Innov Biosyst Bioeng, 2024, vol. 8, no. 2, 69–84
doi: 10.20535/ibb.2024.8.2.297364

Original article

EVALUATING THE INTERACTION BETWEEN SILICON SURFACE AND MICROORGANISMS IN VARIOUS SOLVENTS UNDER THE INFLUENCE OF A STATIC MAGNETIC FIELD USING FRACTAL ANALYSIS

L.V. Marynchenko[1*], O.I. Nizhelska[2], A.S. Shirinyan[2], N.O. Gorchakova[3]

[1]Igor Sikorsky Kyiv Polytechnic Institute, Kyiv, Ukraine
[2]Institute of Applied Physics of NAS of Ukraine, Sumy, Ukraine
[3]Bogomolets National Medical University, Kyiv, Ukraine
*Corresponding author: lolitamar@ukr.net

Received 25 January 2024; Accepted 10 May 2024

Background. Peculiarities of the interaction of microorganisms with the surface are important from the point of view of the functionality of this surface (implants, chips, electrodes with biofilm for producing electric current). The orderliness of organic particles and cells on different surfaces can be assessed by determining the fractal dimension and lacunarity and indicate the structural state or efficiency of the system.
Objective. Investigation how different solvents and the application of a magnetic field affect the texture of suspensions containing microorganisms when dried on various types of silicon surfaces and quantitatively assess the dimensions of the structures formed using fractal analysis.
Methodology. After mixing, the cell suspension was applied to the polished, degreased surface of silicon wafers arranged horizontally and left to dry completely. In a static magnetic field (MF) with an induction of 0.17 T, the induction lines were perpendicular to the surface of the sample. Micrographs of dried cells were processed in software package ImageJ and fractal analysis was performed using the FracLac software application and "Box counting" technique.
Results. Significant differences in the self-organization of various types of microorganism cells during drying on silicon surfaces under the influence of a MF and in different solvents have been found. The tendency for various types of microorganisms was the formation of pseudofractal shapes and an increase in the average fractal dimension D under the action of a MF. As D increased, lacunarity L decreased. However, in the case of yeast suspended in a physiological solution, pseudofractal shapes were observed even in the absence of a MF.
Conclusions. Using fractal analysis of pseudofractal figures consisting of cells of microorganisms on the surface of a silicon plate under the influence of MF, it is possible to evaluate the functionality of cells interacting with the surface, as well as the quality of this surface.

Keywords: yeast; algae; probiotic lactocultures; magnetic field; silicon wafer; solvents effect; dried cells; fractal dimension; lacunarity.

Introduction

Spontaneous structuring is inherent in non-equilibrium thermodynamically open complex systems. Self-organized structures can form in crystals, powders, liquids, gases, and plasma, provided that external fields such as temperature, electromagnetic forces, mechanical oscillations, etc., are applied. This redistribution of substances from a uniform or chaotic state to a pronounced structure can be attributed to energy considerations. Changes in conditions can cause the system to transition to another quasi-stable state with lower energy, as seen in phenomena such as liquid crystals, sand dunes in deserts, and cloud formations [1]. These self-organized structures often exhibit fractal self-similarity. Well-known examples include the growth of ice crystals, tree branches, mushroom mycelium, the formation of blood vessel systems, nerve cells, and riverbeds [2]. Therefore, the question arises regarding the correlations between fractal structures and the physicochemical and biological properties of these systems.

The need for the development of materials with controlled characteristics for practical applications is currently relevant. The interactions of microorganisms with surfaces are important both from a fundamental scientific perspective and from a practical standpoint, such as the functionality of these surfaces for implants or prostheses, or for electrodes with a biofilm for electricity production [3]. Specifically concerning silicon surfaces, solar panels coated with modified *Escherichia coli* cells containing the pigment lycopene, capable of converting sunlight into electricity, are being developed [4]. Data suggest that the efficiency of the system may depend on the ordered distribution of organic components. Therefore, experiments with different types of solvents and processing methods to create textured films of microorganisms on silicon surfaces may have practical significance for solar energy, microelectronics, and materials science in construction.

Analysis of works on this topic reveals the ambiguity and multifactor nature of determining the results of fractality in biological objects and biopolymers. For example, textured films formed on solid surfaces after the evaporation of solutions (geological rocks or sediment at the bottom of a coffee cup) can be considered as a type of fractal structure. As demonstrated in the study [5], the structure of the solid phase of films formed as a result of the evaporation of biopolymer solutions and biological fluids characterizes the interaction between substances in the solution, namely, the dehydration self-organization of particles. In some cases, the external factor acting during solution evaporation leads to the formation of a film with different optical properties than during free drying [6].

Using solutions of biopolymers as an example, the influence of the chemical composition of the buffer, Na^+ and Cl^- ions, as well as heating and γ-irradiation of solutions on the fractal structure of films dried in glass thermostated cuvettes was revealed [7]. Fractal dimension and characteristics of zigzag-like patterns were employed for numerical description of the texture structure. It was noted that in a dried solution of 20 mmol/L NaCl in the absence of biopolymers, no zigzag-like structures were observed.

Research on mineral water containing orthosilicic acid has shown that natural water or water concentrated using reverse osmosis on flat inert surfaces is capable of forming a film consisting of fractal dendrites, with dendrites being larger in concentrated water. In samples of water reconstituted from powder mineral salts, fractal structures did not appear. The presence of organic impurities in natural mineral water may promote the growth of fractal objects by fixing aggregates [8].

In review [9], a series of applications of fractal analysis in biology and medicine was described, highlighting its ability to detect even the smallest changes in structure or shape. This is valuable for analyzing both normal and pathological cells. Texture analysis of films is successfully used in disease diagnosis, product quality assessment, and can serve as the basis for developing rapid tests [10]. Biological tissues exhibit changes in self-similarity associated with cancer progression, leading to alterations in their fractal dimension. Image analysis of tissue sections has shown the potential for accurate differentiation of cancer stages [11]. Besides differences in shape, other properties can also demonstrate fractal behaviour, such as the distribution of adhesion strength on cell surfaces [12].

The formation of fractal structures during the growth of microorganisms has been noted for a long time [13], and now computer analysis of colonies is used instead of laborious counting of viable cells [14]. Fractal parameters of kefir fungal biofilms cultivated at different sugar concentrations have been studied in [15].

Despite the progress made in this field, the influence of surface, solvent type, and external fields on the ability of microorganisms to form ordered structures during droplet drying on a surface has not been sufficiently studied. Initial qualitative considerations without numerical calculations were discussed in works [16, 17]. Therefore, there is a need to move from description to quantitative assessment, which became the subject of the present study.

Of particular interest is the interaction of widely used silicon surfaces with microorganisms that form dried biofilm textures under the influence of different solvents and static magnetic fields (further denoted as MF with induction B). Such information is crucial for materials science, biotechnology and regenerative medicine, as considerations of both targeted immobilization and desired surface anti-adhesiveness are important in modern technologies. Currently, there is ongoing research on various test systems, including those for machine learning and research automation, so the ability to process information in digital format can be beneficial.

Thus, the *aim of the study* is to investigate the influence of solvents and static magnetic fields on the texture of microorganism suspensions dried on the surfaces of various types of silicon, and to numerically assess the dimensionality of the formed structures based on fractal analysis.

Materials and Methods

The research was conducted using washed cultures of *Saccharomyces cerevisiae* yeast produced by PrJSC Enzym Company (located in Lviv, Ukraine), with probiotic strains of bacteria *Lactobacillus bulgaricus* (*Lactobacillus delbrueckii ssp. bulgaricus* IMB B-7123) and *Streptococcus thermophilus* (*Streptococcus thermophilus* IMB B-7176), which are used for obtaining dairy products, from the collection of industrial microorganisms of the Institute of Food Resources of the National Academy of Agrarian Sciences of Ukraine. Additionally, freshwater unicellular green microalgae *Chlamydomonas* from the Collection of Algal Cultures of

Taras Shevchenko National University of Kyiv (ACKU 751, 759, 761), which grow in soil and shallow freshwater reservoirs, were used.

For the research, a rejuvenated one-day culture of yeast was used, meaning pressed yeast was cultivated on malt wort at a temperature of 30 °C, and then washed to remove residual medium.

