Characterisation of functional biomaterials obtained by immobilisation of microorganisms by sol-gel method using isobutyltriethoxysilane

Olga Kamanina *, Pavel Rybochkin, Elizaveta Lantsova, Vitaliy Soromotin

Encapsulation of living cells into the inert biocompatible silica matrix could provide significant benefit in heterogeneous biocatalysts development. Silicate shells of diatoms are the natural examples of such structures. The main function of that systems is the protection of genetic material from adverse conditions. Previously, we showed that under certain conditions of sol-gel synthesis formation of silica shell around yeast cells is possible [Ponamoreva, 2015]. This work continues our investigations. Impact of reaction conditions on 3D structure of biomaterials based on encapsulated in organosilicate matrixes yeast was revealed.

The use of biological agents in biotechnological processes is limited by the strict dependence of their biological activity on environmental conditions: pH, UV radiation, heavy metal salts, temperature, etc. Among the methods of protecting bioagents from negative conditions, the method of encapsulating living cells in a silicon matrix stands out. The cell is surrounded by a porous shell through which an effective exchange of substances between the cell and the environment can take place, but at the same time, the cell is not exposed to aggressive environmental factors. An important component in the sol–gel synthesis of biohybrids are alkylalkoxysilanes, which include tetraethoxysilane, dimethyldiethoxysilane and isobutyltriethoxysilane. The use of alkylalkoxysilanes allows the number of bonds formed between molecules to be varied. Isobutyltriethoxysilane is a substance in which there is a non-hydrolysable bond from an Si atom to a branched isobutyl radical. It is thought that a small amount of it will form a less rigid but stronger shell around microbial cells.

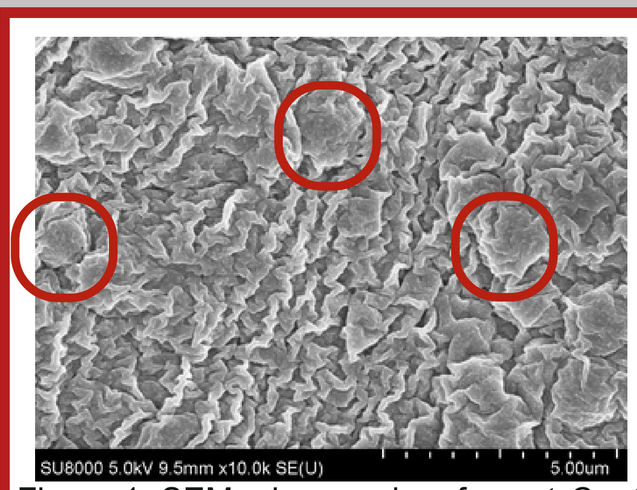
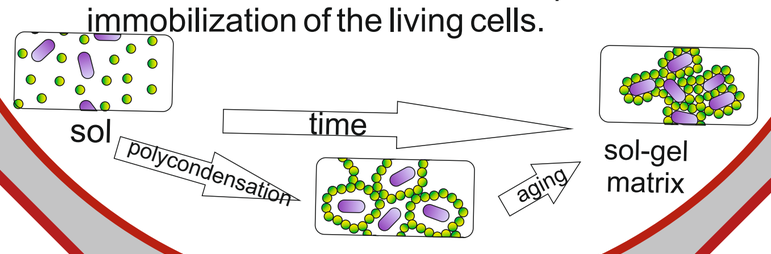


Figure 1. SEM micrographs of yeast *Ogataea polymorpha* BKM Y-2559 encapsulated in silica sol-gel matrix
Insert: SEM micrograph of yeast *Ogataea polymorpha* BKM Y-2559

The formation of the sol-gel matrix

Immobilized cells are able to maintain their viability for a long time, which pro-vides the additional advantages when they are used in industrial and environmental biotechnology [5]. One of the modern approaches for the immobilization of microor-ganisms is their encapsulation. The sol-gel method can be used to obtain such materials, since the method does not require energy-intensive, expensive equipment, it is eco-nomical and environmentally friendly, and most importantly, the sol-gel synthesis re-actions proceed under the mild conditions, which is important for the immobilization of the living cells.



