Biosynthesis of three-dimensional diatomaceous biosilica doped with titanium ions

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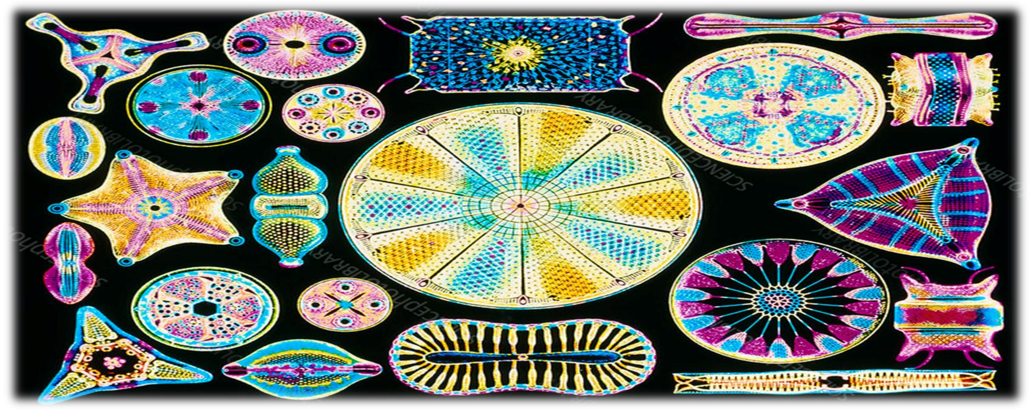
Introduction



The unique structure of the diatom frustule

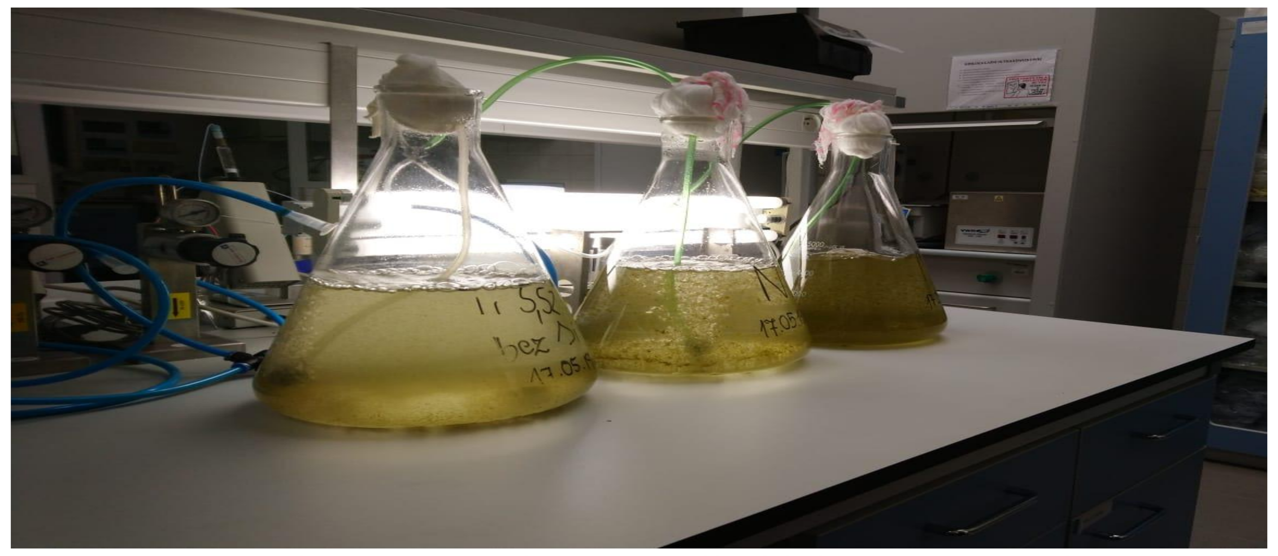
In the search for innovative solutions to modern technologies, and especially in the design and manufacture of new nanocomposite inorganic materials, microorganisms as "natural microtechnologists" may be rich inspiration. In this aspect, diatoms seems to be one of the most spectacular examples thanks to their outstanding ability to synthesize amorphous silica (silica exoskeletons) with hierarchical three-dimensional structure.

The amazing variety of precision 3D silica structures with their own original openwork morphology is represented by siliceous frustules of more than 100 000 known species of diatoms. These unique capabilities of diatoms produce considerable interest because of the prospect of controlled biosynthesis of three dimensional ordered silica structures by growing microorganisms in artificial conditions.

