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FEATURES OF FORMATION OF COLONIAL SETTLEMENTS OF MARINE BENTHIC DIATOMS ON THE SURFACE OF SYNTHETIC POLYMER

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The topic of interactions between plastic and natural communities is now more relevant than ever before. Gradual accumulation of artificial polymer products and their fragments in the natural environment has reached a level at which it is already impossible to ignore the affect of these materials on living organisms. First and foremost, microorganism colonies inhabiting different biotopes, both aquatic and terrestrial, have been affected. These species are at the front-end of interaction with plastic, including those present in marine ecosystems. Nevertheless, in order to understand these processes, it is necessary to take into account several aspects of such interactions: the impact of different types of plastic on microbial community through the release of their decomposed products into the environment, the forms of plastic usage by microorganisms themselves, including mechanisms for surface colonization, as well as possible biodegradation processes of polymers due to the actions of microorganisms. At the same time, types of plastic may differ not only in mechanical strength, but also in their resistance to biodegradation caused by microorganisms. Experiments with surface colonization of types of plastic, which are different in composition and mechanical strength, provide a wide range of results that are not just relevant for understanding modern natural processes involving plastic: these results are also important for application in certain areas of technology development (for example, when creating composite materials). In particular, researches into the forms and mechanisms of sustainable colonization of particularly strong polymers by diatoms from natural communities are of great interest. Due to the fouling of surface of particularly strong synthetic polymers by diatoms, it is possible to form a single diatom-polymeric composite with general properties being already substantially different from those of the polymer itself. For example, when a polymer is fouled with diatoms that are firmly held on its surface due to physiological mechanisms that ensure their reliable fixation, total surface area of the composite increases by 2–3 orders of magnitude compared with this of bare polymer. Such composites and their properties are formed due to mechanisms of substrate colonization used by diatoms from natural marine cenoses – during the transfer of these mechanisms to a new material being prospective for diatom settlement. The practical applications of these composites lie in the sphere of heat and sound insulation, as well as in the field of creating prosthetic tissues for bone operations. In our experiments, we tracked the sequence of development of a stable composite when diatoms colonized the surface of samples of a particularly strong synthetic polymer being resistant to corrosion. In this case, the sample population process took place on the basis of colonies formed in accumulative cultures from the natural marine environment. Samples of ultra-high molecular weight polyethylene (UHMWPE)

with a smooth and porous surface structure (with an open cell, bulk porosity up to 80 %) were colonized by diatoms *Karayevia amoena* (Hust.) Bukht., 2006, *Halamphora coffeaeformis* (C. Agardh) Levkov, 2009, and *Halamphora cymbifera* (W. Greg.) Levkov, 2009. These laboratory experiments lasted for three weeks. Accumulative microphyte cultures, on the basis of which the experiments were conducted, were obtained from the Baltic Sea (Baltiysk area, Russia) and the Arabian Sea (Mumbai area, India). The types and stages of development of colonial settlements on various elements of the frontal surface microrelief and in the underlying caverns were studied using a scanning electron microscope on samples subjected to stepwise thermal drying. Individual cells of *K. amoena*, *H. coffeaeformis*, and *H. cymbifera*, their chain-like aggregates, and outstretched colonial settlements occupied varying in degree non-homogeneous microrelief surface elements, forming structures with a thickness of 1–2 layers with an average settlement height of 1–1.3 single specimen height. *K. amoena* cells were tightly fixed to the polymer substrate using the pore apparatus of the flap of the frustule. Observations using scanning electron microscope revealed shell imprints on the substrate, which were signs of a polymer substrate introduction into hypotheca areoles. The spread mechanisms of diatoms of three mentioned species on various elements of UHMWPE surface were explored, as well as the formation of the characteristic elements of colonial settlements, including for *K. amoena* – consecutively in the form of "pots" and spheres, by means of interaction with polymer surface and its extension with the increase in the number of tightly attached cells in the colonial settlement.

Keywords: diatoms, diatom algae, Bacillariophyta, plastic colonization, UHMWPE, sustainable materials, plastic in the marine environment, aquaculture

For many decades, diatoms attract the attention of a wide range of scientists because of their role in the ecology of the biosphere as a whole: as producers of about ¼ of terrestrial organic matter and almost ⅓ of all oxygen generated on the planet. More recently, in the field of materials science, the study of a hierarchical multilevel organization observed in diatom shells structure and, as a consequence, of their biomechanical characteristics has begun. Many issues of cell interaction with various substrates have been studied in detail [6 ; 7 ; 14 ; 15 ; 16 ; 17 ; 24], but a number of questions still have no adequate response. A deeper understanding of these aspects is expected to be obtained using modern FIB-SEM methods (focused ion beam scanning electron microscopy) [6 ; 20 ; 25]. The use of aquaculture technologies can expand the use of diatoms as a sustainable resource for biofuels, biomineralization, and material production. The potential biodegradation of hydrosphere-contaminating polymers by fouling with diatoms is also considered as an important environmental problem [2 ; 6 ; 22 ; 23 ; 26].

Ultra-high molecular weight polyethylene (hereinafter UHMWPE) commercialized by Celanese [9] is a polymer with high mechanical properties that has been used in marine practice for the manufacture of ropes and sails since the 1990s. Due to bioinertness, as well as acceptable mechanical properties and wear resistance, the field of UHMWPE application in surgery is growing: when creating implants for bones and joints and, more recently, in reconstruction processes at the cellular level, as scaffolds for tissue engineering [13]. Being colonized by mesenchymal stromal cells, UHMWPE scaffolds with open porosity demonstrate a high ability to osseointegration and vascularization [21].

The main idea of this study is as follows: if certain diatom species from natural marine communities are able to colonize the surface of various types of plastic [4 ; 5 ; 6], including UHMWPE, in some cases this process can be classified as a way to create a new class of biocorrosion- and strain-resistant materials – diatom-polymer hybrids. Theoretically, a number of processes accompanying synthetic polymer colonization can be considered:

A. "Surface single-layer colonization" will take place without significant proliferation of diatoms in the depth of the substrate due to the lack of porosity. If the colonized surface is destroyed as a result of biodegradation, over time this process will end with disintegration of polymer products into fragments of various size, and it can be used as a technology for combating environmental pollution with macroscopic plastic.

B. In contrast to “surface single-layer colonization”, formation of a sufficiently thick, dense, and mechanically strong multilayer coating with barrier or other properties, valuable for practical application, is possible on the surface of biostable polymers.

C. “Colonization of volume” due to intensive proliferation in depth of the porous (cellular) polymer is expected to create a stable bulk diatom-polymer composite with a wide range of technical characteristics, that are important for solving structural, tribological, filtration, and thermal problems, as well as for use in the field of vibration- and sound insulation. The expansion of the spectrum of possible applications is due to a significant increase of the total surface of the composite – by 2–3 orders of magnitude compared with the non-colonized surface of the polymer.

Since UHMWPE has significant chemical and biological stability, it is an important candidate material for studying the process of colonization of its surface and volume. In this article, we discuss the first results on the structural aspects of the interaction of marine diatoms from natural microphytobenthos communities with porous UHMWPE surface.

