsorption results at pH values above 8: under more alkaline conditions (pH 8.28), but with an increased adsorption duration of 30-40 minutes, the survival rate was 4 times higher compared to pH 8 with a stirring duration of 10 minutes.

Conclusion. It has been shown that immobilized *Lactobacillus plantarum* 3206 cells on the "Enterosgel" preparation were better protected against the overall negative impact of a constant magnetic field with intensities of 0.03 and 0.09 T. The acidity of the clot formed after 16 hours was approximately 90-95% of the acidity of the product fermented with the native preparation, and the fermentation time increased by only 15-24 minutes. It should be noted that the clot formation time was shorter when exposed to a magnetic field with an intensity of 0.09 T compared to 0.03 T. Unfortunately, the data for clot acidity of the preparation immobilized on the enterosorbent "Toxin.NET" with inulin were unacceptable, and the sample acquired an unpleasant bitterness.

It was determined that the survival of cells immobilized on high-dispersion silicon dioxide preparations was slightly lower compared to the survival of bacteria obtained without the influence of a magnetic field: the CFU count had the same order as the adsorbed preparation. This fact, along with the results of milk fermentation experiments, confirms the previous assumption regarding a methodological flaw in this approach: sorbed cells do not have physical access to the nutrient medium of hydrolyzed agar, hence hindering colony growth. It means that immobilized cells were better protected not only from the effects of extreme conditions of the gastrointestinal tract (low pH and harmful action of bile [4,5]), but also from external physical factors, such as electromagnetic fields. Thus, the immobilization of microorganisms on highly dispersed silica is a favorable factor for their vital activity and synthesis of metabolic products in industrial biotechnology, which does not contradict the generally accepted theory.

Statistical analysis of the obtained data revealed a lack of correlation between survival and functional activity of the culture. The Pearson correlation coefficient was 0.39, and the coefficient of determination was 0.15, indicating that there is no dependence between the values.

It was also determined that conducting adsorption at a pH below 4 is impractical, as the cells quickly perish or lose functionality before immobilization. According to the survival results, the optimal pH range for *L. plantarum* 3206 adsorption was between 6 and 7.

References

1. Dodoo, C. C., Wang, J., Basit, A. W., Stapleton, P., Gaisford, S. (2017). Targeted delivery of probiotics to enhance gastrointestinal stability and intestinal colonisation. *International Journal of Pharmaceutics*, 530(1-2), 224–229. <https://doi.org/10.1016/j.ijpharm.2017.07.068>.
2. Li, Z., Behrens, A. M., Ginat, N., Tzeng, S. Y., Lu, X., Sivan, S., Langer, R., Jaklenec, A. (2018). Biofilm-Inspired encapsulation of probiotics for the treatment of complex infections. *Advanced Materials*, 30 (51), e 1803925. <https://doi.org/10.1002/adma.201803925>.
3. Geng, W., Jiang, N., Qing, G.-Y., Liu, X., Wang, L., Busscher, H. J., Tian, G., Sun, T., Wang, L.-Y., Montelongo, Y., Janiak, C., Zhang, G., Yang, X.-Y., & Su, B.-L. (2019). Click reaction for reversible encapsulation of single yeast cells. *ACS Nano*, 13(12), 14459–14467. <https://doi.org/10.1021/acsnano.9b08108>.
4. Danylenko S., Romanchuk I., Marynchenko L. (2021) Immobilization of probiotic cultures with enterosorbents based on highly dispersed silica. *Journal of Microbiology, Biotechnology and Food Sciences*, 11(2). <https://doi.org/10.15414/jmbfs.3334>.
5. Danylenko, S., Marynchenko, L., Bortnyk, V., Potemskaya, O., Nizhelska, O. (2022). Use of highly dispersed silica in biotechnology of complex probiotic product based on bifidobacteria. *Innovative Biosystems and Bioengineering*, 6(1), 16–24. <https://doi.org/10.20535/ibb.2022.6.1.256179>.
6. Marynchenko L., Nizhelska O., Kurylyuk A., Naumenko S., Makara V. (2020) Observed effects of electromagnetic fields action on yeast and bacteria cells attached to surfaces. 2020 IEEE 40th International Conference on Electronics and Nanotechnology (ELNANO), Kyiv, Ukraine, 22-24 April. 603-608. <https://doi.org/10.1109/ELNANO50318.2020.9088883>.
7. Nizhelska, O., Marynchenko, L., Makara, V., Naumenko, S., & Kurylyuk, A. (2018). The stabilizing effect of magnetic field for the shape of yeast cells *saccharomyces cerevisiae* on silicon surface. *Innovative Biosystems and Bioengineering*, 2 (4), 278–286. <https://doi.org/10.20535/ibb.2018.2.4.151881>.
8. Wei, H., Geng, W., Yang, X.-Y., Kuipers, J., van der Mei, H. C., Busscher, H. J. (2022). Activation of a passive, mesoporous silica nanoparticle layer through attachment of bacterially-derived carbon-quantum-dots for protection and functional enhancement of probiotics. *Materials Today Bio*, 100293. <https://doi.org/10.1016/j.mtbio.2022.100293>.
9. Jesionowski, T., Krysztafkiewicz, A. (1999). Properties of highly dispersed silicas precipitated in an organic medium. *Journal of Dispersion Science and Technology*, 20(6), 1609–1623. <https://doi.org/10.1016/j.mtbio.2022.10029>.
10. Pope, E. J. (1995). Gel encapsulated microorganisms: *Saccharomyces cerevisiae* – Silica gel biocomposites. *Journal of Sol-Gel Science and Technology*, 4, 225-229. <https://doi.org/10.1007/BF00488377>.
11. Campostrini, R., Carturan, G., Caniato, R., Piovan, A., Filippini, R., Innocenti, G., Cappelletti, E. M. (1996). Immobilization of plant cells in hybrid sol-gel materials. *Journal of Sol-Gel Science and Technology*, 7(1-2), 87–97. <https://doi.org/10.1007/BF00401888>.
12. Irkitova, A. N., Matsyura, A. V. (2017). Ecological and biological characteristics of *Lactobacillus acidophilus*. *Ukrainian Journal of Ecology*, 7(4), 214-230. https://doi.org/10.15421/2017_109.
13. Brányik, T., Kuncová, G., Páca, J., Demnerová, K. (1998). Encapsulation of microbial cells into silica gel. *Journal of Sol-Gel Science and Technology*, 13, 283-287. <https://doi.org/10.1023/A:1008655623452>.