The suspension of washed yeast cells was prepared in distilled water or in a physiological saline. The optical density of the yeast cell suspension after dilution with distilled water was $D \leq 0.01$ (at a wavelength of 550 nm, optical path length of 0.5 cm). The concentration of the suspension was approximately 10^6 cells per millilitre. A volume of 50 μl of the suspension was applied to the silicon surface. To obtain an ethanol suspension of cells, yeast *S. cerevisiae* (pressed) produced by mentioned Enzym Company was mixed with 96% ethanol in a ratio of 1:10 in a sterile container and the suspension was created by mechanical mixing (shaking) for 10 minutes.

For the investigation of the texture film of a mixed probiotic culture formed on a silicon wafer, a second 10-fold dilution of the culture suspension was used after incubation in an industrial fermenter. For the cultivation of lactic acid bacteria, a production medium for bacterial preparations was utilized, containing dried skim milk, lactose, glucose, yeast extract, vitamins, and mineral components.

The selected strains of *Chlamydomonas* algae (751, 759, 761) were cultivated and stored on solid agarized Bold Basal Medium (BBM) nutrient medium, from which they were subsequently rinsed with distilled water. The concentration of the suspension was approximately 10^6 cells per millilitre [18].

We seeked to understand how solvents and magnetic fields influence the organization of microorganisms on silicon surfaces. The substrates used were plates of monocrystalline p-type silicon for solar energy applications, doped with boron, and n-type silicon for microelectronics, doped with phosphorus, with a thickness of approximately 500 μm. After mixing, the cell suspension was applied using a microdoser onto polished degreased surfaces of silicon plates, placed horizontally, and left to dry completely at room temperature. Control samples were left to dry spontaneously, typically for 20–30 minutes. The yeast cell suspension on the silicon surface (experimental samples) dried in a static magnetic field with an induction of 0.17 T. In such a field, the induction lines were perpendicular to the surface of the sample. The temperature conditions, pressure, and humidity for each series of samples were kept consistent.

Images of dried cell clusters were obtained using a metallographic microscope Zeiss AXIO Observer A1M (Jena, Germany), photographs taken at least 10 times from 50× to 1000× magnification, under reflected light.

Fractal analysis of images is used as a quantitative tool to measure the complexity and irregularity of the formed structures, providing an understanding of their spatial distribution and arrangement. The comparison of images was conducted visually and then using the ImageJ and FracLac software. To download the ImageJ program, one can follow the link [19]. The FracLac plugin was used to compute fractal dimension and lacunarity. Firstly, the acquired images were processed in the ImageJ software to remove artifacts such as, for example, the scale bar. After ensuring satisfactory image quality, the images were converted to binary (black and white) form and subjected to fractal analysis using the FracLac program with the "Box counting" method and standard settings, including determination of the statistical error [20].

Our aim was to translate visually observed results into quantitative geometric parameters and numerical values of fractality, such as self-similarity density, which indicates the completeness of space filling by a fractal upon its enlargement. Therefore, the main characteristic of the obtained images was chosen as the fractal dimension of clusters of dried cells. Using the scaling rule, we calculated the ratio of the logarithm of the number of self-similar structures N_C to the logarithm of the scale C according to the formula [21]

$$D = \lim_{c \to 0} \left[\frac{\ln N_C}{\ln C^{-1}} \right],$$

where D represents the fractal dimension of structures for which the model error is acceptable, N_C stands for the number of details or the number of self-similar structures of linear size to cover the entire structure, and C represents the scale or linear size of the structure.

Additionally, we chose lacunarity as an additional characteristic for the numerical description of textures, which determines the degree of space filling by the fractal structure [22]. The importance of this parameter for image analysis is confirmed by various publications, such as those concerning microglia in the brain [23]. Before analyzing the images, the errors of the method were determined using this software. For standard fractal objects, such as the Koch snowflake and the Sierpinski triangle, their fractal dimensions were calculated and compared with theoretically known values, which are 1.2618 (for the Koch snowflake) and 1.585 (for the Sierpinski triangle). The relative errors were 0.6 and 2.25 percent, respectively.

It is worth noting that the description using fractal dimension is sensitive to limitations in data quantity and noise in experimental data. Therefore, in our studies, we used data from several samples, selecting at least 10 clusters in the analyzed photos. Then, these cluster photos were automatically calculated by the "Box counting" method. All images and calculations presented below are original.

Results

Yeast *Saccharomyces cerevisiae.* Let's start by visually examining the microphotographs of the films, and then we'll propose numerical estimates using fractal analysis (Fig. 1). On the greaseless substrates of the monocrystalline p-silicon plates, drops of the applied suspension wetted the silicon surface and spread somewhat. Drying occurred from the edges of the drop towards the centre. In the microphotographs of the dried suspension droplets, it can be observed that the cells can remain intact, with a clear round shape (usually at the edges of the drop), or appear damaged and merged into amorphous spots. Regarding the overall appearance, within the microimage of the dried drop, clusters (clumps of yeast cells) of yeast cells are arranged chaotically, appearing as individual clusters.

As one can see, magnetic field can influence the behaviour of materials, and in our case, it seems to have helped maintain the integrity of the cells over an extended period. In particular, if the yeast suspension on the silicon surface was dried in a static magnetic field of $B = 0.17$ T, structures with intact cells had persisted across the entire droplet area for more than 2 years (Fig. 2) [16]. It is evident that the cell shape is preserved, and the clusters form a pattern with free spaces inside.

Visual observable effects were evaluated using calculated parameters of fractal dimension and lacunarity (Table 1).

In microbiology, it's common practice to place cells in a buffer solution to avoid osmotic stress. Yeast cells have relatively strong cell walls that can withstand fluctuations in osmotic pressure in their environment, but experiments were conducted to control this, suspending yeast in a sterile physiological saline. Initially, a drop of the physiological solution was applied to the surface of both p- and

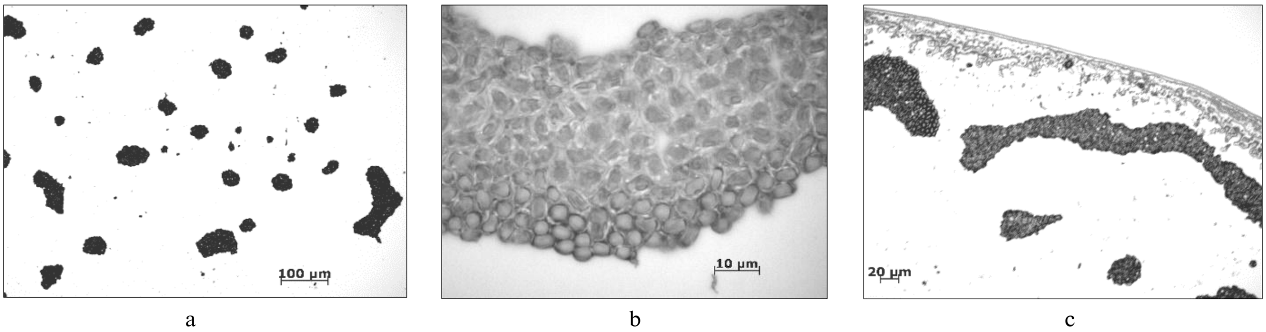

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Figure 1: Microphotographs of the film of spontaneously dried yeast suspension in distilled water on the surface of p-silicon (control): cells clustered into solid clumps (a), with better preservation of individual cells at the edges (b), (c)

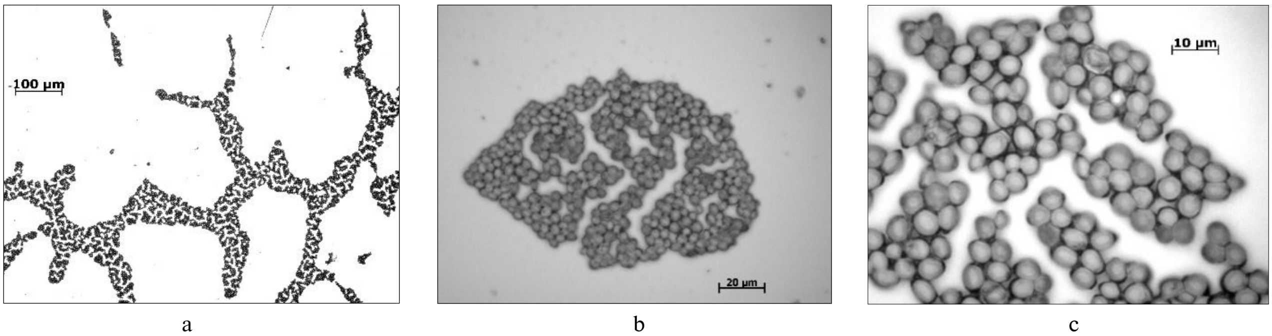

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Figure 2: Microphotographs of the textured film of yeast suspension in distilled water on the surface of p-silicon, dried in a static magnetic field of $B = 0.17$ T. Cells with mostly preserved shapes formed pseudo-fractal patterns, visible at different magnifications. Scale: (a) 100 µm, (b) 20 µm, (c) 10 µm

n-type silicon and observed under a microscope after drying. Microphotographs depict the crystalline structures formed by NaCl (Fig. 3).