The main aim of this study was to evaluate the ability of the diatom species *Pseudostaurosira trainorii* to metabolically insert soluble titanium from culture medium into the structure of their amorphous silica cell walls, by cultivation of selected diatom species under laboratory conditions. The idea of incorporating selected elements in silica exoskeletons of diatoms is based on known capabilities of silicon to isomorphous substitution of these elements in the structure of silicate minerals as well as the opportunities of diatom cells to metabolic inserts of the different metals in their silica frustules.

Materials and Methods

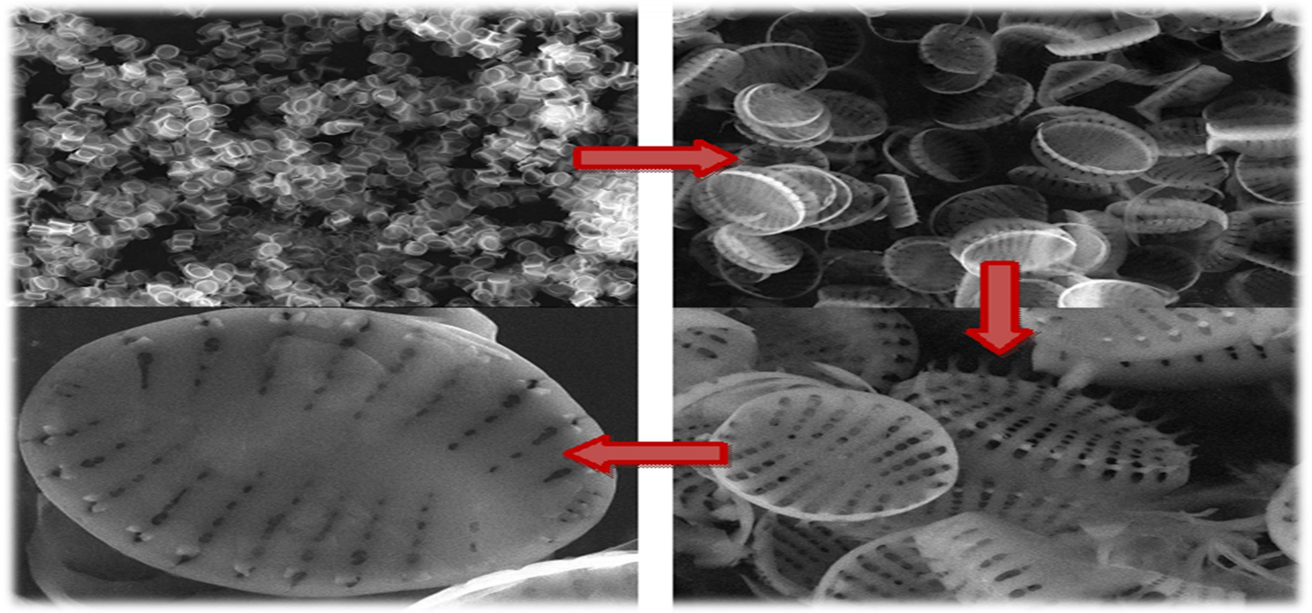
The cultures of diatom species was obtained from Culture Collection of Baltic Algae Institute of Oceanography, University of Gdańsk. The Erlenmeyer flasks (3000 ml) with diatoms culture were incubated using sterilized synthetic sea water and fresh water with f/2 growth medium, under a regime of 12 h light and 12 h darkness illuminated by two fluorescent lamps having intensity of 1500 lux at 20°C. Cultivation was carried out depending on amount of biological inoculums and concentration of the doped silicon. Initial soluble silicon concentrations ($Na_2SiO_3 \cdot 5H_2O$) varied from 1 to 500 mg Si/L were added separately to culture medium. After growth using selective medium, the diatoms were washed five times with distilled water using a centrifugation at 10000 rpm for 10 min and treated by hydrogen peroxide at 80°C for 2 h in order to silica frustules isolation from diatom cell wall. The treated frustules were centrifuged with distilled water to remove salt contents, dried and stored in 70 % ethanol. The growth dynamics was evaluated by optical density measuring of diatom biomass daily in the cultures using Densytometr Biosan DEN-1B at a wavelength of 565 nm. Diatom cells were monitored for growth using a light microscope at 40X - 100X magnification.



Diatoms cultivation under controlled laboratory conditions.