MATERIAL AND METHODS

UHMWPE samples of two types were used to study the surface colonization process: smooth and porous ones. Samples of both types were exposed in the accumulative cultures of diatoms isolated from the sandy littoral: I – in Mumbai area (the Arabian Sea); II – in Baltiysk area (the Kaliningrad Bay, the Baltic Sea). In both accumulative cultures, diatoms grew under natural diffused light, in the conditions of alternating day and night (on the windowsill in the laboratory of P. P. Shirshov Institute of Oceanology), in the temperature range of +5 to +30 °C (from the coldest winter months to the warmest summer ones), covering the walls of 1-L laboratory vessels of polyethylene terephthalate (hereinafter PET) and high density polyethylene (hereinafter HDPE). Culture growth occurred without additional aeration, in the same volume of water into which they were transferred from natural biotopes. The salinity of seawater in the first vessel was of 30 ‰, in the second – of 5 ‰. The age of culture I at the time colonization experiments began was of 21 months, of culture II – of 20 months. In culture I, representatives of the genus *Halamphora* (Cleve) Levkov dominated: *Halamphora coffeaeformis* (C. Agardh) Levkov, 2009 (Fig. 1c–e) and *Halamphora cymbifera* (W. Greg.) Levkov, 2009 (Fig. 1f); in culture II – *Karayevia amoena* (Hust.) Bukht., 2006 (Fig. 1a, b).

The choice of cultivation conditions – sufficiently rigid for marine benthic diatoms taken from the natural environment – was dictated by the need to obtain mixed accumulative cultures from several species, maximally adapted for joint development over a long period of time with no additional aeration and no feeding with biogenes from the outside, as well as with significant changes of lighting conditions and ambient temperature. The act of colonization of vessel walls surface by different diatom species deserved special attention and became the basis for experiments with UHMWPE colonization.

Samples of smooth UHMWPE were obtained by thermal cutting of a dense (non-porous) cylinder with a diameter of 26 mm into 2–3-mm high “tablets” with surfaces smoothed due to reflow.

Samples of porous UHMWPE were prepared in accordance with the method presented in [12]. UHMWPE powder (4120 GUR Ticona®) was mixed with edible rock salt (NaCl) with a particle size of 80–700 μm. The dry mixture with a weight ratio of 1 : 9 was carefully mixed using planetary ball mill Fritsch Pulverisette 5 (Fritsch GmbH, Germany) in 500-ml agate centrifuge filled with corundum balls with diameter of 8 mm. Thermal pressing was carried out under a load of 70 MPa at +180 °C. Salt removal was then carried out using distilled water at +60 °C, using an ultrasonic cleaner. This process resulted in the formation of porous structures with open pores, with a bulk porosity of about 80 %.

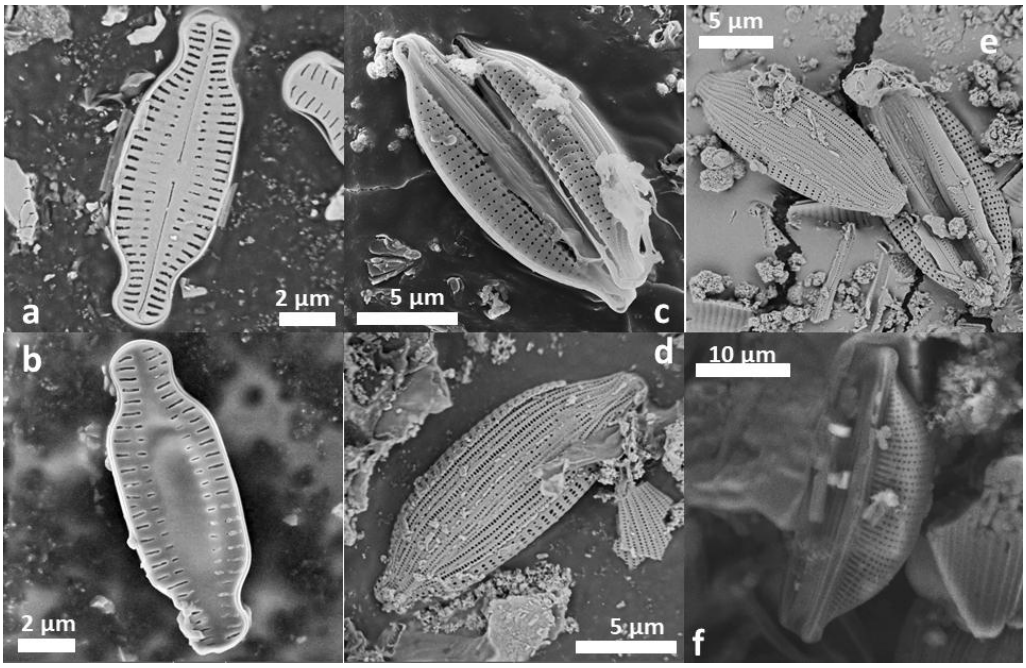

Fig. 1. The most common diatom species that formed fouling on the surface of porous UHMWPE samples: a, b – *Karayevia amoena* (in storage culture II); c–e – *Halamphora coiffeaeformis*; f – *Halamphora cymbifera* (in storage culture I)

To obtain experimental fouling of smooth and porous UHMWPE surface (with different surface microreliefs), various samples of this material – three replicates for each sample – were exposed in accumulative cultures over a period of 21 days under constant diffused light with LED1106 G2 2.3 W, 18 mA, 35 lm/W. The dimensions of rectangular samples of porous UHMWPE were of 40×19×3 mm, and the diameter of smooth samples was of 25 mm. Laboratory vessels made of colorless PET and HDPE with a capacity of 1 L (2 pieces each) with accumulative cultures were located at a distance of 45 cm from the light source. Light intensity was of 135 lx.

Three-week duration of the experiment is explained by the fact that by the end of this period, extensive placers of brown fouling spots had formed on the surface of porous UHMWPE samples, which were clearly visible to the naked eye. This allowed us to proceed to the stage of material microscopy.

Colonial settlements of diatoms from accumulative culture II grew on the walls of vessels 1 and 2 (PET); on the walls of vessels 3 and 4 (HDPE) – colonial settlements of diatoms from accumulative culture I. In vessel 1, samples of porous UHMWPE No. 8 and 9 were exposed in three replicates, in two rows, three in a row. In vessel 2, sample of porous UHMWPE No. 10 were exposed in three replicates, the upper row, and "tablets" of smooth UHMWPE – three replicates, the lower row. In vessel 3, samples of porous UHMWPE No. 1 and 2 were exposed, by analogy with vessel 1. In vessel 4, a sample of porous UHMWPE No. 3, in three replicates, and three "tablets" of smooth UHMWPE, by analogy with vessel 2, were exposed. General scheme of the experiment is shown in Fig. 2.

During the experiment, UHMWPE samples were suspended in water column, on threaded loops made of copper wire (Glorex, 20 m × 0.4 mm, with anti-corrosion coating) at a distance of 5–10 mm from aquarium walls covered with diatom fouling, at an angle of 30–40° to light source. Fouling of *Halamphora* species was obtained on samples No. 1, 2, and 3 (three replicates for each), and of *Karayevia amoena* – on samples No. 8, 9, and 10 (also in triplicate). During the experiments, control extracts of samples were not performed in 21 days of exposure; therefore, it is not possible to determine time and place of appearance of the first diatom cells on specific samples.