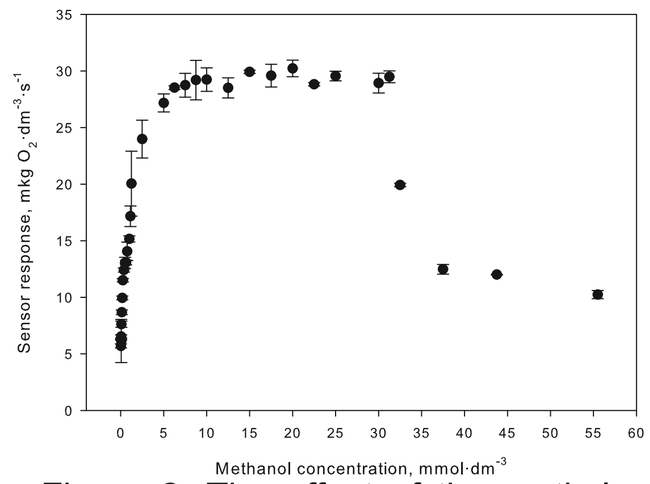


Figure 2. The effect of the methyl alcohol content in the solution on respiratory activity of the yeast cells

The size of the largest number of pores in the sample is in the range from 20 to 80 nm, so mesopores and macropores below 100 nm in diameter predominate in the sample. Thus, bacteria (whose size is 1-2 μm) are securely fixed in the sol-gel matrix and are not able to be washed out, while their metabolic products and substrates can diffuse without restrictions through pores of such sizes.

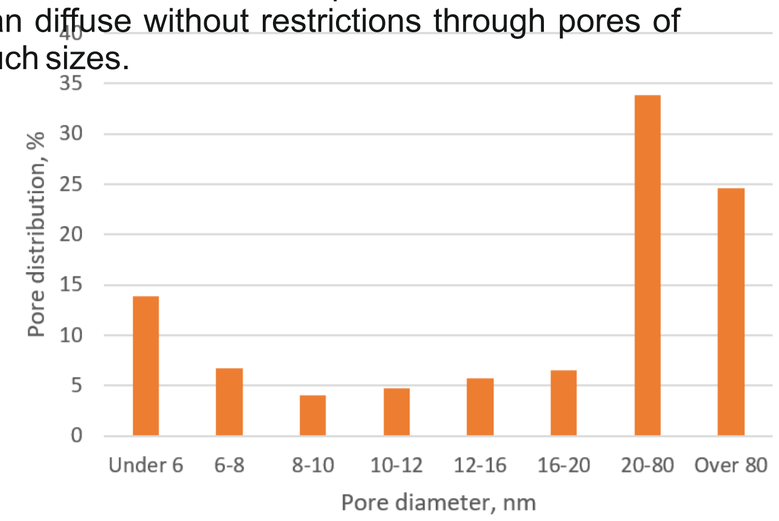


Figure 3 Pore distribution in the sample

The analytical and metrological performance of the biosensitive element with encapsulated yeast cells remained virtually unchanged before and after UV irradiation (5 h, λ = 254 nm) (Figure 4).

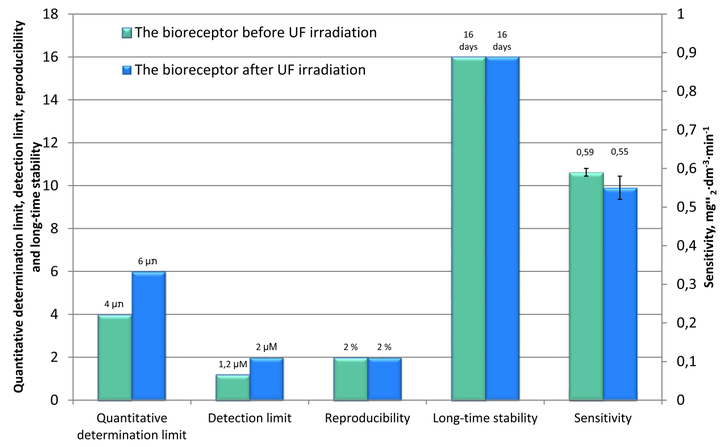


Figure 4. Protection from UV irradiation

In this work, we have characterised functional biomaterials obtained by immobilising microorganisms by means of the sol–gel method using isobutyltriethoxysilane. It is shown that the catalytic activity of the obtained hybrid material is not high, but the use of insignificant amounts of isobutyltriethoxysilane leads to the reliable encapsulation of microorganisms. Therefore, such a material can be used not only in environmental monitoring for the production of biofilters and biosensors, but also in the production of porous materials as carriers of substances.

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