As seen in Fig. 3, the microimages of dried salt crystals on both types of silicon substrates, in control and with the presence inducing 0.17 T magnetic field, did not visually differ. However, upon drying the physiological solution with yeast cells, the images drastically changed (Fig. 4), with a grid-like pattern forming even in the control sample. It's evident that the conductivity type of silicon also did not influence the texture of these films.

A chimeric yet fairly structured distribution pattern of yeast cells for the enlarged 50× image was analyzed using fractal dimension calculation. Fragments without NaCl crystals were taken for image analysis. The calculated fractal dimension and lacunarity indices do not demonstrate a significant difference (Table 2).

In further experiments aimed at detecting weak effects (such as the influence of an external magnetic field during droplet drying), we attempted to minimize the presence of salts in the cell suspension.

Therefore, similar studies were conducted with yeast cells in ethanol. For control samples (without yeast), ethanol was applied to the degreased surface of p-type silicon and immediately subjected to a magnetic field, where the samples remained for 10 days. Under the microscope, we observed a uniform distribution of microdroplets of impurities, probably getterized by alcohol from the silicon wafer during drying (Fig. 5). As can be seen, the distribution of impurities on the surface is chaotic on both p- and n-type silicon, without forming any patterns. The inclusions of impurities appear to be small, in the form of microdroplets.

The addition of yeast to ethanol, without the influence of a magnetic field, did not alter the overall distribution pattern on the surface (Fig. 6). In the alcoholic environment, unlike the saline one, yeasts do not form any distinct figures.

Under the influence of a static magnetic field with an induction of $B = 0.17$ T within the field of view around individual yeast cells surrounded by a rim of sediment, ray-like formations appeared (Fig. 7), dendritic structures appeared on the "old" p-type silicon substrate (Fig. 8), and chains formed on the n-type silicon wafer (Fig. 9). "Old" silicon is designated silicon that has been stored for a long time after manufacture and had an increased surface impurity content (had accumulated an increased content of impurities on the surface).

Table 1: Fractal dimension and lacunarity of the texture of dried droplets of aqueous suspension of *S. cerevisiae* on the surface of a silicon wafer under the influence of a magnetic field with an induction $B = 0.17$ T and without a magnetic field

Sample	Magnetic Field Induction *T*	Average Fractal Dimension *D*	Average Lacunarity *L*
S. cerevisiae,100×	0	1.29 ± 0.02	1.16 ± 0.10
S. cerevisiae,100×	0.17	1.50 ± 0.03	0.61 ± 0.02
S. cerevisiae, 500×	0.17	1.68 ± 0.05	0.49 ± 0.06
S. cerevisiae, 1000×	0.17	1.67 ± 0.03	0.49 ± 0.06

The source: the author's calculations based on the conducted fractal analysis.

Table 2: Fractal dimension and lacunarity of the texture of dried droplets of *S. cerevisiae* suspension in physiological solution on the surface of silicon wafers under the influence of magnetic field with an induction $B = 0.17$ T and without magnetic field

Sample	Magnetic Field Induction *T*	Average Fractal Dimension *D*	Average Lacunarity *L*
S. cerevisiae, p-type silicon, control, 50×	0	1.78 ± 0.03	0.26 ± 0.03
S. cerevisiae, p-type silicon, 50×	0.17	1.77 ± 0.03	0.25 ± 0.04
S. cerevisiae, n-type silicon, 50×	0.17	1.78 ± 0.02	0.26 ± 0.03

The source: the author's calculations based on the conducted fractal analysis.

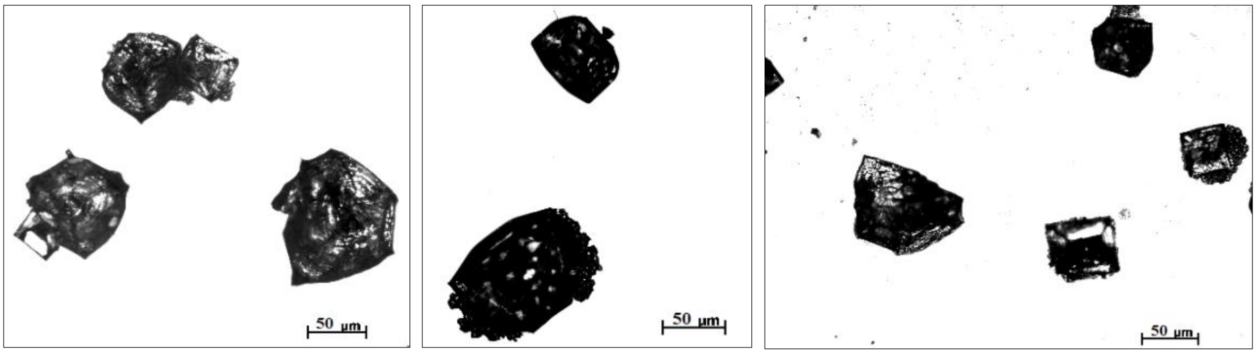

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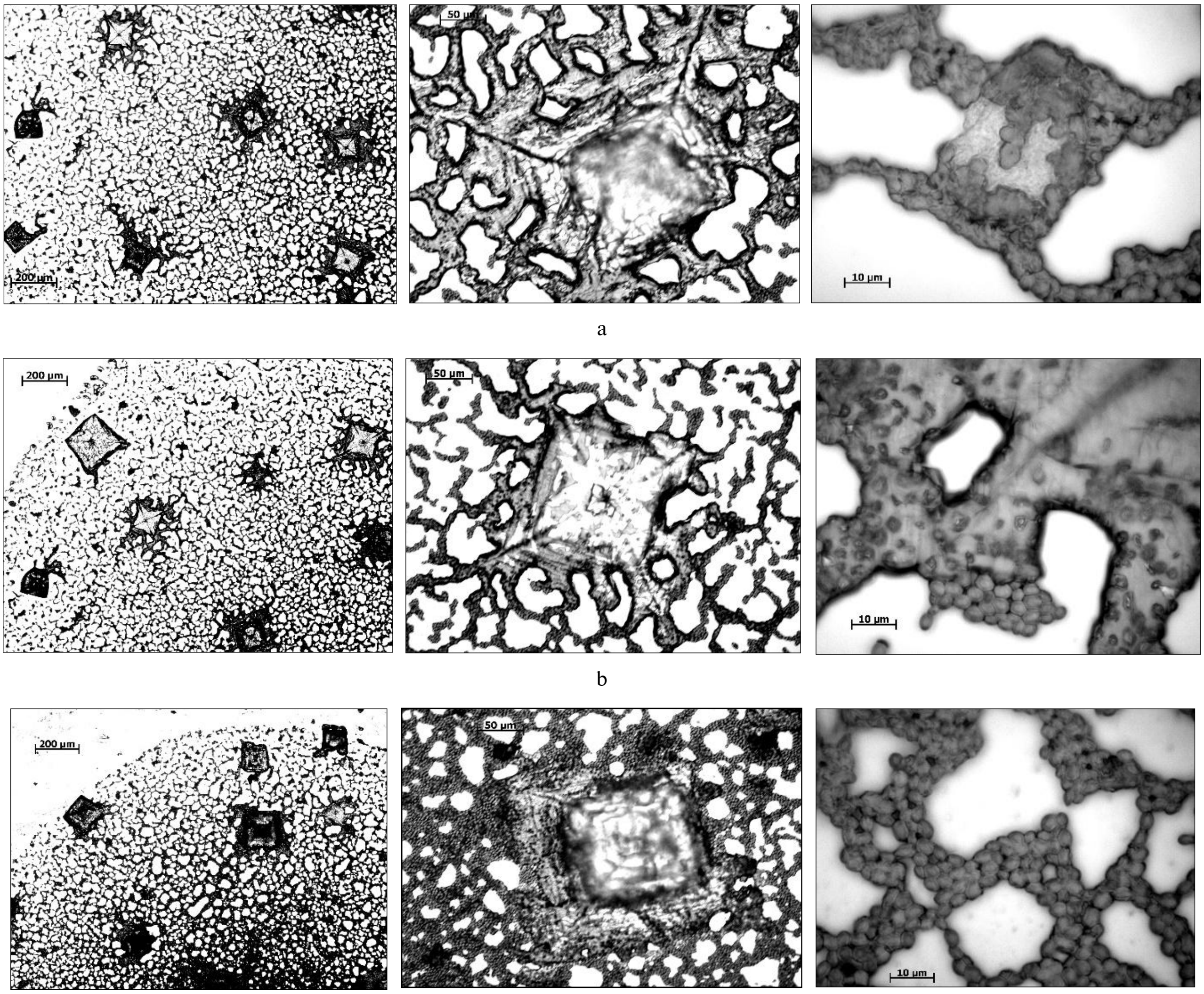
Figure 3: Crystals of the physiological solution dried on the surface of (a) p-type silicon, control, (b) p-type silicon embedded in magnetic field, (c) n-type silicon embedded in magnetic field. The distribution of crystals on the surface is random and uniform