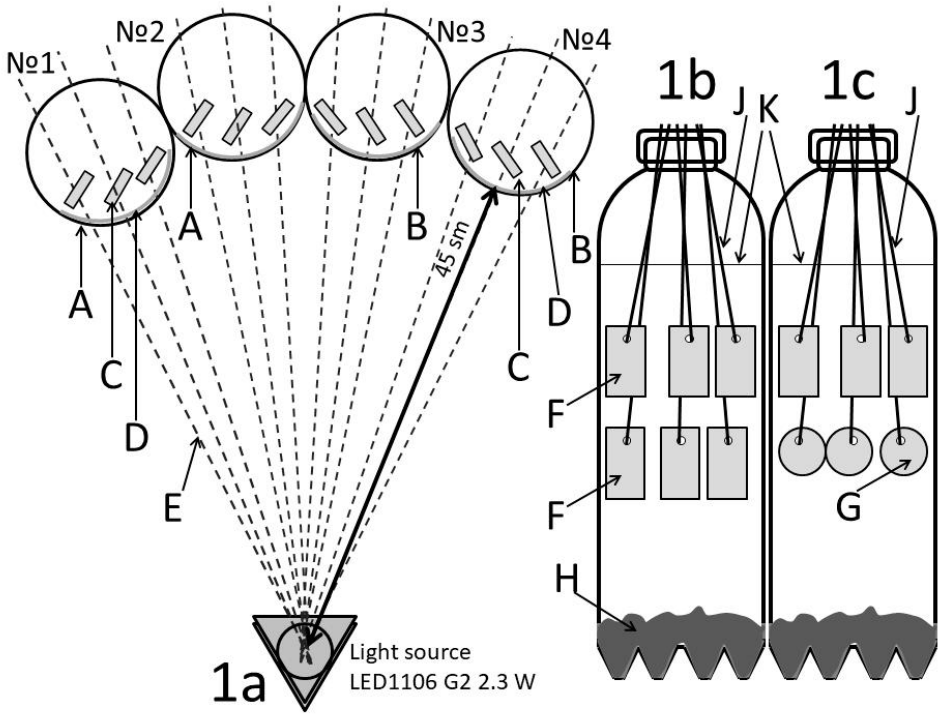

Fig. 2. General scheme of the experiment with colonization of the surface of porous and smooth UHMWPE samples by marine diatoms from various storage polycultures. **1a** – scheme of the experiment, top view: A – PET-mini-aquariums (1-L bottles); B – HDPE-mini-aquariums (1-L bottles); C – samples located at an angle to light source; D – layer of diatom colonial settlements on the bottle wall; E – vector of the direction of the light flux from the source. **1b** – layout of samples in bottles No. 1 and 3: F – porous UHMWPE samples; H – sea soil; J – copper wire fixing UHMWPE sample in water, near the bottle wall; K – sea water level. **1c** – layout of samples in bottles No. 2 and 4: G – smooth UHMWPE samples

When preparing samples for microphotography using a scanning electron microscope, we used a new author's method of three-stage drying: exposure in a drying cabinet at +50 °C for 8 hours, at +80 °C – for 3 hours, and at +100 °C – for 1 hour. The methodology proposed, never published before, was based:

1) on the results of Ph. V. Sapozhnikov for drying diatom periphyton on filamentous algae in a drying cabinet, obtained in 1996 at Belomorsky Biological Station of Moscow State University, whose goal was to create permanent preparations from dried diatom shells on filamentous algae surface without loss of periphyton spatial organization;

2) on the data on changes in UHMWPE properties upon heating, which made it possible to estimate the degree of density of shell association with sample surface.

The temperature of +80 °C is the limit, beyond which UHMWPE begins to soften, acquiring the properties of thick resin. However, small diatoms, such as *K. amoena* (up to 15 μm long), cannot immerse into the thickness of this polymer due to their own weight, since their mass is too small, the specific surface area of wide ovaloids of revolution, which geometrically are their frustules, is quite large, and the softness of the substrate itself is insufficient for this. This is also evidenced by the fact that larger cell diatom species used in the experiments (*Halamphora coffeaeformis* and *H. cymbifera* having length of less than 30 and 50 μm, respectively, and the geometric shape of a wide ovaloid of revolution) did not immerse in the polymer thickness when heated above +80 °C. Moreover, at +90 °C UHMWPE samples begin to be affected by the shape memory effect (common materials science designation of this process is "cylinder narrows and extends"), due to which small objects, immersed in it under their own weight, are pushed out.

Thus, after the final stage of drying for one hour at +100 °C, one should not expect the effect of spontaneous fusion of diatom shells into the surface of this polymer. Rather, if loosely associated with the polymer, they would separate from the surface due to buoyancy shape memory effect.

After primary drying, the samples exhibited in accumulative culture I were heavily coated with salt; therefore, they were additionally washed by two-day exposure in the distillate, and then dried again for 4 hours at +60 °C. Microphotography of diatom fouling was carried out at a magnification ×500 to ×700 using three different scanning electron microscopes: Hitachi TM 1000, Tescan LYRA, and Tescan MAIA3.

Counting of the shells on the surface of UHMWPE samples was carried out manually using microphotographs, marking the specimens counted as a part of both chains and "cloak-like" settlements. When isolating discrete spots, markers of different colors were used. The number of intervals when separating the size classes of spots was approximately calculated using the formula:

$$h = 2(IQ)n^{-1/3},$$

where h is the length of the interval;

(IQ) is the difference between the upper and lower quartiles (according to Freedman – Diaconis formula [8]).

RESULTS AND DISCUSSION

Surface colonization of smooth UHMWPE samples did not occur in any of diatom accumulative cultures. The result obtained is important because of its potential application in the design of marine antifouling underwater structures of UHMWPE with a smooth surface.

Experiments with surface colonization of porous UHMWPE samples showed a number of important features of this process, including common ones, for various diatom species. So, in accumulative cultures, where many diatom species developed on a sandy substrate, and only a few species settled on the walls of experimental vessels (with the predominance of mentioned above), only certain taxa transferred to UHMWPE samples.

In particular, accumulative culture II consisted of 10 species of benthic diatoms. Of them, *Karayevia amoena* formed numerous and dense colonial settlements on the walls of PET vessels and sparse settlements – on grains of sand at the bottom of the vessel; *Melosira nummuloides* C. Agardh formed few short chains; other species from the genera *Amphora* Ehr. ex Kütz., *Diploneis* (Ehr.) Cleve, *Nitzschia* Hassall, and *Fallacia* Stickle et D. G. Mann were often found in the sand and occasionally – on the walls of the vessel. Only the first two species mentioned – *K. amoena* and *M. nummuloides* – have moved on to living on a new substrate (porous polyethylene).

Scanning electron microscope studies did not reveal the development of bacterial colonies on UHMWPE samples. In turn, *K. amoena* formed different types of colonial settlements on the surface of various UHMWPE samples.

In accumulative culture I, three species from the genus *Halamphora* were noted, two – from *Karayevia* Round et Bukhtiyarova ex Round, two – from *Nitzschia*, and one – from *Navicula* Bory. All benthic diatoms lived not only in sand at the bottom of the vessel, but also on the walls, forming on them dense spots of colonial settlements with a predominance of *H. coffeaeformis*. Only *H. cymbifera*, *H. coffeaeformis*, and *K. amoena* settled on porous UHMWPE samples. The third species in culture I had morphological differences from that in culture II; it was seen rarely and by separate cells, while the first two species formed

colonial settlements of various types. The dominant species forming the most extensive colonial settlements on the porous UHMWPE was *H. coffeaeformis*. No bacterial colonies were observed on the surface of UHMWPE samples in this experiment either, but individual cells of rod-shaped bacteria were found.

