ISSN 0006-2979, Biochemistry (Moscow), 2007, Vol. 72, No. 11, pp. 1261-1269. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © T. A. Safonova, V. V. Annenkov, E. P. Chebykin, E. N. Danilovtseva, Ye. V. Likhoshway, M. A. Grachev, 2007, published in Biokhimiya, 2007, Vol. 72, No. 11, pp. 1548-1558.

Aberration of Morphogenesis of Siliceous Frustule Elements of the Diatom *Synedra acus* in the Presence of Germanic Acid

T. A. Safonova, V. V. Annenkov, E. P. Chebykin, E. N. Danilovtseva, Ye. V. Likhoshway*, and M. A. Grachev

Limnological Institute, Siberian Branch of the Russian Academy of Sciences, ul. Ulan-Batorskaya 3, 664033 Irkutsk, Russia; fax: (3952) 425-405; E-mail: yel@lin.irk.ru

Received May 10, 2007
Revision received July 2, 2007

Abstract—Addition of germanic acid into the culture medium of the diatom *Synedra acus* subsp. *radians* (Kutz.) Skabitsch. had nearly no influence on the culture growth at the Ge/Si molar ratio 0.01, but stopped it at ratios 0.05 and higher. It was shown by mass-spectrometry that at the Ge/Si ratio 0.01 germanium was incorporated in both the cytoplasm and siliceous valves, whereas at Ge/Si 0.05 it was incorporated into the cytoplasm but almost failed to accumulate in the valves. At Ge/Si 0.1 germanium was accumulated in the cytoplasm, but its incorporation into the valves terminated. Studies on the cell morphology by light, epifluorescence, and transmission electron microscopy showed that high concentrations of germanic acid induced disorders in morphogenesis of the siliceous frustule and accumulation of large rhodamine-stainable electron-dense inclusions. Model chemical experiments with over-saturated solutions of silicic acid in the presence of polyallylamine revealed that addition of 5% germanic acid considerably accelerated coagulation of silica. Hence, the toxic effect of germanic acid on diatoms could be caused by changes in coagulation of silica.

DOI: 10.1134/S0006297907110132

Key words: diatoms, silica, germanium, polyallylamine, morphogenesis, epifluorescence and transmission electron microscopy

Diatoms are unicellular eukaryotic algae responsible for about 40% of the primary production of organic matter on Earth. Building their cellular walls from silica, they dehydrate and remove from the oceans 10^{10} tons of dissolved silicic acid every year [1]. The ability of diatoms to build siliceous frustules with genetically programmed complicated nanostructural patterns is the most enigmatic [2]. During the last decade, progress has taken place in understanding the molecular mechanisms of silicic acid uptake by diatoms and its transfer from the environmental water into the cytoplasm. This transfer is realized by special membrane proteins, or silicon transporters (SIT) [3-6]. But how silicic acid is transferred from the cytoplasm into silica deposition vesicles (SDV), where siliceous valves are synthesized, and how the sophisticated siliceous cell walls are "molded" from silicic acid are still very poorly known [7].

Chemical properties of germanium, which is a chemical analog of silicon, are similar to those of silicon, and it can be assimilated by all silicon-accumulating organisms. In low doses (~1% of the silicon content in the culture medium), germanic acid is assimilated by diatoms similarly to silicic acid and does not influence their growth and morphology [8]. An increase in the germanic acid concentration is toxic for development of diatoms [9, 10]. The mechanism of the toxic action of germanic acid on diatoms is unknown, but some findings [10, 11] suggest that it inhibits not the transfer of silicic acid across the membrane but its intracellular transformation.

This work was designed to study the influence of germanic acid on the growth and synthesis of siliceous structures in the freshwater diatom *Synedra acus* subsp. *radians* (Kutz.) Skabitsch.

MATERIALS AND METHODS

Isolation of cultures. Two cell cultures of the diatom *S. acus* subsp. *radians* were isolated from phytoplankton

Abbreviations: PAA) polyallylamine; SDV) silica deposition vesicles; SIT) silicon transporter; TEM) transmission electron microscopy.

* To whom correspondence should be addressed.

of Lake Baikal: K1 from the Listvennichny Bay in June, 2003 and K2 from the Chivyrkuy Bay in July, 2005. The isolation, accounting of cell number, and cultivation of diatoms were performed on DM liquid medium [12] prepared with distilled water and containing (mg/liter): $Ca(NO_3)_2 \cdot 4H_2O$, 20; KH_2PO_4, 12.4; $MgSO_4 \cdot 7H_2O$, 25; $NaHCO_3$, 16; $Na_2 \cdot EDTA$, 2.25; H_3BO_3, 2.48; $MnCl_2 \cdot 4H_2O$, 1.39; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1; $Na_2SiO_3 \cdot 5H_2O$, 42.6; $FeCl_3$, 1.6; cyanocobalamin (vitamin B12), thiamine hydrochloride (vitamin B1), and biotin, 0.04 each. Accumulating cultures of purified cells were grown in glass flasks of 100-1000 ml volume. The K1 culture was grown at room temperature with periodical stirring at the natural day-night alternation. The K2 culture was grown in a thermostat (New Brunswick G25, USA) with constant stirring at 7-8°C and illumination of 13-16 μmol/(m^2·sec) with alternating 12-h dark and light phases.

Silicon starvation. Logarithmic phase cells were precipitated by settling in glass tubes under sterile conditions and washed thrice in silicon-free DM medium. The washed cells were placed into plastic Petri dishes with silicon-free DM medium and maintained for two days under the same conditions of cultivation as before the synchronization. Afterwards the cells were placed in the dark for 1 h, and then sodium silicate and germanate were added.

Cell growth. The effect of germanium on the diatom growth was studied on plastic plates with 400-μl flash-bottom cells for cell microcultures (Linbro Biomedical, INC, Denmark). The cells were grown in a microthermostat at 13°C and illumination of 16 μmol/(m^2·sec) with alternating 12-h dark and light phases [13]. The cell culture was suspended in silicon-free DM medium to the final concentration of 1000-3000 cells/ml, and 90 μl of the suspension was placed into each cell and then supplemented with 10 μl of a mixture of sodium silicate and germanate solutions (20 mM) at the molar ratios of 0, 0.01, 0.05, 0.1, and 1. The silicate and germanate concentrations together were equal to the silicon content in DM medium (0.2 mM). For each concentration, the experiments were performed in five or six repeats. The culture growth had been observed for 11 days, and the cells were counted automatically using an Axiovert 200 inverted microscope (Zeiss, Germany) and special software [13].