a

b

c

Figure 4: Physiological saline with yeast cells dried on the surface of (a) p-type silicon, control, (b) p-type silicon placed in magnetic field, (c) n-type silicon placed in magnetic field. The cells formed pseudo-fractal figures-chambers both in the presence and absence of magnetic field on both types of silicon

Probiotics *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The influence of magnetic fields on the probiotic microorganisms *Lactobacillus bulgaricus* and *Streptococcus thermophilus* is evidenced by the alteration of the texture of dried cells on a silicon substrate under the action of a static magnetic field. Control samples of a suspension of such mixed probiotic culture after drying show a uniform and chaotic distribution of cells on the droplet surface (Fig. 10a). In the case of magnetic field exposure during drying, cells form fractal-like structures (Fig. 10c). The average values of fractal dimension and lacunarity of the mixed probiotic culture on silicon are provided in Table 3.

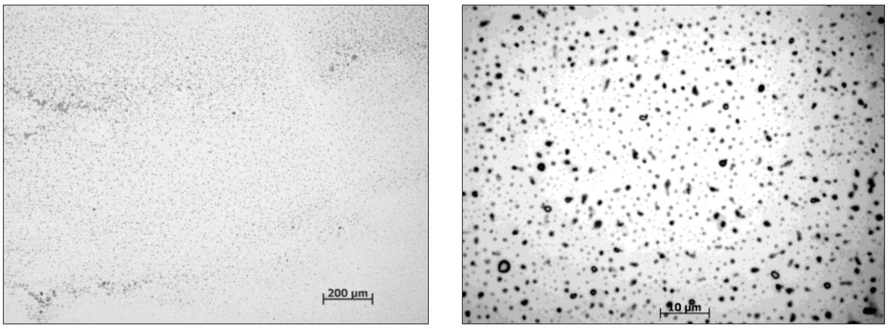


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Figure 5: Microphotographs of the surface of p-type silicon (a) and n-type silicon (b) with applied droplets of alcohol, subjected to a static magnetic field of $B = 0.17$ T for 10 days, at various magnifications. The distribution of impurities dissolved in ethanol is uniform

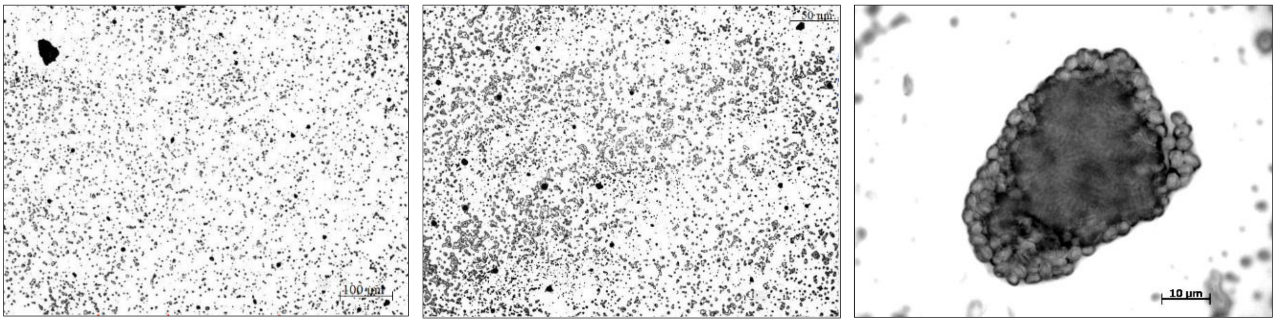

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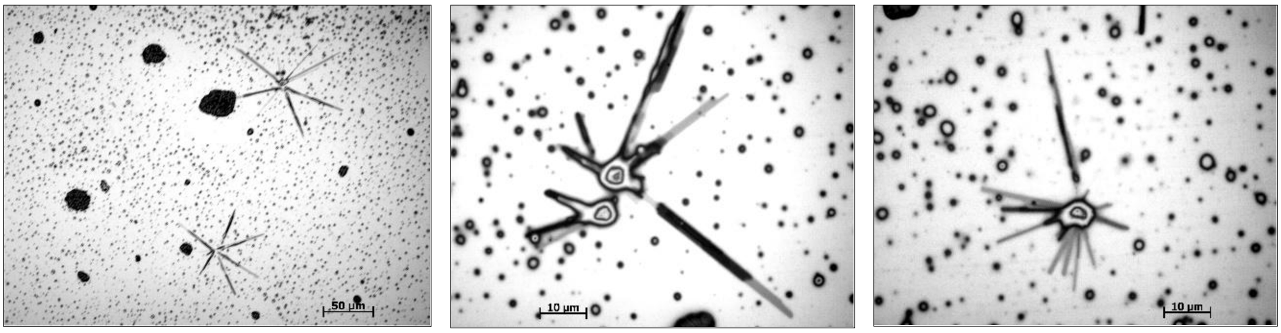
Figure 6: Microphotographs of samples of yeast ethanol suspension on the surface of p-type silicon, control, various magnifications. Scale: (a) 100 μm, (b) 50 μm, (c) 10 μm. The distribution of particles on the surface is almost uniform



a b c

Figure 7: Microphotographs of the surface of p-type silicon with applied suspension of yeast in alcohol, subjected to a magnetic field for 7 days, at various magnifications. Scale: (a) 50 μm, (b) and (c) 10 μm. Clumps and individual yeast cells, as well as impurities from the silicon wafer, are unstructured. Individual yeast cells became centres of "stars"

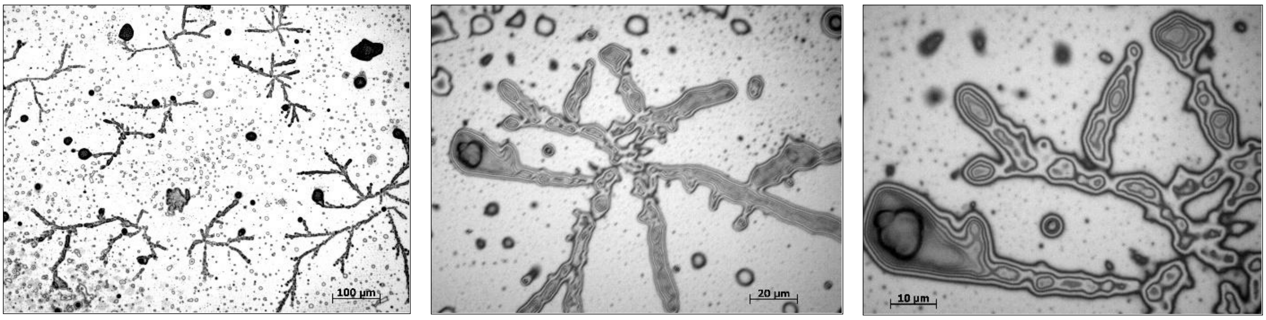


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Figure 8: Microphotographs of the surface of "old" p-type silicon with applied suspension of yeast in alcohol, subjected to a magnetic field for 11 days, at various magnifications. Scale: (a) 100 μm, (b) 20 μm, (c) 10 μm. Branched dendritic structures formed in the magnetic field, along with the presence of damaged cell contents, indicate surface defects