All three species that showed active growth on porous UHMWPE (*K. amoena*, *H. coffeaeformis*, and *H. cymbifera*) are benthic ones and lead an attached lifestyle in nature, colonizing various substrates (surface of mineral grains of sand and plant debris, chitinous shells of dead invertebrates). Moreover, according to shell macro- and micromorphology, ability to move actively, and way of fixing on the substrate, representatives of the genus *Karayevia* differ significantly from those of *Halamphora* [3 ; 10 ; 11 ; 19]. Extremely inactive *K. amoena* attaches very tightly to substrate surface, and all movements of its cells are reduced to upper daughter cell crawling away from the lower one after division over a distance not exceeding, as a rule, its length. To date, no independent movement of *K. amoena* over distances significantly exceeding its shell length has been reported. The transfer of cells of this species to new habitats, significantly remote from the previous ones, occurs solely due to action of external factors during water movement or during the movement of substrate particles already populated by them. In particular, we assume the possibility of transferring cells to UHMWPE surface (from the composition of colonial settlements on the walls of the vessels and from the surface of sandy soil) using bubbles of oxygen released by microphytes, because the walls of these bubbles, being separated from the fouling, often had a brownish color. On the contrary, *H. coffeaeformis* and *H. cymbifera* cells, although they lead an attached lifestyle and are inactive, are still able to transfer over distances many times larger than the length of their shell, which can affect the nature of the settlements formed by them [18].

We observed three main types of colonial settlements with transitional forms between them. This suggests that the process of populating porous UHMWPE with *K. amoena* occurs in three successive stages. First, the cells of this diatom propagate along the substrate, forming primary colonization chains (Fig. 3). To do this, they use the tops (ridges) or the edge areas of folds and surface flocculent fragments. Chains of this type are characterized by terminal (apical) growth; they are formed as a result of cell division and the subsequent movement of each upper daughter cell from the lower one over a small distance along the surface of the highest protruding microrelief elements of the sample. Intercalar doubling of cells in such a chain occurs locally, and only in places of microrelief "branching": lateral processes are added to the main growth direction, also elongating terminally. It can be assumed that the formation of these chains is not only the primary surface colonization, but also the process of searching for areas, where the formation of more compact colonial settlements is possible.

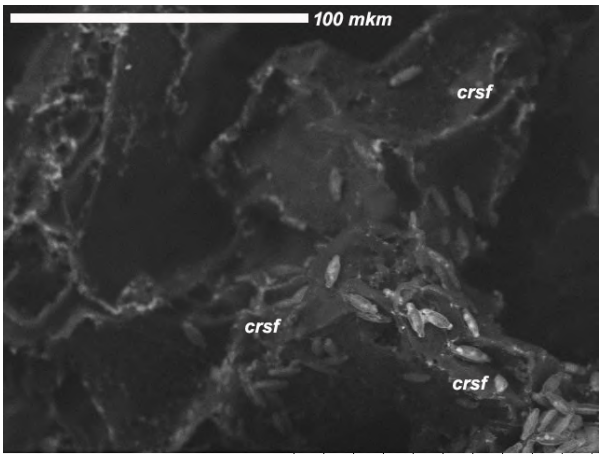

Fig. 3. Primary colonization chains of porous UHMWPE by *Karayevia amoena* cells. In the foreground, the chains pass along the crests of substrate folds (*crsf*)

When such chains reach relief areas characterized by either a high density of folds (especially on hill-shaped elevations), or, conversely, by the relative surface smoothness (including the bottom of small gaps), the formation of secondary colonization chains begins. These structures are formed due to the doubling of not only the terminal cell in the chain, but also of all its other cells that have reached such an area. As a result, oblong sinuous or branching structures are formed from cells arranged in two rows according to the "herringbone principle" (parquet "herringbone") (Fig. 4). If primary colonization chain has significant

intervals (gaps) (at least of 1–1.5 cells long) then several secondary colonization chains can form from it. Given the quasihomogeneous nature of the microrelief, primary and then secondary colonization chains are able to cover substrate surface with a rather dense net (Fig. 4).

Secondary colonization chains give rise to "nuclei", or to the most dense, initial groups of cells, during the growth of colonial settlements. Continuing to double frequently in secondary colonization chains, *K. amoena* cells efficiently spread over an area with a high density of folds (often along a "hill") or over a limited area on a relatively flat surface, filling its entire available area. This forms the third stage of colonization, or "cloak-like" settlement (Fig. 5).

The area covered by such a settlement depends on the space scale of the elements of microrelief, that ensures its development. Such vast settlements, often formed from hundreds and thousands of cells, consist of smaller fragments of mosaics, or "spots" of a similar configuration [1]. "Spots" can be clearly distinguished by narrow winding gaps between them, as well as by the direction of the axes of the cells of which they consist. As a rule, these "spots" look like tubercles in the composition of the settlement, are located at different angles in relation to each other, and correspond to centers of intense cell division. With the development of a particularly dense "cloak-like" settlement, they reflect the features of microrelief surface on which they are formed. On UHMWPE samples of different porosity, such "spots" differ in the abundance of cells. When the maximum packing density is reached and the cells of the settlement already cover the substrate in 1–1.3 dense layers and begin to rise above it in the form of a knoll, they cease to massively divide, as noted in the composition of extensive open "cloak-like" settlements. It was also registered that periodically, in conditions of a small area of accessible relatively flat surface, the cells continue doubling and begin to actively transform the substrate, as it will be described below.

On the surface areas of sample No. 8, represented mainly by relatively smooth 20(30)–80 μm wide flakes being torn along the edges, the size of primary

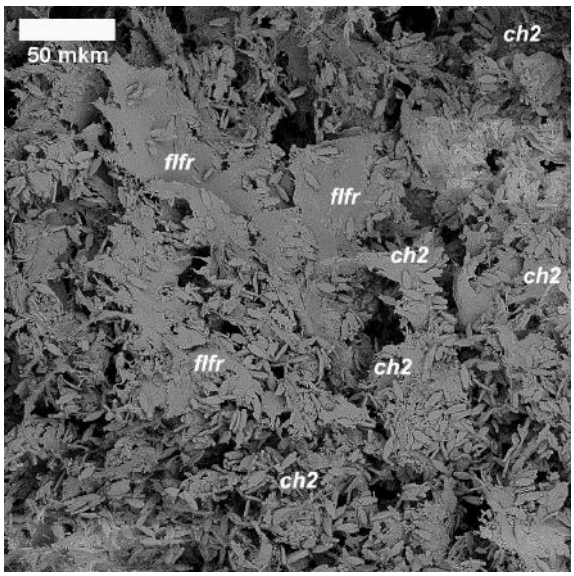

Fig. 4. Secondary colonization chains (*ch2*) of porous UHMWPE by diatom *K. amoena*: cell structures arranged in two rows according to "herringbone principle" ("herringbone" parquet). The substrate is represented by small flocculent fragments (*flfr*) of a relatively flat surface