For transmission electron microscopy (TEM) and mass-spectrometry, the cells were grown in glass flasks. To 30 ml of the cell suspension in silicon-free DM medium, 300 μl of the mixture of sodium silicate and germanate solutions was added. The cells were cultured at 20-25°C under natural illumination.

Light and epifluorescence microscopy. Three days after the addition of silicic and germanic acids, the *S. acus* subsp. *radians* cells grown at different Ge/Si ratios were supplemented with rhodamine 6G (Berezniki Chemical Factory, Russia) to the final concentration of 0.5 μg/ml. Three days later the cells were studied by fluorescence microscopy using an Axiovert 200 inverted microscope with an HBO 50W/AC ASRAM ultraviolet lamp equipped with a green filter (wavelength 546 nm).

Transmission electron microscopy. To prepare ultrathin sections, the cells were fixed in 2.5% glutaraldehyde for 2 h. The cells were concentrated by successive centrifugation at 4000*g* for 5 min. After three washings in phosphate buffer (pH 7.4), the cells were postfixed in 1% solution of osmium oxide for 2 h at room temperature. Then the cells were washed in buffer and mounted in 1.5% agar (Wako, Japan). Specimens were dehydrated in a series of ethanol solutions (10, 30, 50, 70, 80, 96, and 100% ethanol, 10 min in each solution) and then washed twice for 30 min in acetone dehydrated above copper sulfate (Reakhimkomplekt, Russia). Epoxy resins Epon 812, DDSA, and MNA (Fluka, Switzerland) were mixed in the amounts of 4.5, 2.2, and 2.2 ml, respectively. Upon impregnation, the material was transferred into polypropylene capsules Beem™, then into a fresh mixture of epoxy resins with a catalyst (five drops of DMP-30 per 10 ml of the resins), and polymerized with open lids in a thermostat successively at 37°C (12 h), 45°C (12 h), and 60°C (48 h).

Sections prepared on an Ultracut R microtome (Leica, Austria) with an Ultra 35° diamond knife (Diatom, Austria) were mounted onto palladium grids. The preparations were contrasted with lead citrate as described in [14] and washed first in 0.02 M NaOH and then in distilled water. The sections were studied with a Leo 906E TEM (Zeiss) at the accelerating voltage of 80 kV.

Mass-spectrometry with inductively-coupled plasma (ICP-MS). *Preparation of specimens.* Aliquots of the medium of the diatom cultures were centrifuged at 4000*g* and diluted seven-eight times (by weight) with 2% HNO_3 (70.4%, high purity 27-5 GOST 11125-84; Mosreaktiv, Russia) containing 10 μg/liter indium (Fluka; 1000 μg/liter, 0.5 M HNO_3) as an internal standard. The medium without Si and Ge was used as a reference sample. The specimens were taken after 0, 1, 3, 5, and 7 days of cultivation.

After the experiment termination (on the seventh day), the valves were centrifuged, washed five times in 1.5 ml of the medium without Si and Ge, and then heated two times for 15 min in 0.5 ml of distilled water on a boiling water bath. The resulting extracts were diluted twofold with 2% HNO_3 supplemented with indium (10 μg/liter).

After the extraction with water, the diatom valves were incubated two times for 30 min at 100°C in 0.2 ml of 2% SDS, treated for 2 h with 0.5 ml of concentrated HNO_3 at 80°C, and left overnight at room temperature. The valves were washed five times in 1.5 ml of distilled water and placed into 15-ml polypropylene tubes. The

tubes were supplemented with 0.2 g 50% HF (high purity 27-5 TU 6-09-3401-88; Galogen, Russia) and were irradiated five times for 10 sec by microwaves (LG MS 1902H domestic microwave oven, 700 W). The volume of the solution was adjusted to 15 ml with 2% HNO_3 supplemented with indium (10 μg/liter by weight).

ICP-MS-measurement. The resulting solutions were measured by ICP-MS using an Agilent 7500ce quadrupole mass-spectrometer (Ultramicroanalysis Center at the Limnological Institute, Siberian Branch, Russian Academy of Sciences, Irkutsk). The samples were loaded with a flow nebulizer (0.1 ml/min) and a lead-in system of aggressive solvents. The device was calibrated with standard solution Tune (Li, Co, Y, Tl, and U – 10 μg/liter) (Agilent, USA). The device drive was followed using the internal indium standard.

Effect of germanic acid on silicic acid condensation in the presence of polyallylamine (PAA). As sources of silicic and germanic acids we used 0.1 M solutions of $Na_2SiO_3 \cdot 9H_2O$ and Na_2GeO_3. PAA hydrochloride (Aldrich, USA) with molecular weight of 15 kD was predissolved in water to the concentration of 0.1 M. The experiments were performed in 0.1 M acetate buffer. Addition of solutions of PAA and sodium silicate (or its mixture with germanate) increased the pH of the medium to 6.0. Therefore, in the final experiments, before the addition of sodium silicate, the solution was supplemented with 1 M HCl in the previously determined volume. Thus, pH of the solutions before beginning of the silicic acid condensation was 5.5.

RESULTS

The isolated cultures of *S. acus* subsp. *radians* were different in cell size (Fig. 1). On average, the K1 culture cells were of 150 μm in length and 3 μm in width (Fig. 1a) and the K2 culture cells were, respectively, of 360 and 5 μm size (Fig. 1b). The valves of *S. acus* subsp. *radians* were elongated little troughs speckled with figures of areolar pores (Fig. 1c).

The valves—epitheca and hypotheca—of a mature living cell are connected by a system of siliceous girdle-like thin borders (Fig. 1d and Fig. 2 (stages *4-8*, according to [15]; see color insert)). On both ends of the valve there are rimoportulae and an apical pore field (Fig. 1e). Holes in the siliceous frustule provide the interaction of the cell with the environment and excretion of polysaccharide mucus and other organic substances.