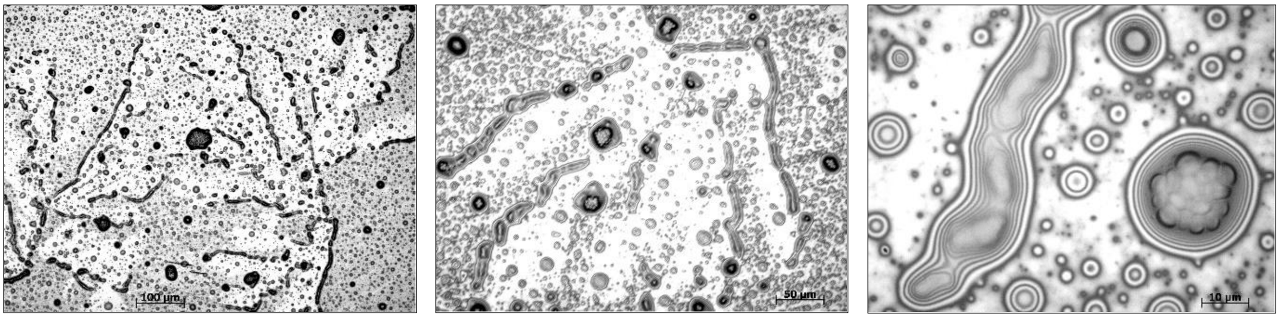

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Figure 9: Microphotographs of the surface of n-type silicon with applied suspension of yeast in ethanol, subjected to a magnetic field for 11 days, at various magnifications: Scale: (a) 100 μm, (b) 50 μm, (c) 10 μm. The pattern of formed capsules with yeast and impurities resembles chains rather than "stars" or "branches" as seen in p-type silicon

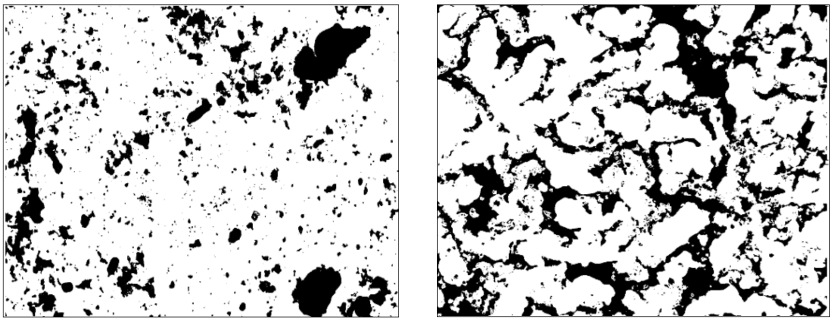

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Figure 10: Binary microimages of the mixed probiotic association of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* cultures on p-silicon wafer: (a) control, (b) under the influence of a magnetic field of 0.17 T. In the magnetic field, cells structured during drying, forming pseudo-fractal patterns

Table 3: Fractal dimension and lacunarity of the dried droplet texture of water suspension of *S. thermophilus* and *L. bulgaricus* on the surface of a silicon wafer under the influence of a magnetic field with an induction of 0.17 T and without a magnetic field

Sample name	Magnetic Field Induction T	Average Fractal Dimension D	Average Lacunarity L
S. thermophilus and *L. bulgaricus*, 200×	0	1.70 ± 0.01	0.87 ± 0.10
S. thermophilus and *L. bulgaricus*, 200×	0.17	1.83 ± 0.01	0.34 ± 0.07

The source: the author's calculations based on the conducted fractal analysis.

***Chlamydomonas* algae**. For cells of flagellate microalgae, differences in the of cells from different strains can be observed after free drying of the suspension droplet on a silicon surface (20–30 minutes), as shown in Figs. 11, 12, and 13. According to microbiological data, the highest number of motile algae is observed in strain *Chlamydomonas* 751, whereas strains 759 and 761 exhibit lower activity.

Samples on p-silicon, prepared simultaneously with the control and subjected to drying in a static magnetic field with an induction of 0.17 T, were also investigated. For strain 751, the cell arrangement significantly differed from the control: they rearranged, forming patterns with pronounced voids and appeared as figures-chambers (Fig. 14).

In contrast, such figures-chambers were not observed in the low mobility *Chlamydomonas* algae strain 759, only uniformly formed clusters were observed along the edges and inside the dried droplet (Fig. 15).

For *Chlamydomonas* algae strain 761, the appearance of arrangement of cell suspensions dried under the influence of the magnetic field was similar to the control.

The results of the fractal analysis of *Chlamydomonas* strains 751 and 759 are provided in Table 4.

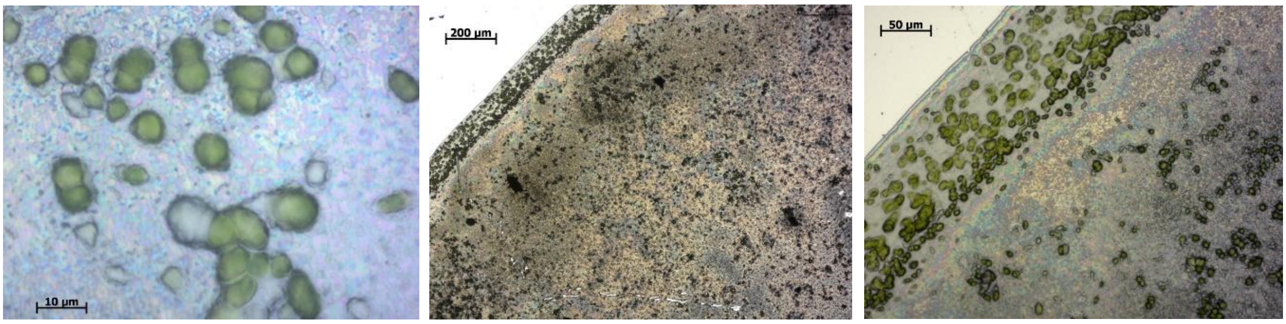

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Figure 11: Microphotographs of the p-silicon surface with deposited cells of the *Chlamydomonas* algae strain 751, control, at different magnifications: (a) general view, (b) edge of the droplet, (c) view of individual cells with flagella. The arrangement of cells on the droplet surface is chaotic, without any formed patterns

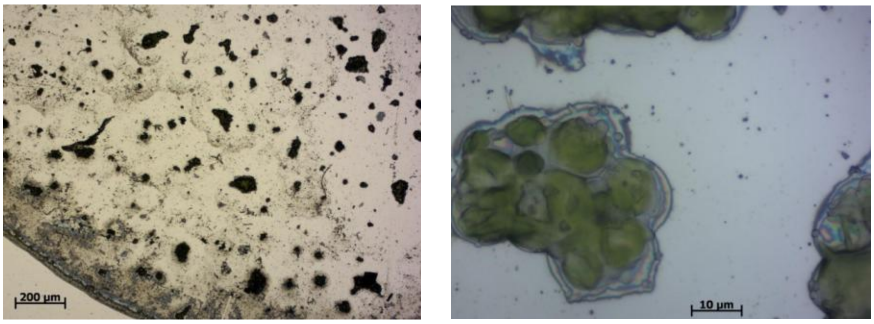

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Figure 12: Microphotographs of *Chlamydomonas* algae strain 759 on p-silicon, control, at different magnifications. Scale: (a) 200 µm, (b) 10 µm. Cells in clusters are not mobile, the aggregates remain inside the dried droplet