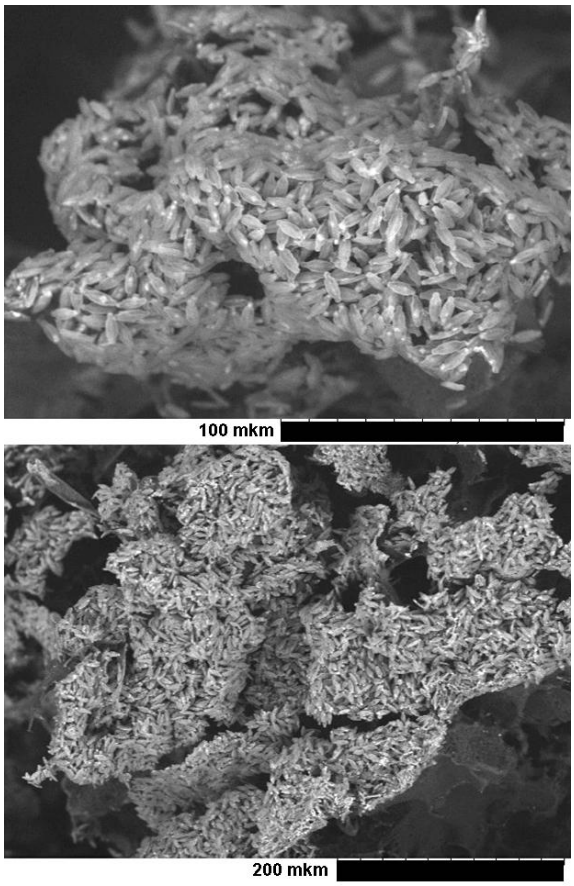

Fig. 5. View of "cloak-like" areas of *K. amoena* settlements on the surface of porous UHMWPE (at different magnification)

colonization chains ranged from 2 to 12 cells (3 on average), with a predominance of chains of 3–4 cells. The size of secondary colonization chains at the stage of the abundant formation of “herringbones” and the coating of the substrate with a dense net varied from 3 to 23 cells (9.94 on average), with a predominance of chains of 6–11 cells (they accounted for a total of 48.75 %) (Fig. 6). In turn, the size of “spots” ranged from 9 to 73 cells (31.01 on average), and the largest of them had branched outlines.

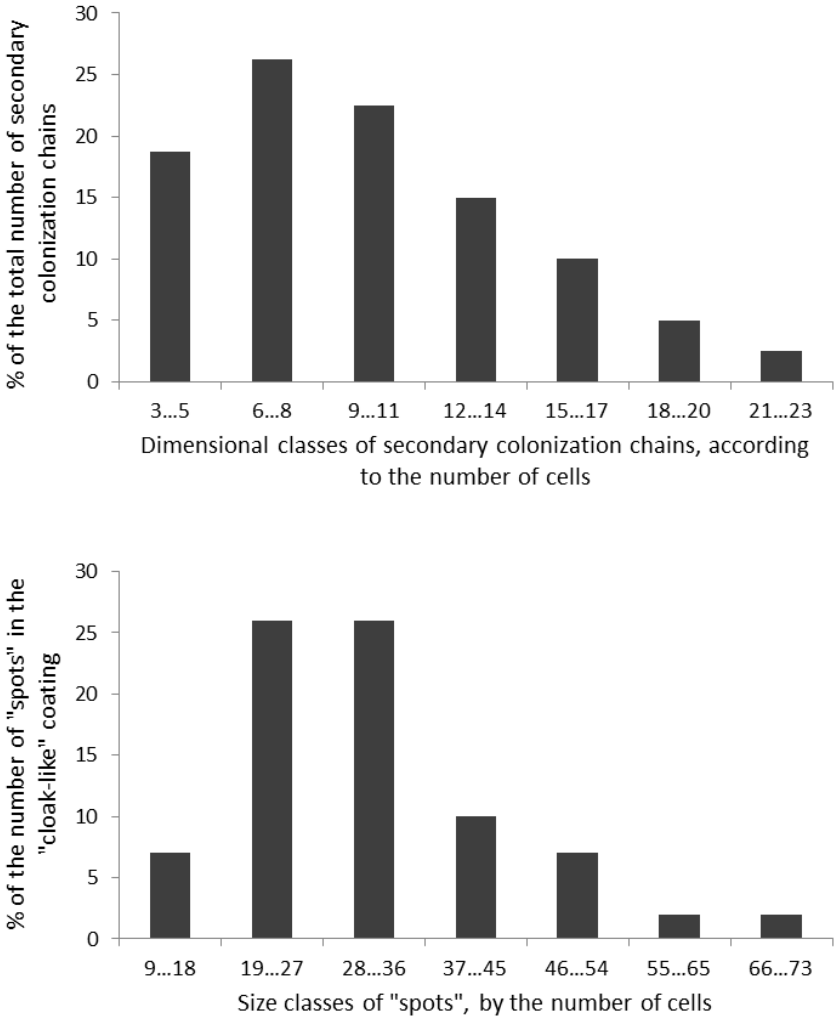

Fig. 6. Frequency distribution of size classes of “spots” in “cloak-like” colonial settlements on the surface of sample No. 8

However, in the structure of “cloak-like” settlements, “spots” with a size of 19–35 cells prevailed (65 % in total) (Fig. 6). Totally, location of 12,404 shells in 400 “spots” was taken into account.

On the surface of sample No. 10, the folding was significantly higher: the microrelief was sinuous and finely folded; it consisted of three-dimensionally branching structures covered with a mosaic of small flat sections (of 40–60 μm along the largest axis), located in different planes and separated by thin low folds-barriers. Primary colonization chains were 2–8 cells long (3–4 cells on average); secondary colonization chains consisted of 3–15 cells (5–8 cells on average; chains of this size accounted for 60 %) (Fig. 7). “Spots” consisted of 5–26 cells (17 on average), but structures of two types prevailed among them: formed of 14–16 and 19–21 cells, depending on characteristics of the surface microrelief (Fig. 7). In this sample, 9520 shells in 560 “spots” were taken into account.

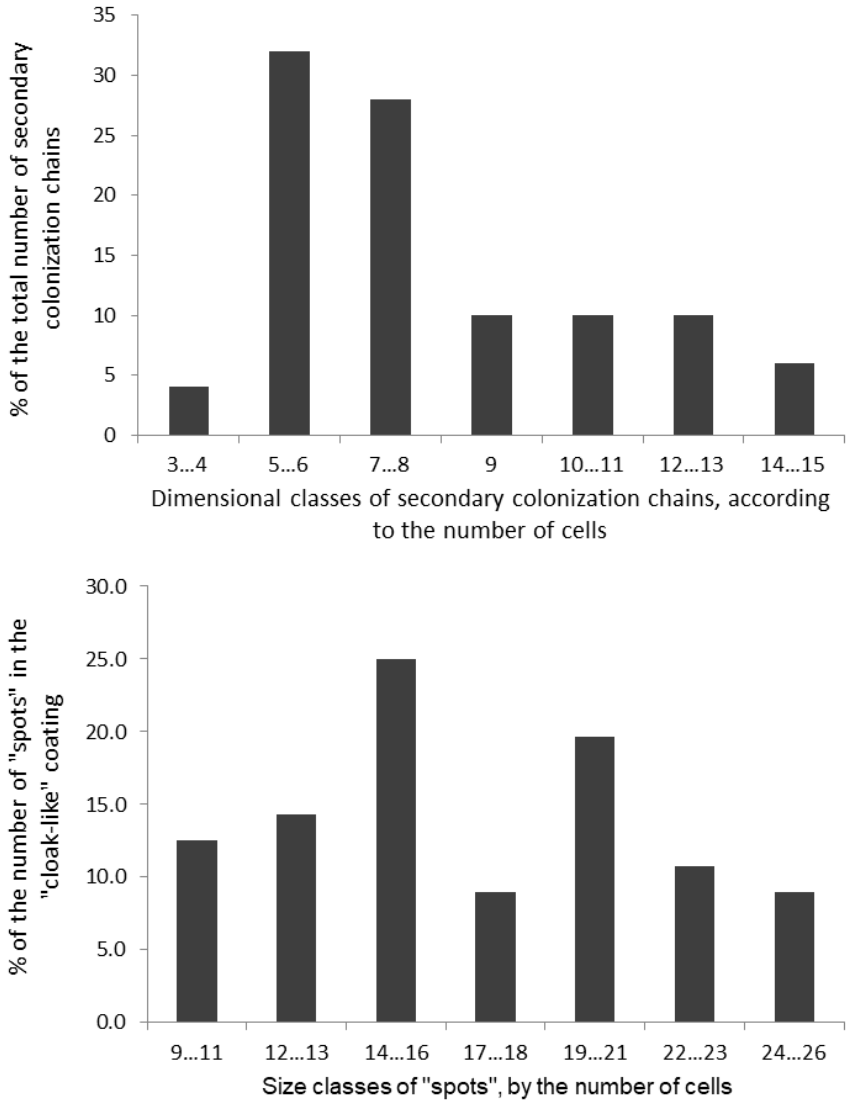

Fig. 7. Frequency distribution of size classes of “spots” in “cloak-like” colonial settlements on the surface of sample No. 10

It is important to note the ability of *K. amoena*, which we established, to modify UHMWPE microrelief: on the one hand, due to a very tight attachment of cells to sample surface, and on the other, due to their synchronous division in rows. When examing fragments of the developed fouling at a magnification ×3000, the following types of deformation of polyethylene surface by diatom cells were noted.