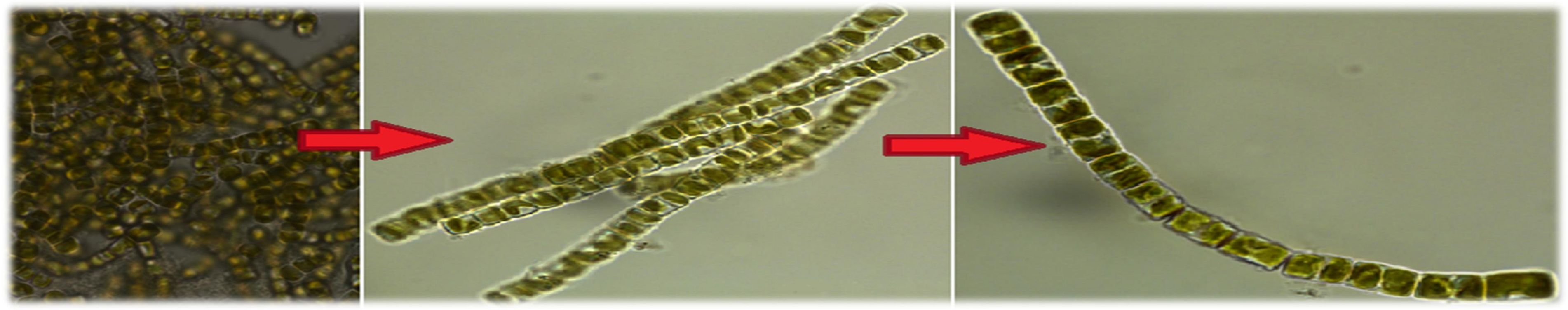
The morphological features of the diatom-biosilica was investigated by scanning electron microscopy (SEM/FIB - Quanta 3D FEG). The micro- and nanoscale structures of diatom frustule, porous structure and frustule chemical composition were analyzed by transmission electron microscopy (TEM) and scanning transmission electron microscopy/X-ray dispersive analysis (STEM-EDS) using TEM (FEITecnai F20 X-Twintool) coupled with the Energy Dispersive X-Ray detector (EDX) with the placement of the sample on a carbon-coated copper grid (Lacey Carbon Support Film 400 mesh).The photoluminesence spectra of the biosilica samples were obtained by using an JASCO FP-8300 spectrofluorometer.

Results

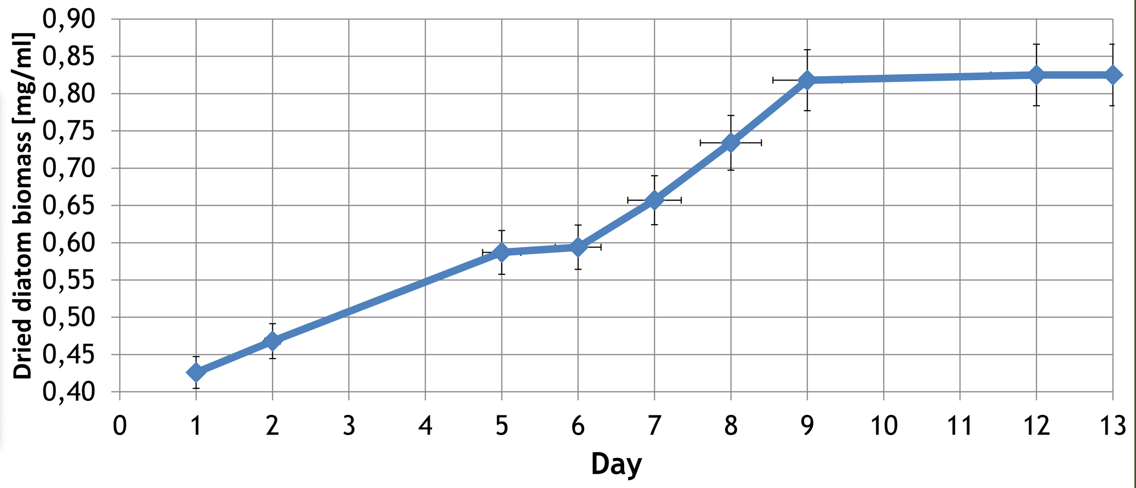


Scanning Electron Microscopy (SEM) images of the biosilica frustules doped with titanium ions

Scanning electron microscopy (SEM) gave a view of the structure and morphology of a cleaned diatom frustules at different magnifications The figure presents the purified frustules doped witht titanium ions after drying. One can observe the uniformity and well preserved forms of the diatom frustules with silica cell walls elegantly patterned by spatially periodic porous network. The valves of the frustules are round to slightly elliptical with an average diameter 4-5 µm. A solid strip of biosilica (raphe) divides the valve into two halves which surface consists eight parallel rows of pores with oval form. The size of pores is in nanometer range.