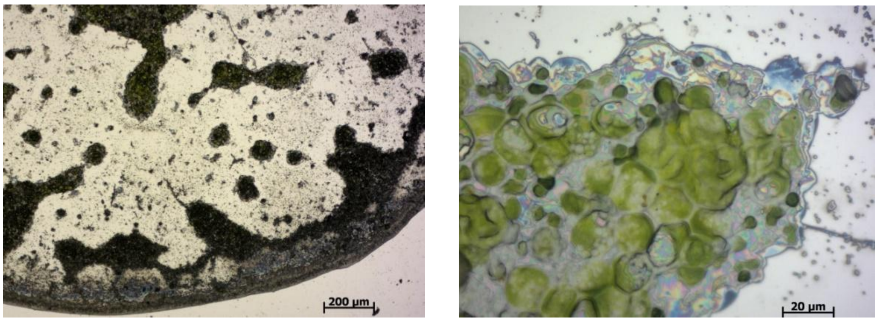

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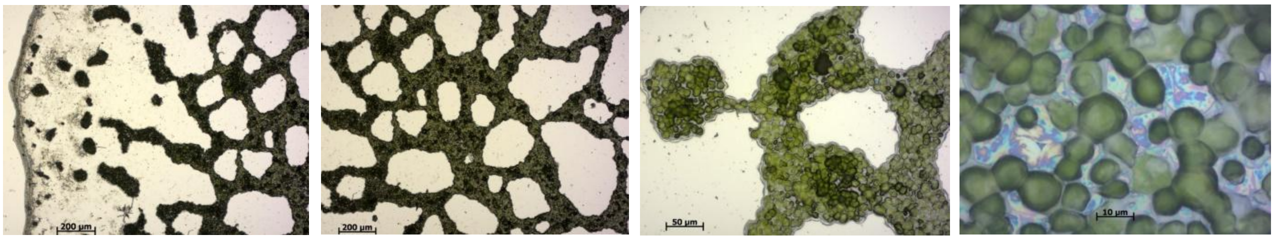
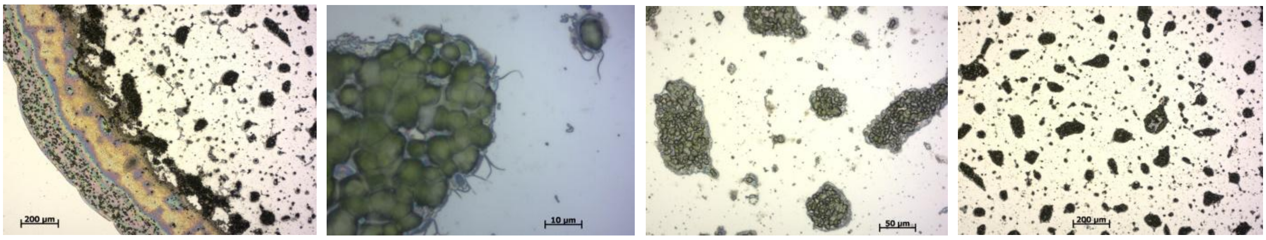
Figure 13: *Chlamydomonas* algae strain 761 on p-silicon, control, at different magnifications. Cell clusters are even more massive (a), enclosed in a mucous capsule (b), and randomly distributed inside or near the edge of the droplet



a b c d

Figure 14: Microphotographs of the p-silicon surface with cells of *Chlamydomonas* algae strain 751 under the influence of a magnetic field of 0.17 T, at various magnifications. Scale: (a) and (b) 200 µm, (c) 50 µm, (d) 10 µm



a b c d

Figure 15: Microphotographs of the p-silicon surface with cells of *Chlamydomonas* algae strain 759, after exposure to a magnetic field with an induction of 0.17 T, at various magnifications. Scale: (a) 200 µm, (b) 10 µm, (c) 50 µm, (d) 200 µm. Cells are arranged in massive clusters that do not form any patterns regardless of the state of the silicon wafer surface

Table 4: Results of fractal analysis of *Chlamydomonas* algae strains 751 and 759

Sample name	Magnetic Field Induction T	Average Fractal Dimension D	Average Lacunarity L
Chlamydomonas algae strain 751, 50×	0	1.65 ± 0.07	0.75 ± 0.11
	0.17	1.74 ± 0.03	0.40 ± 0.09
Chlamydomonas algae strain 759, 50×	0	1.64 ± 0.04	0.70 ± 0.07
	0.17	1.67 ± 0.03	0.68 ± 0.08

The source: the author's calculations based on the conducted fractal analysis.

Discussion

Understanding interactions between microorganisms and surfaces is crucial for comprehending the functionality of surfaces, and as a result, researchers actively investigate them from both applied and fundamental perspectives. Assessing the arrangement of organic particles and cells on a surface is important for understanding the structural integrity or efficiency of a system. The primary focus is on identifying correlations between surface structure and its physicochemical and biological properties. One method for evaluating this arrangement is determining parameters such as fractal dimension and lacunarity. These indicators help understand the degree of orderliness and distribution models, providing valuable insights into the system's state and efficiency.

Efforts to apply the fractal concept to visually observable figures formed by biological objects were made as early as the last century, typically focusing on changes in the shape of pathological cells or tissues within the organism. This could have practical significance for diagnosis through automated detection of changes for machine learning when processing microphotographs of cytological specimens.

Regarding microorganism cells, the global structure of highly branched mycelium of two microbial species, *Streptomyces griseus* and *Ashbya gossypii*, during their growth process, was analyzed using "Box counting" methods [13]. Fractal analysis of microbial colonies is also known [14], allowing for the estimation of the number of colony-forming units automatically by calculating fractal dimension.

Fractal-like figures in dried suspensions were observed in our previous experimental studies [16, 24]. The corresponding influence of electromagnetic fields on biological objects was perceived as a kind of "side effect" that lacked precise explanation and quantitative measurement. However, comparing our results with the work of other researchers, certain common patterns can be established. For instance, there must be an organic component in the suspension. In our studies, these were microorganisms: yeast, green algae, and probiotic cultures. In other similar works, organic components included blood components, biopolymers, impurities in natural mineral water. Conversely, dried solutions of NaCl in distilled water in the absence of biopolymers did not exhibit fractal patterns [7], and artificially recreated mineral water from a set of salts also did not produce fractal structures after drying [8]. An example of a dried physiological solution on a silicon surface can be seen in Fig. 3. Comparing the results allows us to conclude that they correlate with the findings in works [7, 8] indicating that biopolymers directly participate in the formation of structures, and the presence of organic impurities may contribute to the growth of fractal objects by capturing aggregates.

Let's consider the microscopic textures obtained in the presented study, formed by dried suspensions on the silicon surface. The figures of overall appearance and cell clusters at various magnifications in Figs. 1 and 2 are similar but not identical, indicating pseudo-fractal figures. There is a tendency of increasing fractal dimension and decreasing lacunarity when the droplet dried in a static magnetic field (see Table 1).

During the drying of yeast suspension droplets in a physiological saline, a structure formed with NaCl crystals and yeast cells attached to them, resembling a grid with periodic cells-chambers. Some cells ended up inside the salt crystals due to their rapid growth, but then yeast cell chains joined the edges of the already formed crystal and "extended" the crystallization over the entire surface of the droplet.

Since the figures formed from yeast cells appeared in both the control sample and under the influence of the magnetic field, as well as on the surface of both types of silicon, it can be assumed that the solvent (presence of dissociated salts) has a much stronger influence on the type of texture than the external magnetic field or the type of silicon conductivity. There is likely an interaction of charges on the surface, which is fixed at the moment of final drying of the suspension.

The texture of the film from the alcohol-yeast suspension on the surface of the "old" p-type silicon in the magnetic field may visualize substrate material defects that were not evident without the addition of yeast cells. The same chaotic dispersion of impurities was observed both without the influence of the magnetic field and under the magnetic field's action on the surface of n-type silicon. However, the nature of the formed structures from the alcohol-extracted yeast content on p- and n-type silicon significantly differed: "stars" or complex dendritic figures formed on p-type silicon, while chains formed on n-type silicon. These figures may indicate the presence of microcracks or other defects in the silicon wafer structure, where the alcohol extract from yeast cells "spreads." The ring sediment around yeast cells and microimpurities is similar to the ring sediment of a large droplet, indicating rapid drying of the alcohol solvent and a more gradual drying of the extract solution. We can speak of self-similarity of structures "droplet within a droplet."