The culture growth insignificantly decelerated as compared to the control at the Ge/Si molar ratio equal to 0.01 and completely stopped at Ge/Si ratio equal to 0.05 and higher (Fig. 3).

In the following series of experiments kinetics of the Si and Ge decrease in the nutritional medium and the accumulation of Si and Ge in the cytoplasm and valves were studied during the growth of the K1 and K2 cultures. At the Ge/Si ratio 0.01, concentrations of both Ge and Si in the medium decreased (Fig. 4). At the higher values of the Ge/Si ratio, the uptake of these elements terminated, and their concentrations remained the same as initially during all the time of the experiment (seven days).

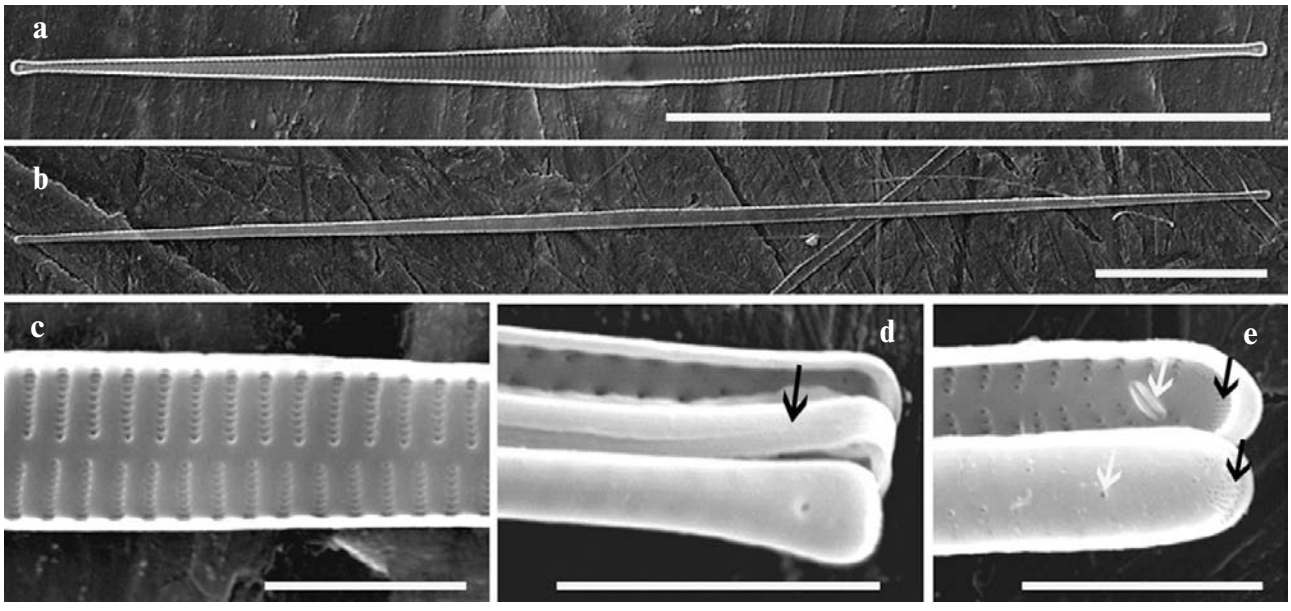


Fig. 1. Scanning electron microscopy. The *S. acus* subsp. *radians* cells: a) the K1 culture; b) the K2 culture; c) areolar rows; d) girdle bands between two valves (black arrow); e) ends of valves, the rimoportula on the inner side of the valve and its external opening (white arrows), the apical pore field (black arrows). Scale: a, b) 50 μm; c-e) 5 μm.

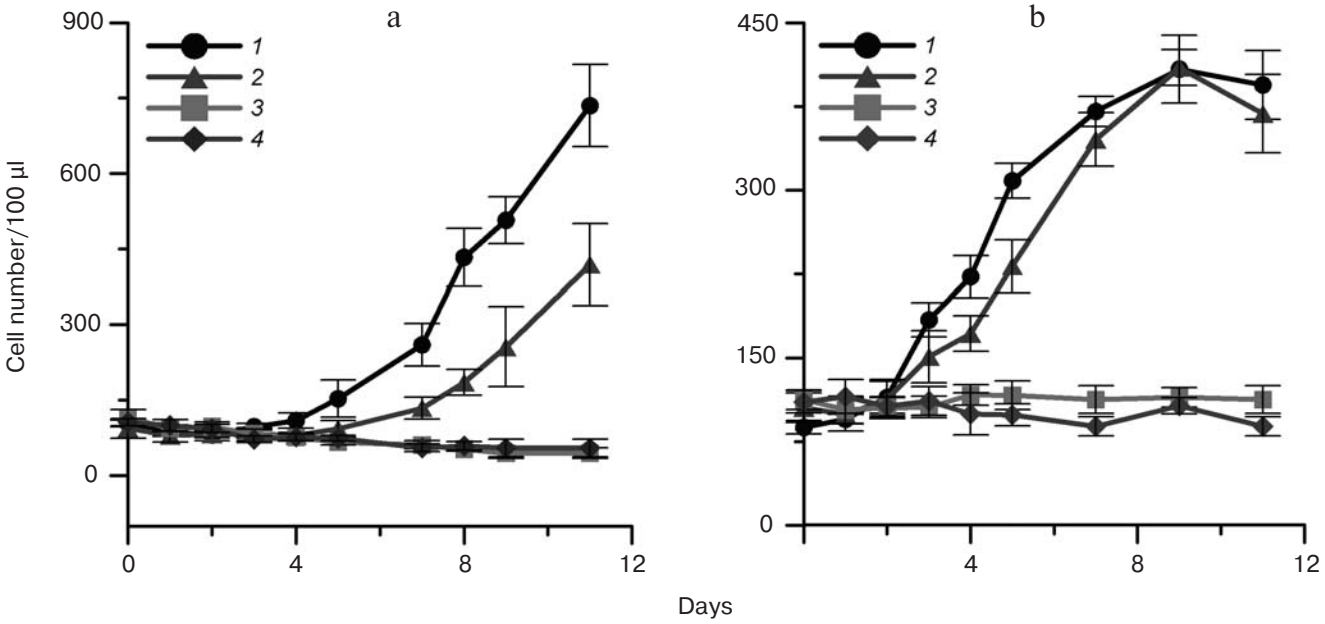


Fig. 3. Growth of the *Synedra acus* subsp. *radians* cells at varied molar ratios Ge/Si. a) K1 culture; b) K2 culture. *1*) Without Ge; *2-4*) Ge/Si ratios equal to 0.01, 0.05, and 0.1, respectively.