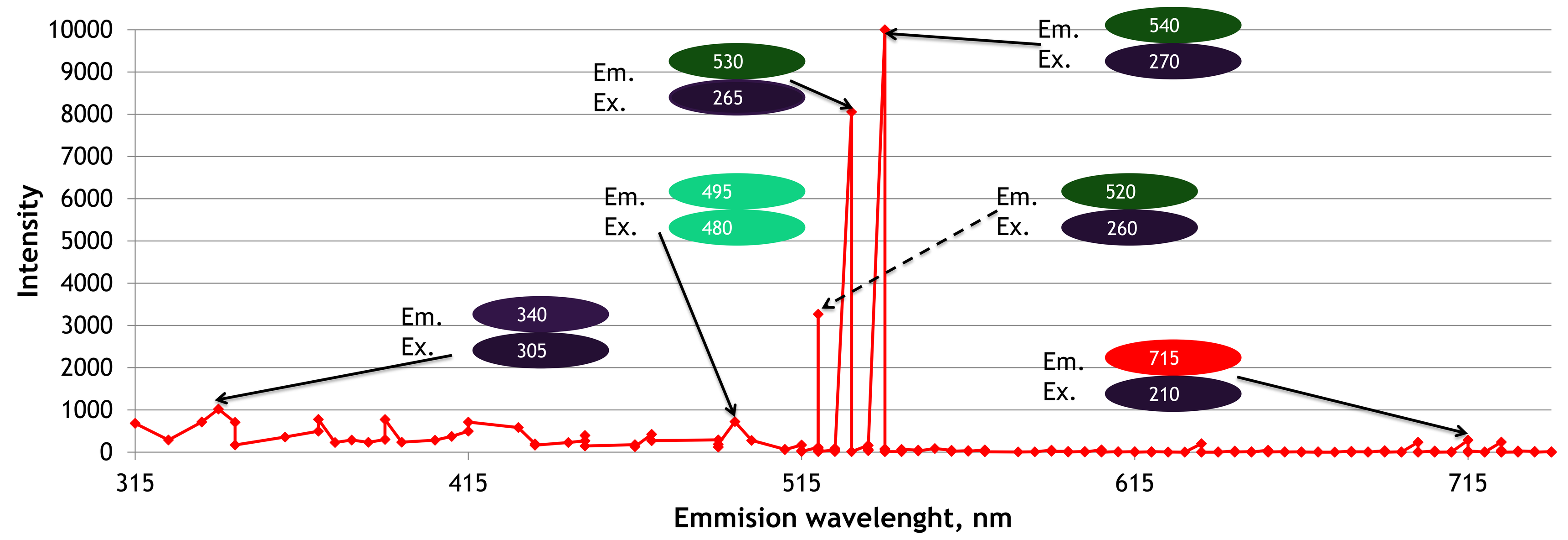
Light microscopy images of living diatoms in form of long colonial chains