The first type consisted of ridges with a length of 20–30 μm, squeezed out by diatom shells due to fouling compaction on both fold sides. Successive doubling of cell rows tightly attached to the substrate, on both sides of a low but wide fold, with the integration of newly formed rows between them, led to stretching of the substrate itself: the fold was stretched into a narrow ridge. On such ridges, several cells grew, which were in the substrate in the wells depicting the outlines of the shell (Fig. 8). The wells could be formed due to the compression of these cells, which continue to hold tightly in place, while raising the edges of the crest apex due to its extension in height, accompanied by squeezing the edge into the fold.

The second type was formed by squeezed and thinned edges of flat surface areas, along the edges of which there were diatoms in the wells in the shape of a shell (Fig. 8).

Both types of elements of large (in relation to the shells) deformation were formed due to the growth of cell chains in width (doubling), and due to the fact that the shells were tightly attached to the surface, and the newly formed cell rows were embedded between already attached rows, thus stretching the substrate.

Absolutely special secondary structures formed by *K. amoena* from UHMWPE were noted on the surface of sample No. 8 (Fig. 9). Upon reaching the maximum population density of an even and relatively small area, the cells began to transform its surface using tight attachment to the substrate, as well as increasing of the number of adjacent rows, thereby stretching the polymer substrate.

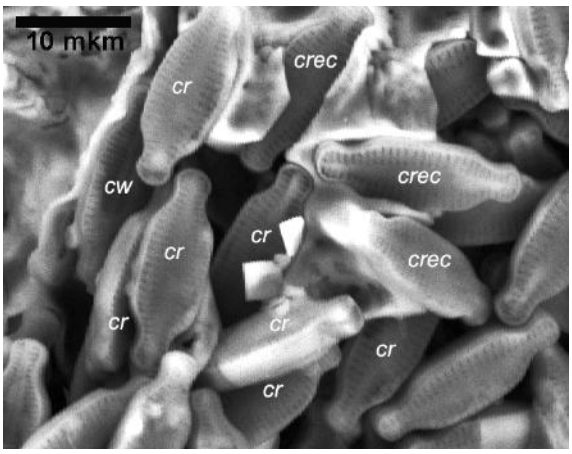

Fig. 8. UHMWPE surface deformations by *K. amoena* cells during the formation of a "cloak-like" settlement. Cells on the edge of the crest (*crec*), squeezed from a wide fold of the substrate in dense cell rows (*cr*), sit in the wells formed by extruding the brow in the form of a fold. The cell in the well (*cw*) is visible on the left in the wrung-out part of the marginal area (the squeezed fold frames the cell on the left)

First, an annular row located along the area edge was formed (Fig. 9a), and its doubling began on both sides with a gradual extrusion of the substrate surface into a low annulated fold. Then, separating more and more new rows in both directions – in and out of the annular row – the diatoms squeezed the fold already into the annular wall (Fig. 9b). Rows inside such a "well" under construction received obviously less light and biogens than rows outside, because a semi-enclosed space was formed. As a result, the number of rows outside grew faster, including due to their intercalary doubling up and down along the entire height of the "well". Because of this, the walls of the "well" bent outward, forming a "pot" (Fig. 9c).

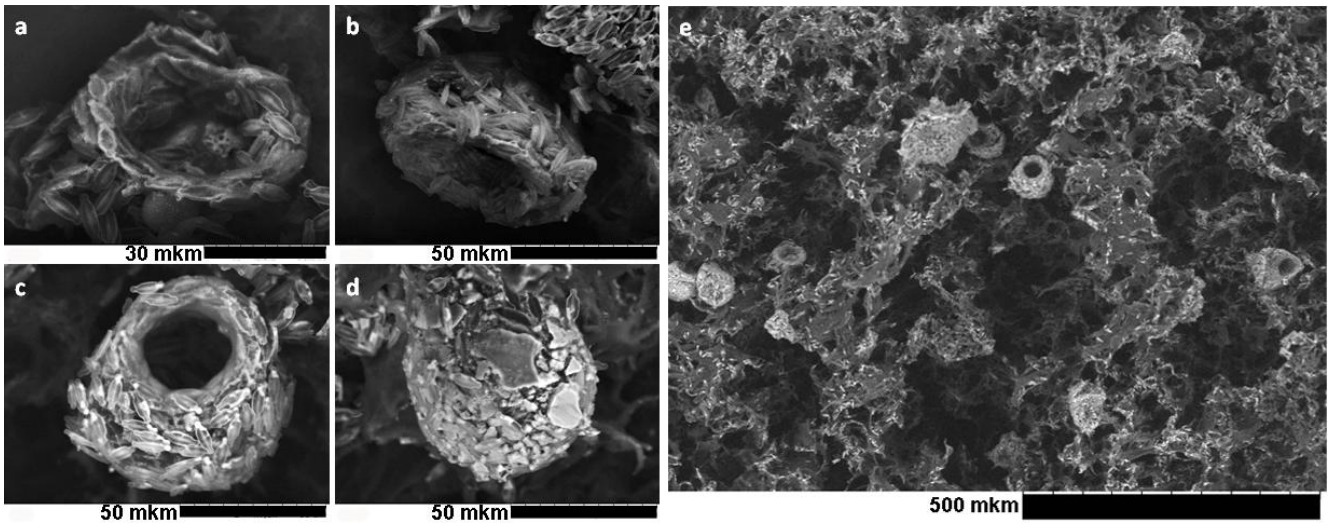

Fig. 9. Deep deformation of the surface of porous UHMWPE by the growing *K. amoena* colonial settlement: a – formation of an annular fold; b – stage of fold extrusion in the annular wall; c – stage of the "pot"; d – formation of a fragment of a "cloak-like" settlement in the form of a sphere, inside the fouling there is a fragment of UHMWPE surface extruded by dense cell rows in the form of a "pot"; e – location of the protruding fragments of the "cloak-like" settlement in the form of "pots" and balls on frontal surface of porous UHMWPE

In the late stages of the formation of such a structure, light and biogens almost ceased to enter its internal space through a narrow gap. During this period, the increase in the number of cells continued only outside, and the diatoms in the rows already formed short sequences of 3–4 cells, forming "end-specks" of the membrane. Such "end-specks" were located at different angles to each other. In the final stage, a similarity of a sphere was formed (Fig. 9d): the terminal hole of the bloated "pot" covered the "spot" of the diatoms of the outer membrane. The diameter of these extruded structures was of ≈ 60 μm at the stage of fold and of ≈ 80 μm at the stage of "pot"; the volume formed was of ≈ 270–290 μm^3. The distance between the "pots" on the front surface of the sample reached 300(400)–600(700) μm with their rare location and 10(40)–700 μm – with frequent location, including doubles (Fig. 9e).