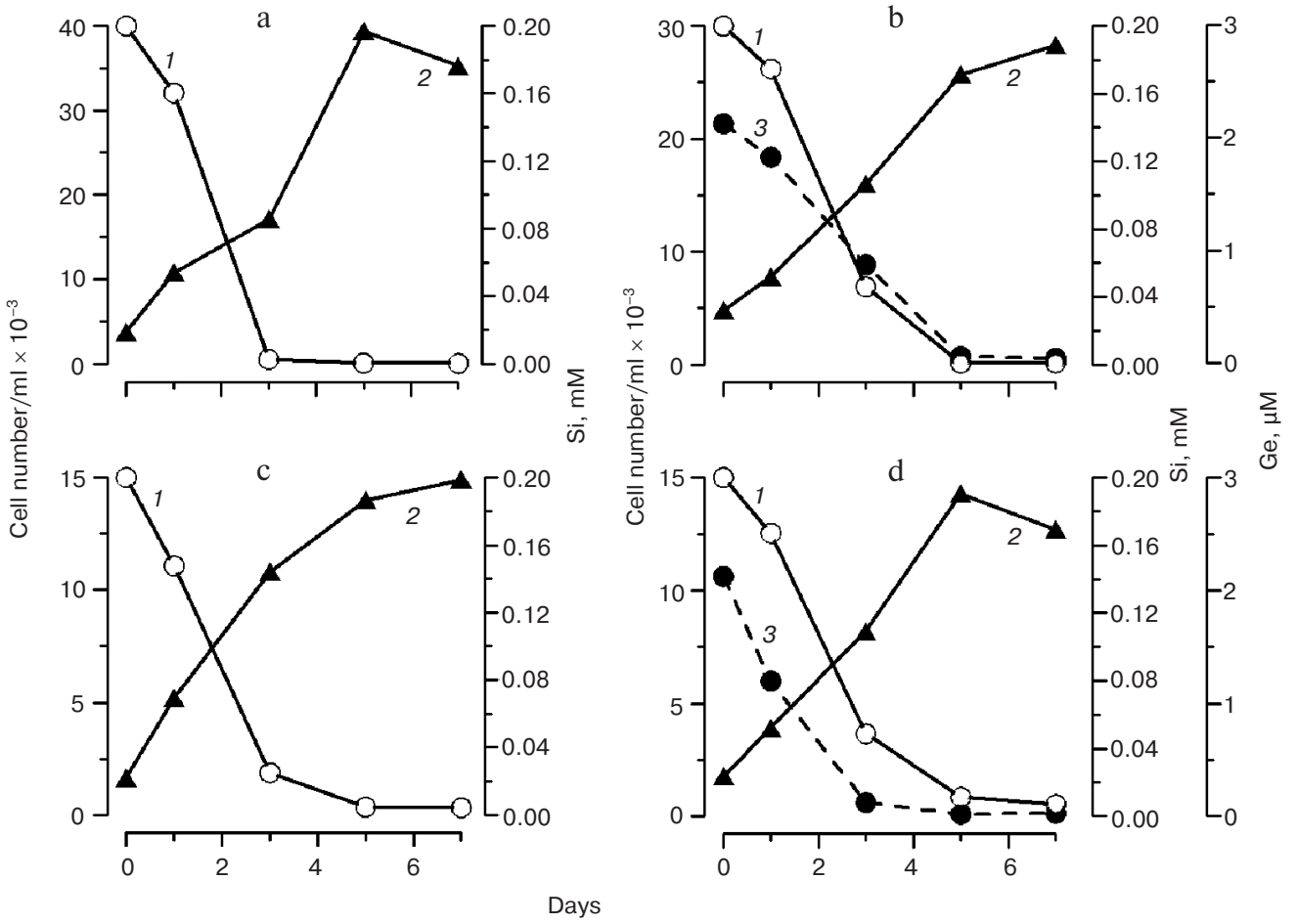


Fig. 4. Uptake of Ge and Si from the medium by the diatom *S. acus* subsp. *radians* cells (by data of ISP-MS). K1 culture: a) control; b) Ge/Si ratio 0.01. K2 culture: c) control; d) Ge/Si ratio 0.01. *1*) Concentration of Si; *2*) cell number per ml; *3*) concentration of Ge.