Kinetic of diatom growth

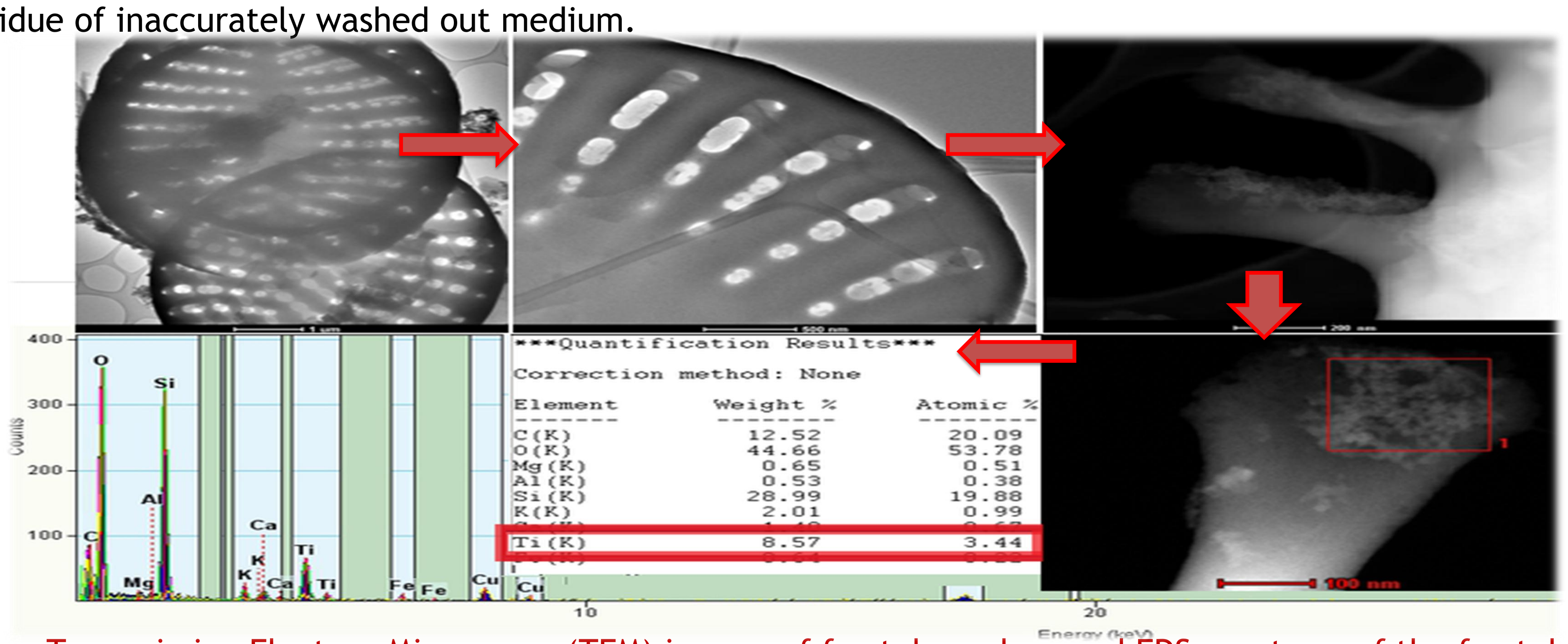
The dynamics of growth of the density of selected *Pseudostaurosira trainorii* diatoms species can be characterized in four phases: I- intensive growth phase in the first five days; II - the growth slowdown phase on the sixth day; III - intensive growth phase from the seventh to the ninth day; IV - stationary phase with constant cell density.

Transmission electron microscopy (TEM) images show the intricate design of the diatom valves in detail. The architecture of the entire valve of diatomaceous shells is visible, as well as details of the structure of individual pores. It can also be seen that the rows of pores are elongated by two or three pores on the upside down side of the valve (frustule). TEM-EDS analysis confirmed that the insulated diatomaceous frustules doped with titanium ions consist mainly of silica in the form of SiO_2. As can be seen in the TEM-EDS spectrum (quantitative elemental analysis), the dominant peaks come from oxygen and silicon, and over 73% of the elemental composition of biosilica is Si and O. TEM-EDS analysis confirmed the presence of "flake" titanium in the frustrates (8.57wt.%). The carbon content (12.52wt.%) may be related to the residual organic materials in the cleaned shells even after digestion with hydrogen peroxide. The presence of Cu is due to the copper TEM mesh. The remaining first grids may be a residue of inaccurately washed out medium.



Photoluminescence spectrum of cleaned diatom biosilica doped with titanium ions

The photoluminescence spectrum of the biosilica doped with titanium ions allows to distinguish four main types of photoluminescence activity. I- excitation and emission in the ultraviolet region. In this region, the strongest photoluminescence is manifested by a peak wavelength of 340 nm and an excitation of 305 nm. This activity is very characteristic of titanium ions. II- emission of excitation in the blue region of the visible spectrum (Ex. 480 nm and Em. 495 nm). III - very intense emission in the green region of the visible spectrum centered at 540 nm when excitation occurs in the ultraviolet region at 270 nm. IV - emission in the red region of the visible spectrum centered at 715 nm when excitation occurs in the ultraviolet region at 210 nm.



Transmission Electron Microscopy (TEM) images of frustules valves and EDS spectrum of the frustules biosilica doped with titanium ions

Conclusions

The results obtained in the study clearly indicate that the biosilica doped with titanium ions has unique optical properties and a three-dimensional, hierarchical structure, which makes it an attractive source of solutions for the development of modern materials engineering. There is a wide range of possibilities of using such materials, including in the production of biosensors, optical devices, catalysts, semiconductors, effective adsorbents, nanolithography templates, as well as in the design of drug carriers and bone implants.