The surface of porous UHMWPE in sample thickness was colonized up to a depth of 150–200 μm – both in the form of primary colonization chains along the bottom of the caverns and due to the wide "cloak-like" settlements in the areas of deep folding of the front surface.

The successive formation of the same three stages of the colonization of porous UHMWPE was noted for *H. coffeaeformis*, but with its own characteristics. Firstly, primary colonization chains of this species were significantly sparser (due to the movement of daughter cells over longer distances after division). Secondly, intercalular cell division was more often observed in their composition, and this was not always accompanied by the growth of lateral "branches" along suitable microrelief areas. Thirdly, primary colonization occurred not only in the protruding, multi-folded areas (on samples No. 1 and 2), but also in concave relief elements – as on the surface of sample No. 3 (Fig. 10). Here, wide, being devoid of additional folding, and smoothly curving "blades" (with the width of 200–400 μm) often interspersed with wide caverns (of 200–700 μm along the largest axis), the depth of which reached 200–500 μm. In turn, the blades themselves, due to their bends, could reach a height of 300–700 μm. Nevertheless, the formation of secondary colonization chains occurred along "ridges" and edges of caverns in the front UHMWPE surface according to the same principle of parquet row doubling as for *K. amoena*, or by an increase in the number of cells in the chain in the form of bundles. Fourthly, the development of particularly large "cloak-like" *H. coffeaeformis* settlements was most often observed along the edges of large caverns (Fig. 11), and of smaller ones – along the upper edges of the "blades" (on sample No. 3) or along the "hills" (on samples No. 1 and 2) (Fig. 12). Due to the sparseness of the chains of primary and secondary colonization, as well as the net nature of "spots", it is not possible to reliably distinguish their characteristic sizes for *H. coffeaeformis*.

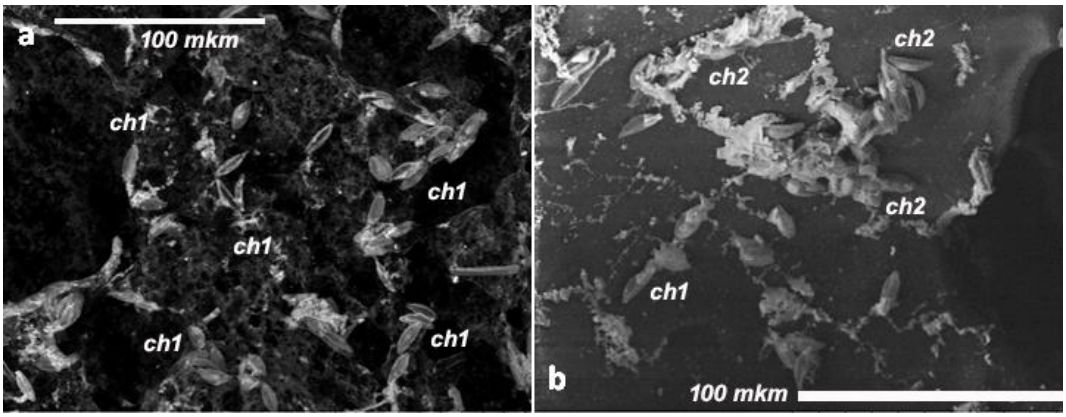

Fig. 10. Primary (*ch1*) and secondary (*ch2*) colonization chains of surface of porous UHMWPE by *Halamphora coffeaeformis* cells

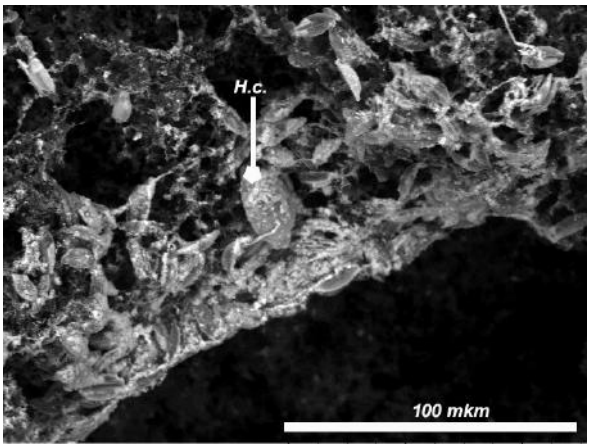

Fig. 11. Large "cloak-like" settlement of *Halamphora coffeaeformis* along the edge of a large cavern on the frontal surface of porous UHMWPE. In the center of the image there is a separate large cell of *H. coffeaeformis* (*H.c.*)

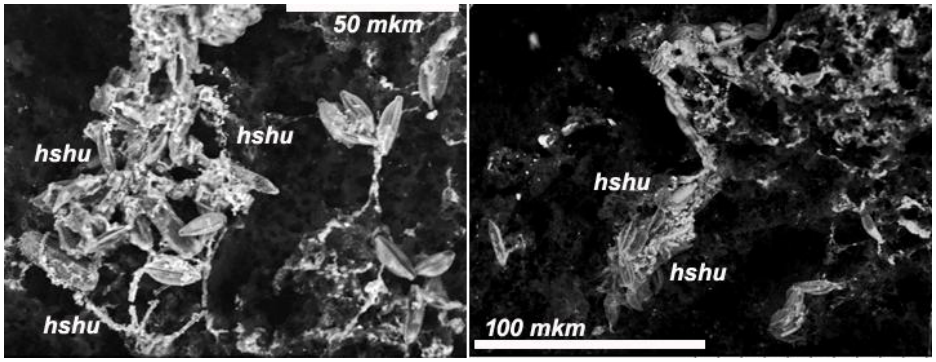

Fig. 12. Small "cloak-like" *Halamphora coffeaeformis* settlements along the tops of hill-shaped uplifts (*hshu*) of the densely folded surface of porous UHMWPE

Finally, *H. cymbifera* did not form primary colonization chains at all to search for topologically suitable surface areas. Large cells of this species either settled on shaded microrelief areas and gave rise there to very dense, compact colonial settlements (on sample No. 3) (Fig. 13), or settled alone along the edges of caverns, among dense *H. coffeaeformis* settlements (on samples No. 1 and 2) (Fig. 11).

The size of compact *H. cymbifera* populations varied from 6 to 32 cells (17.08 on average). Groups of 14–18 cells prevailed (a total of 32 %).

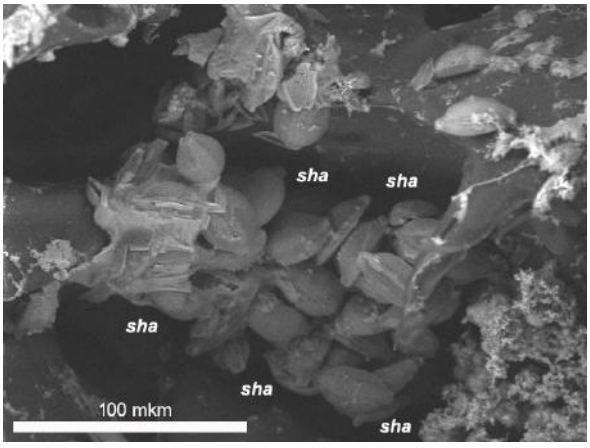

Fig. 13. Dense and compact *Halamphora cymbifera* colonial settlement in the shaded ("cavernous") area (*sha*) of porous UHMWPE microrelief

Conclusion. The results of the experiments on the study of diatom fouling of UHMWPE samples with different surface microrelief structures revealed a number of general characteristics of the process. Surface fouling of smooth UHMWPE did not occur. Samples of porous UHMWPE were colonized by cells with different morphologies: achnantoids (*Karayevia amoena*) and amphoraids (*Halamphora* spp.), which have different mechanisms of adhesion to the substrate surface, but have shown common strategies for accustoming samples differing in microrelief. For species of similar sizes (*K. amoena* and *H. coffeaeformis*, size class of ≈ 10–18 μm), three common successive stages of colonial settlements formation were revealed: 1) primary colonization chains, with the help of which the cells settled on the substrate; 2) secondary, or planar, colonization chains; 3) open, or "cloak-like", settlements formed from "spots".