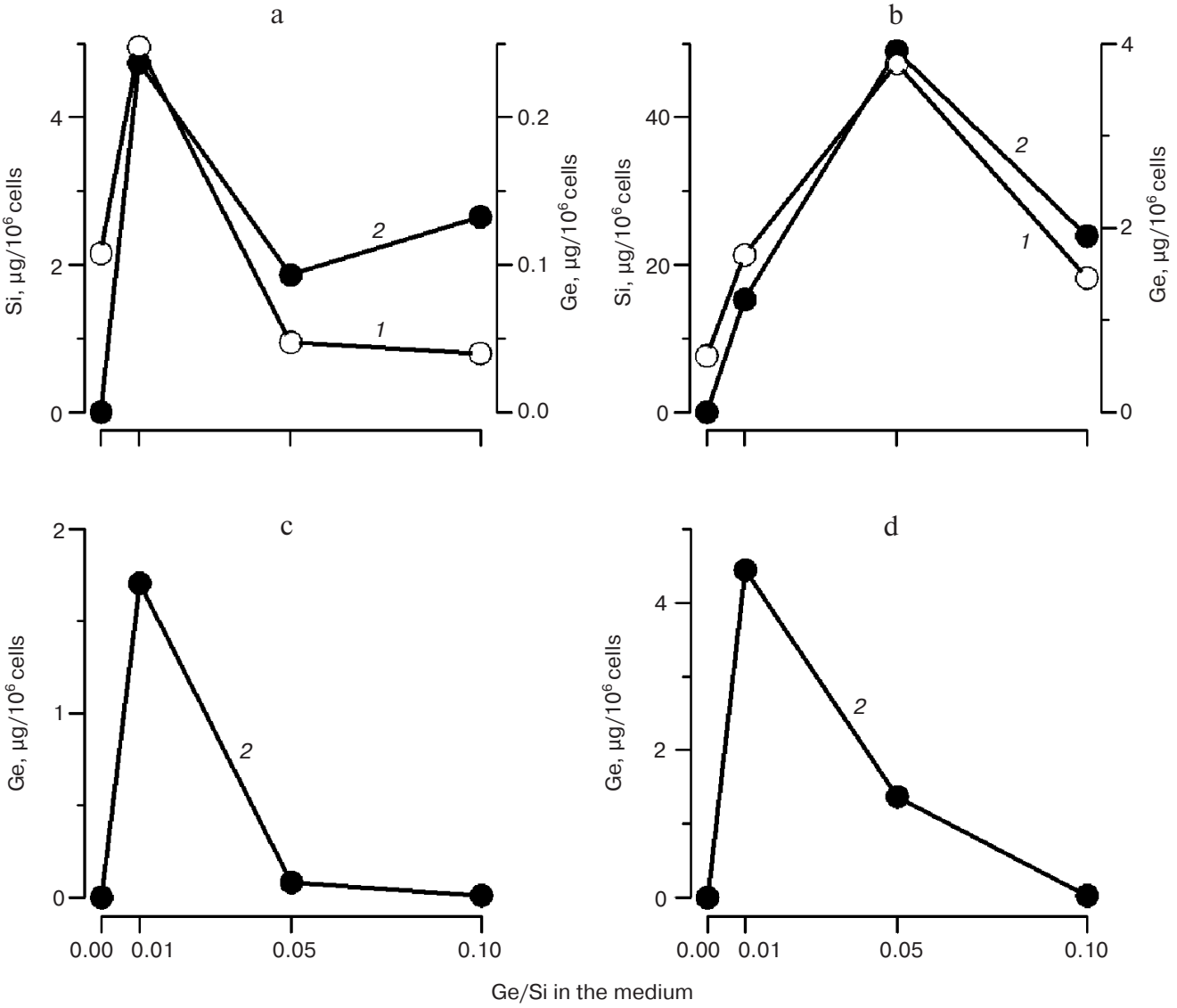


Fig. 5. Contents of Si and Ge in the cytoplasm (a, b) and valves (c, d) of the *S. acus* subsp. *radians* cells grown in medium with different Ge/Si ratio (by ISP-MS data). K1 culture (a, c); K2 culture (b, d). *1*) Si content; *2*) Ge content.

The incorporation of Si and Ge into the cytoplasm of the two cultures with the cells different in size and volume was studied at varied ratio of Ge/Si (Fig. 5). The volumes were calculated for a geometrical figure corresponding to the alga under study (two cones) [16]. At all Ge/Si ratios studied the incorporation into the K1 culture cells (Fig. 5a) (average cell length, 150 μm; volume, $0.35 \cdot 10^{-12}$ liter) was several times lower than into the K2 culture cells (Fig. 5b) (360 μm, $2.36 \cdot 10^{-12}$ liter, respectively).

Recalculations of the values have shown that the concentration of "cytoplasmic" silicon is many times higher than the solubility limit of silicic acid in water (2-3 mM) [17]. In the control (without addition of Ge) for the K1 culture cells 2.2 μg Si per 10^6 cells correspond to ~220 mM and for the K2 culture cells 7.7 μg Si per 10^6 cells correspond to ~120 mM (Fig. 5a). But this does not mean that silicon is present in the cytoplasm as free silicic acid. Extraction of diatoms with hot water [11] indicates that the cytoplasm contains no free silicic acid but some yet unknown substances, possibly oligosilicates [18], which have entered the aqueous solution during the extraction. The concentration of Si including that in these substances within the diatom cytoplasm can be even higher (e.g. 340 mM for the species *Stephanopyxis turris* Greville *et* Arnott) [11, 19].

Surprisingly, the addition of Ge into the medium caused an increase in the intracellular concentration not only of Ge itself but also of Si. At the Ge/Si ratio 0.01, the Si concentration increased to 500 and 320 mM in the K1 and K2 culture cells, respectively. At Ge/Si 0.05, the Si