Let us use the obtained results for comparing and analyzing the surface electric potential in silicon crystals. Dynamic changes in the distribution and magnitude of the surface electric potential in silicon crystals for solar energy production (p-type) and microelectronics (n-type), stimulated by the influence of static magnetic fields oriented perpendicular to the silicon wafer, were described in the article [24]. It was found that the control p-type silicon crystals contained three times more carbon atoms than n-type crystals, which affected the relief character of the surface, as observed using atomic force microscopy (AFM) and magnetic force microscopy (MFM) methods. Under the influence of magnetic fields, a phenomenon of magnetostimulated enrichment of the surface and surface layer with impurities was observed due to the gettering of impurities from the crystal volume and the process of impurity adsorption from the surrounding oxygen-containing environment. The surface roughness index increased. However, the nature of the changes in different types of silicon also differed: the amount of carbon increased nearly twice on the surface of p-type silicon compared to n-type silicon; conversely, the oxygen concentration on the surface of n-type silicon decreased by 2 times, while on the surface of p-type silicon, it increased by 2.5 times. Additionally, the electrical potential value for p-type silicon was recorded to be twice that of n-type silicon after the influence of magnetic field.

Based on the results of these studies, the difference in the nature of the formed figures after applying a droplet of yeast suspension in the magnetic field shown in Figs. 7, 8 and 9, is not surprising: the appearance of the formed structures (dendrites or chains) indicates the different nature of p- and n-silicon impurities present on the plate surface under the influence of magnetic field.

Since ethanol is a more active solvent than water, the solution of inorganic and organic substances (from yeast cells and substrate) is structured under the influence of the magnetic field. The formed structures of microimpurities and adsorbed cells act as indicators of silicon surface defects and charge distribution under the influence of the magnetic field. In this way, it is possible to assess the purity of silicon crystals and, perhaps, the internal stresses in the crystal, which are visualized in lines at the locations of microdroplets, and impurity concentrations. In "old" silicon samples stored for a long time, the impurity content is higher, and the structures under the influence of the magnetic field are more complex.

Thus, the suspension of yeast cells in ethanol interacts very actively with the silicon surface in the magnetic field, dissolving impurities, partially extracting them from the yeast, and forming needle-like, dendritic, or filamentous structures. In the absence of the magnetic field influence during droplet drying, such effects of structure formation were absent. Under the influence of the static magnetic field, small bacterial cells (smaller than yeast) formed pseudo-fractal figures (see Fig. 10). Here, as with yeast in distilled water, we observe a tendency towards increased fractal dimension and significant reduction in lacunarity compared to the texture of the film formed without the influence of the magnetic field (see Table 4).

As seen, *Chlamydomonas* strain 751 cell suspension is finely dispersed (see Fig. 11), as individual algae cells can move freely and are mostly uniformly distributed within the droplet. Typically, cells have a negative ζ-potential, which impedes aggregation [25, 26]. It is also worth noting the clear edge of the droplet resembling a rim. The presence of a rim-like structure with varying cell sizes could indicate some intriguing dynamics at play: the inner radius of the rim is characterized by cells with smaller sizes. This observation could be significant in understanding the structure and behaviour of the droplet. The algal cells on the plane of the droplet are positioned separately from each other. At a magnification of ×1000, algal flagella can be observed.

In contrast, in the *Chlamydomonas* strain 759, most cells are inactive in terms of mobility. This may also be associated with a decrease in the ζ-potential modulus of the cells, causing them to aggregate. The sizes of the clusters are approximately 40–120 μm. After the suspension dries, the cells mostly remain inside the droplet across the entire plane but in dense groups surrounded by a layer of mucus. Apparently, this makes them less mobile (see Fig. 12). For *Chlamydomonas* strain 761, it was also observed that the algae were less active during drying. They tended to form solid clusters, ranging in size from hundreds of micrometers, either inside the droplet or unevenly distributed near the droplet's edge (see Fig. 13).

It is noteworthy that the patterns formed under the influence of static magnetic fields (see Fig. 14) were located inside the plane of the dried droplet rather than at its edges. This indicates a different nature of cell movement compared to the usual concentration of impurities at the edges, or the wave-like distribution of impurities concentration observed during droplet evaporation, which determines the uneven concentration distribution during drying.

Instead, in the *Chlamydomonas* algae strain 759, after the suspension dried in the magnetic field, cells in large groups shifted to the edges of the droplet, while other individual clusters, approximately 50–150 μm in size, remained inside the droplet across its entire plane, but fractal-like chains did not form (see Fig. 15). The edge of the droplet is represented by separate, apparently functionally active cells, and their appearance does not differ much from the edge formed in the dried droplet of *Chlamydomonas* algae strain 751. This suggests some continuity or consistency in the structure and function of the droplet's edge across different states. In other words, some characteristics persist through the drying process, which could have implications for various applications or studies involving droplets. For strain 761 under the influence of the magnetic field, the appearance was similar to the control.

Thus, the dispersity of the cell suspension and the ability of cells to move freely during droplet evaporation are important for the reaction to the magnetic field action and the formation of figures. This may be due to the presence of surface charge on the cells and physicochemical interactions between the surface of silicon plates and individual cells. Therefore, inactive cells grouped together and moving as a whole do not react to the magnetic field under these conditions and do not form figures. This is also clearly demonstrated by the calculation of the fractal dimension and, especially, lacunarity (see Table 4). For mobile individual cells of *Chlamydomonas* algae strain 751, which form a pronounced chamber pattern, the fractal dimension significantly increases, and the lacunarity index decreases by almost half. In contrast, for clusters of cells of *Chlamydomonas* algae strain 759, even those with flagella, these indicators change within the

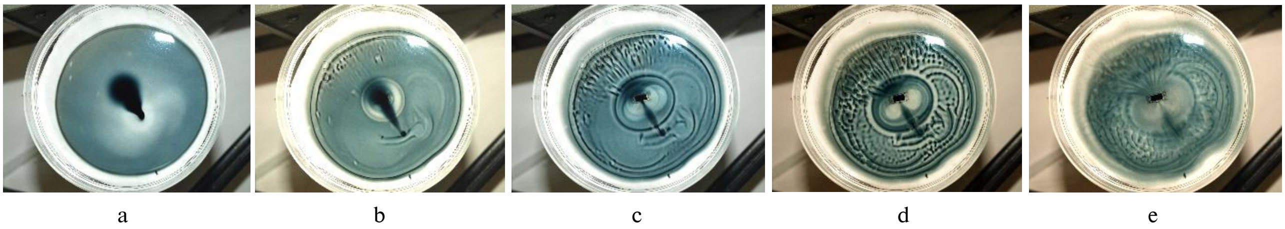


Figure 16: "Rayleigh-Bénard cells" formed by convection currents in a flat horizontal layer of liquid under the influence of EHF-EMF: observations over time from (a) to (e). The last photo (e) shows the instability of the formed figures due to diffusion after the field is turned off [27]

margin of error. This also supports the interaction of negatively charged cells with positive impurities gettering on the surface of the silicon plates during magnetic treatment [24]. It is worth noting that in the study by [7], a decrease in ζ-potential (surface charge of protein particles) was modeled by adding $AlCl_3$ to the medium, which resulted in a reduction in the number of fractal-like patterns.

The formed pseudo-fractal figures with cells (see Figs. 2, 4, 10b, and 14) resemble not dendrites, but "Rayleigh-Bénard cells" – dissipative structures that arise in a flat horizontal layer of liquid due to convection, with the cell sizes being comparable to the thickness of the liquid layer [1]. To explain this, let's consider the evolution of the structure from a drop of Indian ink in water in a Petri dish placed on the waveguide of an extremely high-frequency generator, as described in [27]. In Fig. 16, it is shown how after switching on the extremely high-frequency electromagnetic field (EHF-EMF) in the round drop of Indian ink, a convective vortex forms (a), then a more complex cellular structure forms (b)–(d), which gradually undergoes diffusion decay after switching off the EHF-EMF (e). The whole process takes tens of minutes. In control samples, with the generator turned off, there is usual slow diffusion of the Indian ink droplet in water, without any structured figures. It is worth noting that Indian ink is a dispersed complex of soot with protein in water.