The first and second stages of colonization spread mainly along the protruding elements of the microrelief, and the third stage – in relatively flat areas or areas speckled with tiny, densely arranged folds, where each cell was attached immediately to two or three folds. In the areas with the densest folding or with a relatively smooth surface, both species formed extensive "cloak-like" settlements, being the most abundant on the "hills" and "lobes" rising above the general level of the frontal surface. In all samples, where the minimum repeating area of the folded surface was comparable with the size of the cells [from ≈ 10 : 1 to ≈ (50…100) : 1], the formation of "spots" from a more or less certain number of cells was noted, with their location repeating microrelief features and forming "cloak-like" settlements.

For *K. amoena*, the ability to modify UHMWPE surface was noted for the first time – due to extremely tight attachment of cells to the substrate and, as a result, due to stretching of this surface with an increase in the number of cell rows. In some cases, the modification was expressed in point stretching of marginal zones of the smallest flat areas suitable for the growth of open settlements. In other cases, the transformation consisted of squeezing of area surface in the form of a ring and of further formation of a "pot" with convex walls. On the surface of the sample with minimal folding, *H. coffeaeformis* (size class of ≈ 10–18 μm) used shaded areas for large compact settlements – along cavern edges, in dents along the bends of the "blades", and on narrow isthmuses between the caverns. In this case, minimum relatively homogeneous area significantly exceeded the area of the cells of this size class, and they preferred the areas protected from water movement and direct lighting. Larger *H. cymbifera* cells (size class of ≈ 30–35 μm) with minimal folding also went into "shadow", but used more comparable areas at the bottom of shallow caverns.

Thus, it was revealed that during the colonization of various samples of porous UHMWPE, diatoms form stable settlements, tightly associated with the surface, the morphology of which is closely related to the features of surface microrelief. With appropriate processing of composites obtained, which allows us to get rid of the organic content of diatom cells and to clean their shells, it becomes possible to obtain UHMWPE samples with a stably biomineralized surface, the total area of which is several orders of magnitude larger than that of the original surface.

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ОСОБЕННОСТИ ФОРМИРОВАНИЯ КОЛОНИАЛЬНЫХ ПОСЕЛЕНИЙ МОРСКИХ БЕНТОСНЫХ ДИАТОМЕЙ НА ПОВЕРХНОСТИ СИНТЕТИЧЕСКОГО ПОЛИМЕРА

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Тема взаимодействий пластика и природных сообществ к настоящему времени актуальна как никогда прежде. Постепенное накопление изделий из искусственных полимеров и их фрагментов в природной среде достигло того уровня, при котором уже невозможно не считаться с влиянием этих материалов на живые организмы. В первую очередь воздействию подвергаются сообщества микроорганизмов, населяющих разные биотопы (как водные, так и наземные). Эти существа находятся на переднем крае взаимодействия с пластиком, в том числе в морских экосистемах. Тем не менее для понимания данных процессов необходимо принимать во внимание несколько аспектов таких взаимодействий: влияние разных видов пластика на сообщества микроорганизмов через выделение в среду продуктов их разложения, формы использования пластика самими микроорганизмами, в том числе механизмы колонизации его поверхности, а также возможные процессы биодеструкции полимеров за счёт деятельности микроорганизмов. При этом разные виды пластика могут отличаться не только механической прочностью, но и устойчивостью к биодеструкции, вызываемой микроорганизмами. Эксперименты с колонизацией поверхности видов пластика, разных по составу и механической прочности, позволяют получить широкий спектр

результатов, актуальных не только для понимания современных природных процессов с участием пластика: эти результаты важны и для применения в некоторых областях развития технологий (например, при создании композитных материалов). В частности, представляют большой интерес исследования форм и механизмов устойчивой колонизации особо прочных полимеров видами диатомовых водорослей из состава природных сообществ. За счёт обрастания поверхности особо прочных синтетических полимеров диатомеями возможно формирование единого диатомово-полимерного композита, общие свойства которого уже существенно отличаются от свойств полимера как такового. Например, при обрастании полимера диатомеями, плотно удерживающимися на его поверхности за счёт физиологических механизмов, обеспечивающих им надёжную фиксацию, суммарная площадь поверхности композита возрастает на 2–3 порядка по сравнению с таковой голого полимера. Такие композиты и их свойства формируются за счёт механизмов колонизации субстратов, используемых диатомеями из естественных морских ценозов, — при перенесении этих механизмов на новый, перспективный для заселения диатомеями материал. Возможности практического применения этих композитов лежат в сфере тепло- и звукоизоляции, а также в области создания протезирующей ткани при операциях на костях. В наших экспериментах отслежены последовательности развития устойчивого композита при колонизации диатомеями поверхности образцов особо прочного синтетического полимера, стойкого к коррозии. Процесс заселения образцов происходил на базе сообществ, сформированных в накопительных культурах из природной морской среды. Образцы сверхвысокомолекулярного полиэтилена низкого давления (СВМПЭ) с гладкой и пористой структурой поверхности (с открытой ячейкой, до 80 объёмных % пористости) были подвергнуты колонизации диатомовыми водорослями *Karayevia amoena* (Hust.) Bukht., 2006, *Halamphora coffeaeformis* (C. Agardh) Levkov, 2009 и *Halamphora cymbifera* (W. Greg.) Levkov, 2009. Лабораторные эксперименты продолжались три недели. Накопительные культуры микрофитов, на базе которых проводили эксперименты, были получены из Балтийского моря (район г. Балтийска, Россия) и Аравийского моря (район г. Мумбаи, Индия). Типы и стадии развития колониальных поселений на различных элементах микрорельефа фронтальной поверхности и в подлежащих полостях изучали с помощью сканирующего электронного микроскопа на образцах, подвергнутых постадийной термической сушке. Отдельные клетки *K. amoena*, *H. coffeaeformis* и *H. cymbifera*, их цепочковидные агрегаты и распростёртые колониальные поселения занимают различные по степени неоднородности элементы поверхности микрорельефа, образуя структуры мощностью в 1–2 слоя со средней высотой поселения 1–1,3 высоты единичной особи. Клетки *K. amoena* плотно фиксируются на полимерном субстрате, используя поровый аппарат нижней створки панциря. При этом наблюдения с помощью сканирующего электронного микроскопа выявили отпечатки панцирей на субстрате, являющиеся признаками внедрения полимерной подложки в ареолы гипотеки. Рассмотрены механизмы распространения диатомей трёх указанных видов по различным элементам поверхности СВМПЭ, а также формирования характерных элементов колониальных поселений, в том числе для *K. amoena* — последовательно в форме «горшков» и сфер, посредством взаимодействия с поверхностью полимера и её растяжения по мере нарастания количества плотно прикреплённых клеток в колониальном поселении.

Ключевые слова: диатомеи, диатомовые водоросли, Bacillariophyta, колонизация пластика, СВМПЭ, устойчивые материалы, пластик в морской среде, аквакультура