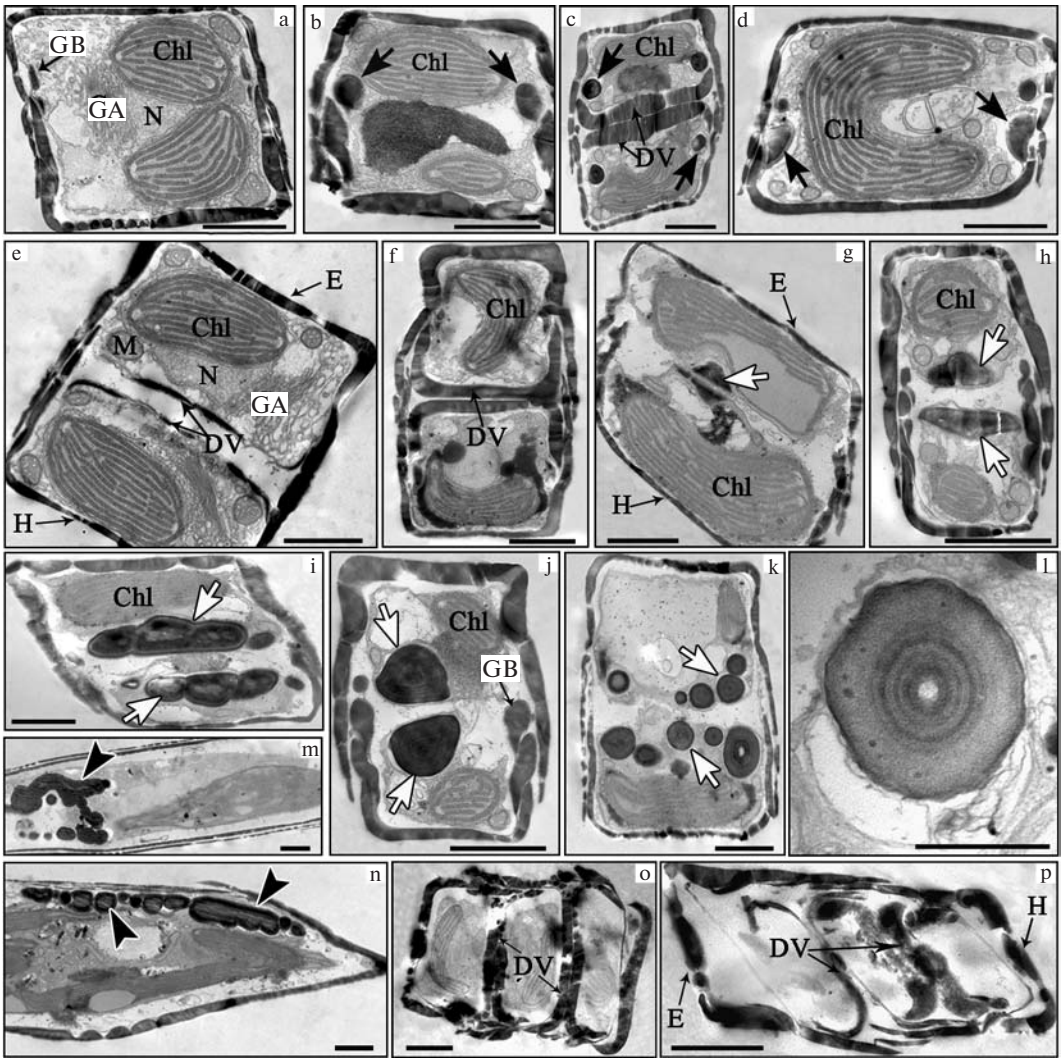


Fig. 7. Deterioration in morphogenesis of siliceous structures as shown by TEM of ultrathin sections of the *S. acus* subsp. *radians* cells (K1) grown at different Ge/Si ratios. a-d) Effect on the formation of girdle bands; e-k) effect on the formation of daughter valves. a, e) Control; f) Ge/Si 0.01; b, g, h, m) Ge/Si 0.05; c, d, i, j, n, p) Ge/Si 0.1; k-m) Ge/Si 1; l) a magnified electron-dense vacuole of the daughter valve; m) longitudinal section of the cell; n) oblique section. E, epitheca; H, hypotheca; DV, daughter valves; GB, girdle band; Chl, chloroplast; GA, Golgi apparatus; N, nucleus; M, mitochondrion. Black and white big arrows indicate deteriorations in the structure of the girdle band and daughter valves, respectively. Black heads of the arrows indicate lamellar inclusions. Scale: a-k, n-p) 1 μm; l) 500 nm.

concentration increased to 720 mM in the K2 culture cells (Fig. 5b). At Ge/Si 0.1, the growth of both cell cultures was completely inhibited (Fig. 3) but the intracellular contents of both Ge and Si were slightly increased compared to the control (Fig. 5, a and b).

To study the Ge incorporation into the diatom valves in dependence on the Ge/Si ratio, the cell cultures were washed initially in a detergent and then in hot HNO_3. This treatment did not destroy mature valves of the diatoms. The washed valves were dissolved in hydrofluoric acid. Then the Ge content was determined in the solution by ICP-MS. The incorporation of Ge decreased with the increase in the Ge/Si ratio from 0.01 to 0.1 (Fig. 5, c and d).

The light and epifluorescence microscopy of the *S. acus* subsp. *radians* cells (Fig. 6; see color insert) revealed that at the Ge/Si ratio 0.01 they similarly to the control (Fig. 6, a-d) had distinctly pronounced chloroplasts with red autofluorescence (Fig. 6, e-h). Similarly to the control, the daughter valves gave a bright green fluorescence on staining with rhodamine 6G, which is a supravital dye binding with silicic acid, depositing in SDV, and staining only the growing valves not finally formed by the moment of the dye addition [20-22]. In the control, only the narrow region was stained where the daughter valves were growing (Fig. 6, a-d). At Ge/Si 0.05 (Fig. 6, i-l) and 0.1 (Fig. 6, m-p) the cells died, the chloroplasts were destroyed, and the daughter valves did not form. The

green fluorescence on addition of rhodamine 6G was observed not in the region of the valve formation in normal cells but was dispersed in the cytoplasm (large rounded inclusions were fluorescent).

Studies by TEM on ultrathin sections of the *S. acus* subsp. *radians* cells (Fig. 7, a-p) indicated that at the Ge/Si ratio 0.01 (Fig. 7f) the cell structure was virtually the same as in the control (Fig. 7, a and e). At Ge/Si 0.05 (Fig. 7, b, g, and h) and higher (Fig. 7, c, d, i-l), the formation of normal cell siliceous structures was affected, as also shown in the longitudinal and diagonal sections (Figs. 7m and 7n, respectively).

The formation of the first girdle bands occurred with deteriorations from normal. These deteriorations looked in the sections like electron-dense rings (Fig. 7, b-d). In the place of the daughter valve laying, one can see either over-inflated valves (Fig. 7, g-i) or rounded vacuoles of different shape filled with an electron-dense substance located in the same silicalemma (Fig. 7, j and k). At the greater magnification vacuoles generated in both the place of the girdle bands and the laying of daughter valves looked like concentric circles with different electron density (Fig. 7l). Note that comparison of longitudinal (Fig. 7, b-d, h-l) and transverse (Fig. 7m) sections suggests that these layers have a cylindrical organization of the layers oriented longitudinally towards the cell ends (Fig. 1e). At the inhibitory Ge/Si ratio 0.05 and higher, a number of daughter valves are produced but the cells do not separate (Fig. 7, m and p).

Our findings suggest that the mechanism of intracellular production of silica is disturbed in the presence of Ge. We have determined the condensation of silicic acid in the presence of PAA $[-CH_2-CH(CH_2NH_2)-]_n$, which is a known accelerator of this reaction used for *in vitro* modeling synthesis of siliceous structures [23]. The reaction mixture contained 36 mM silicic acid (or its mixtures with germanic acid) and 18 mM PAA (pH 5.5). The addition of germanic acid to Ge/Si 0.05 considerably accelerated the precipitate fall-out (Fig. 8), which was observed even in 10 min, whereas in the absence of germanic acid the mixture remained homogenous for 1 h.