The considered pseudo-fractal structures resembling "Rayleigh-Bénard cells" in liquids are unstable and depend on the geometry of the energy flow input to the sample. Therefore, practically utilizing or even precisely fixing such structures is quite challenging. In contrast, dried droplets of cell suspensions on silicon, especially after exposure to a magnetic field, are extremely stable. Thus, although the chambers form within a liquid (suspension) layer, after drying, a solid structure is obtained, which is convenient for photography, preservation, and long-term study.

In study [7], it was concluded that the fractal dimension of film textures decreases in the case of gamma irradiation and heating of biopolymer solutions, which is associated with denaturation, fragmentation, and aggregation of proteins due to the influence of high temperature or radiation. Thus, damage to the native structure of biopolymers manifests itself in a reduction in the number or disappearance of fractal-like structures in dried films.

Our research with live cells of yeast, algae, and probiotic cultures also confirms similar conclusions: fractal-like structures are formed only from intact, visually undamaged cells. Therefore, it can be assumed that the higher value of fractal dimension of cells immobilized on silicon surfaces may indicate their better preservation and functional activity. Further investigation of these effects is planned.

To improve the accuracy of fractal dimension and lacunarity calculations in systemic research, attention should also be paid to the following factors:

– solvent influence: residues from the growth or dilution medium will affect the microfigures of the dried droplet of microbial suspensions; for example, components of the medium may form dendrites, spherolites, etc., and salts may crystallize;

– concentration effect: fractal dimension will change if cells aggregate or layer on top of each other;

– magnification during microscopy: for comparison, the same zoom level should be used, as we are dealing with physical fractality rather than strict mathematical reproduction of the image at different scales;

– field of view influence: avoiding the edges of the droplet is advisable as it may distort calculations;

– fractal dimension assessment method: in our opinion, the "Box counting" method allows users to eliminate small artifacts (such as scale or salt crystals) and has a user-friendly interface of program, which is freely available.

Conclusions

The analysis indicates significant differences both in the preservation of the shape of dried cells under the influence of the magnetic field and in the self-organization of cells during drying on the silicon surface. The formation of pseudo-fractal figures with cells on the silicon surface depends on the solvent. The numerical evaluation of the dimensionality of the formed structures and the influence of solvents and static magnetic fields on the texture of microorganism suspensions are summarized by the following conclusions.

1) The results of the experiments indicate that fractal-like textures in dried droplets can form on a flat surface in the presence of an organic component in the suspension. In our experiments, these were microorganisms: yeast, green algae, and probiotic cultures. In other similar studies, organic components included blood constituents, biopolymers, impurities in natural mineral water. Conversely, a solution of salt in distilled water did not produce fractal structures, which is consistent with the findings of other researchers [7, 8]

2) Previously identified differences in the preservation of the shape of dried cells under the influence of the magnetic field and in the self-organization of cells during drying on the silicon surface [16] can be expressed using the fractal dimension and lacunarity of the texture of the dried suspension. The trend for various types of microorganisms was an increase in the average fractal dimension D (formation of figures). For example, for yeast cells in ×100 images, it increased from 1.29 ± 0.02 for control samples to 1.50 ± 0.03; for the *Chlamydomonas* 751 ×50 strain, the fractal dimension increased from 1.65 ± 0.07 to 1.75 ± 0.11; for the mixed culture of *S. thermophylus* and *L. bulgaricus* in ×200 images, it increased from 1.70 ± 0.01 to 1.83 ± 0.01. It should be noted that the magnitude of D somewhat depended on the scale of the image, indicating a physical rather than purely mathematical nature of fractality.

3) Simultaneously with the increase in D under the influence of the magnetic field, a decrease in lacunarity L was observed, indicating a reduction in voids in the distribution pattern of cells on the surface or an increase in certain orderliness. For example, for the *Chlamydomonas* 751 ×50 strain, L decreased from 0.75 ± 0.11 to 0.40 ± 0.09. Hence, control of lacunarity is important in creating electrically active coatings on the surface.

4) The formation of pseudo-fractal figures with cells on the surface of the silicon wafer depends on the solvent: in distilled water, figures with yeast, bacteria, or algae cells in the form of chambers were formed only under the influence of the magnetic field; in physiological solution, such chambers formed from yeast cells were observed even without the influence of the magnetic field; in ethyl alcohol, chamber-like structures were never observed, instead, needle-like, dendritic, and filamentous structures were formed from yeast cells, and their shape depended on the type and amount of impurities on the silicon wafer.

5) The authors associate the distribution of cells with the interaction primarily of the negative ζ-potential of microorganisms with positively charged impurities on the surface of silicon, which accumulate on the surface under the influence of the static magnetic field. In the presence of large cell aggregates, ranging in size from tens to hundreds of micrometers, surrounded by mucus (which blocks surface charge of individual cells, such as in the strains of *Chlamydomonas* algae 759 and 761), there was no free movement of cells. As a result, the suspension droplet dried, and the formation of pseudo-fractal texture was not observed. Therefore, the dispersion of the cell suspension and the ability of cells to freely rearrange during droplet drying are important for detecting the response to the magnetic field.

Further research will involve a more detailed investigation into the fractality of immobilized probiotic lactocultures. This holds practical value due to the necessity of protecting probiotics from the harmful effects of the gastrointestinal tract. Additionally, comparing different methods of fractal analysis for calculating the dimensionality of textures may also be the subject of separate research.

Interests disclosure

The authors declare that there are no conflicts of interests.

Acknowledgements

The authors express their gratitude to Alla Kuryliuk (PhD, Assistance Professor) from the Department of Metal Physics at Kyiv National University named after Taras Shevchenko, for providing samples of silicon of various types and assistance in conducting experiments.

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Л.В. Маринченко[1], О.І. Ніжельська[2], А.С. Шірінян[2], Н.О. Горчакова[3]

[1]КПІ ім. Ігоря Сікорського, Київ, Україна
[2]Інститут прикладної фізики НАН України, Київ, Україна
[3]Національний медичний університет імені О.О. Богомольця, Київ, Україна

ОЦІНКА ВЗАЄМОДІЇ МІЖ ПОВЕРХНЕЮ КРЕМНІЮ ТА МІКРООРГАНІЗМАМИ В РІЗНИХ РОЗЧИННИКАХ ПІД ВПЛИВОМ СТАТИЧНОГО МАГНІТНОГО ПОЛЯ ЗА ДОПОМОГОЮ ФРАКТАЛЬНОГО АНАЛІЗУ

Проблематика. Особливості взаємодії мікроорганізмів із поверхнею важливі з точки зору функціональності цієї поверхні (наприклад, імплантів, чіпів, електродів з біоплівкою для продукування електричного струму). Впорядкованість органічних частинок і клітин на різних поверхнях може бути оцінена за допомогою визначення фрактальної розмірності й лакунарності та свідчити про структурний стан або ефективність системи.

Мета. Дослідження впливу розчинників і статичного магнітного поля на текстуру суспензій мікроорганізмів, що висохли на поверхні кремнію різних типів, та числова оцінка розмірності утворених структур на основі фрактального аналізу.

Методика реалізації. Суспензію клітин після перемішування наносили на поліровану знежирену поверхню пластинок кремнію, розташованих горизонтально, і залишали до повного висихання. В статичному магнітному полі (МП) індукцією 0,17 Тл лінії індукції були перпендикулярні до поверхні зразка. Мікрозображення висохлих клітин обробляли в програмі ImageJ і виконували фрактальний аналіз за допомогою додатка FracLac методом “Box counting”.

Результати. Виявлено суттєві відмінності в самоорганізації різних типів клітин мікроорганізмів під час сушіння на поверхнях кремнію за дії магнітного поля та в різних розчинниках. Тенденцією для різних типів мікроорганізмів було утворення псевдофрактальних фігур і збільшення середньої фрактальної розмірності D за дії магнітного поля. Зі збільшенням D знижувалась лакунарність L. Однак, якщо дріжджі перебували у фізрозчині, то псевдофрактальні фігури утворювались і за відсутності МП.

Висновки. За допомогою аналізу фрактальної розмірності псевдофрактальних фігур, які складаються з клітин мікроорганізмів, на поверхні кремнієвої пластини за впливу МП можна оцінити функціональність клітин, що взаємодіють із поверхнею, а також якість цієї поверхні.

Ключові слова: дріжджі; водорості; пробіотичні лактокультури; магнітне поле; кремнієва пластина; вплив розчинників; висушені клітини; фрактальна розмірність; лакунарність.