DISCUSSION

The use of germanic acid as an analog of silicic acid was earlier considered as a promising approach for studies on morphogenesis of siliceous valves of diatoms because it allows radioisotopes of Ge to be used. Radioisotopes of Ge are more available and their half-lives are more convenient for biological experiments than that of Si radioisotope. The data published in [3, 10, 11] and the present work findings (Fig. 4) have shown that germanic acid in the concentration of 1% of that of silicic acid can be used by SIT as a substrate and be absorbed by diatom cells with a similar efficiency as silicic acid. The toxic effect of germanic acid is manifested at its higher concentrations and on the stages following the absorption.

Morphogenesis of diatom valves was not disturbed at the low doses (Ge/Si 0.01) [8]. Using two cultures of the same species but different in cell size, we have shown that small cells (K1) are more sensitive to this "substitution" (Fig. 3a).

In both cultures, the addition of germanic acid into the culture medium resulted in a sharp increase in both Ge and Si contents in the cytoplasm as compared to the control cells grown on the usual medium (Fig. 5).

Why does this "excess" pool of intracellular silicic acid appear? The routine extraction of "free" silicic acid from diatoms by keeping them in hot water results in washing out of $Si(OH)_4$ which is present in the cells not only as a monomer but also as dissolved oligomers and of silica particles not transformed to quartz glass state. The staining with rhodamine 6G of the cells cultured at the low content of germanium has revealed the normal termination of morphogenesis (Fig. 6f) along with its slight disorders in some of the cells (Fig. 6h). With increase in the dose of germanium, the cell does not form new daughter valves, but large cytoplasmic rounded rhodamine-stainable inclusions are produced (Fig. 6, j, l, n, p). In ultrathin sections, these electron-dense inclusions are found in the place of formation of girdle bands (Fig. 7, b-d) and daughter valves (Fig. 7, g-k) or are dispersed in the cytoplasm (Fig. 7, m and n). These inclusions are surrounded

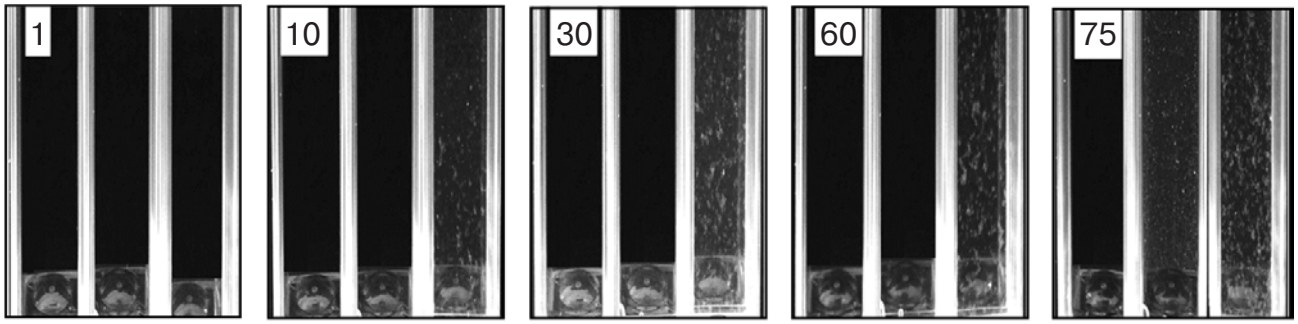
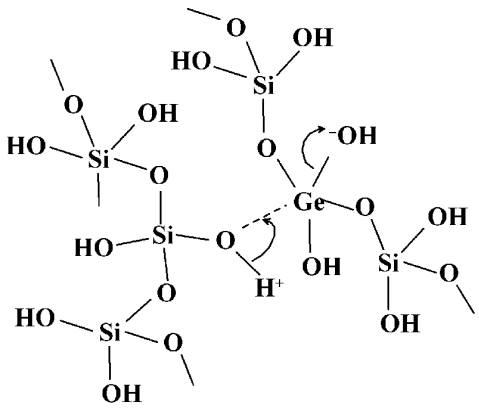


Fig. 8. Condensation of silicic acid in the presence of PAA (central cuvette). In the right cuvette, 5% silicic acid is substituted by germanic acid, and the left cuvette is the control (water). Numbers in the photographs show the time (minutes) from the experiment beginning.

by membranes, i.e. they are silica deposition vesicles. It seems that such inclusions containing "immature" silica are destroyed on extraction with hot water and enrich the cytoplasmic fraction with silicon (and germanium).

Why are "inflated" voluminous structures formed in the presence of Ge? Mechanisms of transport and compactization of silicic acid in the diatom cells after its transfer across the cell membrane are yet unclear. Silicic acid is supposed to be transferred within the cytoplasm from plasmalemma to SDV either by special 30-40-nm silica transport vesicles [24] or as polymerized stable forms [25]. In any case, $Si(OH)_4$ cannot be transferred inside the cell and accumulated as a monomer because its measurable intracellular concentrations (19-340 mM [11, 19]) are many times higher than the equilibrium solubility of silica (2-3 mM [17]). Moreover, a direct determination of silicic acid in living diatoms by NMR [26] has shown its presence only as a polymer. Obviously, intracellular mechanisms of the $Si(OH)_4$ transport and its complete polymerization to quartz glass with the specific density of 2.0 are to be well coordinated to prevent an early formation of solid particles in "wrong" places.

Germanium has more pronounced metal features than silicon and due to its partial positive charge is more easily attacked by the electron pair of oxygen of the reactive silicate residue:



This seems to explain the observed accelerated coagulation of silica in the presence of germanic acid (Fig. 8). It seems that just a high electrophilicity of Ge is responsible for acceleration of silica gel deposition within SDV. This results in an early generation of silica gel, affects its normal dehydration, and robs the cell of controlling the correct shape of daughter valves and girdle-like ring borders.

Why do silica deposits produced in the presence of germanic acid have a layered structure? The observed abnormal silica deposits have a layered structure (Fig. 7, b-d, g-n)—regions of dense silica similar to material of the normal valves alternate with more electron-transparent regions. Usually in thin sections of SDV an electron-dense part of the deposited silica can be seen, and its rarefied edges towards the silicalemma are changed to an "empty" space ([27], Fig. 13d), and SDV gradually broadens to the size of a mature structure (the valve or girdle-like ring border) [27]. It has been shown by TEM ([2, 22], Figs. 14 and 15) that during early stages of synthesis of diatom valves silica is deposited as rounded-edged flash structures with hollow "bubbles" inside some of them (the presence of such bubbles seems to indicate a liquid hardening [18]). The valves and girdle-like ring borders are formed in SDV due to successive deposition of new portions of silica [22, 28], but the resulting material has a unified electron-dense structure. Notwithstanding a high concentration (to 340 mM) of soluble forms of silicic acid in diatoms, the formation of solid silica particles should be accompanied by release of a large amount of water, because 340 mM correspond to 2% SiO_2 and 98% H_2O. In the case of normal functioning of SDV a mechanism should exist responsible for removal of this water (possibly via aquaporins [18]). An early precipitation of silica under the influence of germanic acid seems to affect this mechanism; water is not removed and remains within the more transparent layer.

Thus, on entering into diatom cells germanic acid affects the normal mechanism of formation of new valves and in high concentrations causes the cell death possibly acting on the stage of silica coagulation. We think that dehydration of silica inside SDV is crucial for determination of a "correct" morphology of the elements to be formed.

We are grateful to workers of the Limnological Institute G. I. Popovskaya and A. E. Kuzmina for their help in determination of the cultures and Ye. D. Bedoshvili for preparation of ultrathin sections for TEM and useful discussion concerning this work.

This work was supported by the Russian Academy of Sciences Presidium Program on Molecular and Cellular Biology (project No. 10.3), the Foundation for Native Science Assistance (NSh-4738.2006.4), and the Russian Foundation for Basic Research (project No. 06-04-082240).

REFERENCES

1. Nelson, D. M., Treguer, P., Brzezinski, M. A., Leynaert, A., and Queguiner, B. (1995) *Global Biogeochem. Cycles*, **9**, 353-365.
2. Pickett-Heaps, J. D., Schmid, A.-M. M., and Edgar, L. A. (1990) *Prog. Phycol. Res.*, **7**, 1-168.
3. Hildebrand, M., Volcani, B. E., Gassmann, W., and Schroeder, J. I. (1997) *Nature*, **385**, 688-689.
4. Hildebrand, M., Dahlin, K., and Volcani, B. E. (1998) *Mol. Gen. Genet.*, **260**, 480-486.
5. Grachev, M. A., Denikina, N. N., Belikov, S. I., Likhoshway, Ye. V., Usoltseva, M. V., Tikhonova, I. V., Adelshin, R. V., Kler, S. A., and Sherbakova, T. A. (2002) *Mol. Biol.* (Moscow), **36**, 679-681.

6. Sherbakova, T. A., Masyukova, Yu. A., Safonova, T. A., Petrova, D. P., Vereschagin, A. L., Minaeva, T. V., Adelshin, R. V., Triboi, T. I., Stonik, I. V., Aizdaicher, N. A., Kozlov, M. V., Likhoshway, Ye. V., and Grachev, M. A. (2005) *Mol. Biol.* (Moscow), **39**, 303-316.
7. Zurzolo, C., and Bowler, C. (2001) *Plant Physiol.*, **127**, 1339-1345.
8. Azam, F., Hemmingsen, B. B., and Volcani, B. E. (1973) *Arch. Microbiol.*, **92**, 11-20.
9. Lewin, J. C. (1966) *Phycologia*, **6**, 1-12.
10. Azam, F., and Volcani, B. E. (1981) in *Silicon and Siliceous Structures in Biological Systems* (Simpson, T. L., and Volcani, B. E., eds.) Springer-Verlag, Berlin, pp. 43-67.
11. Martin-Jezequel, V., Hildebrand, M., and Brzezinski, M. A. (2000) *J. Phycol.*, **36**, 821-840.
12. Thompson, A. S., Rhodes, J. C., and Pettman, I. (eds.) (1988) *Culture Collections of Algae and Protozoa*, Kendal, Titus Wilson & Son, p. 18.
13. Safonova, T. A., Aslamov, I. A., Basharina, T. N., Chenski, A. G., Vereschagin, A. L., Glyzina, O. Yu., and Grachev, M. A. (2007) *Diatom Res.*, **22**, 189-195.
14. Reynolds, E. S. (1963) *J. Cell Biol.*, **17**, 208-212.
15. Bedoshwili, E. D., Likhoshway, Ye. V., and Grachev, M. A. (2007) *Izv. Ros. Akad. Nauk*, *Ser. Biol.,* **3**, 367-371.
16. Makarova, I. V., and Pichkily, L. O. (1970) *Botan. Zh.*, **55**, 1488-1493.
17. Iler, R. (1982) *The Chemistry of Silica* [Russian translation], Mir, Moscow.
18. Grachev, M. A., Annenkov, V. V., and Likhoshway, Ye. V. (2006) *19th Int. Diatom Symp.*, Irkutsk, p. 52.
19. Hildebrand, M. (2003) *Progr. Org. Coatings*, **47**, 256-266.
20. Li, C.-W., Chu, S., and Lee, M. (1989) *Protoplasma*, **151**, 158-163.
21. Brzezinski, M. A., and Conley, D. J. (1994) *J. Phycol.*, **30**, 45-55.
22. Kaluzhnaya, O. V., and Likhoshway, Ye. V. (2007) *Diatom Res.*, **22**, 81-87.
23. Patwardhan, S. V., and Clarson, S. J. (2002) *J. Inorg. Organometal. Polymers*, **12**, 109-116.
24. Schmid, A.-M. M., and Schulz, D. (1979) *Protoplasma*, **100**, 267-288.
25. Sumper, M. (2004) *Angew. Chem.*, **116**, 2301-2304.
26. Bertermann, R., Kroger, N., and Tacke, R. (2003) *Analyt. Bioanalyt. Chem.*, **375**, 630-634.
27. Round, F. E., Crawford, R. M., and Mann, D. G. (1990) *The Diatoms*, Cambridge University Press, N. Y.
28. Hildebrand, M., York, E., Kelz, J. I., Davis, A. K., Frigeri, L. G., Allison, D. P., and Doktycz, M. J. (2006) *J. Mater. Res.*, **21**, 2689-2698.