SILICON POLYETHYLENE GLYCOLATES AND SILICON GLYCEROLATES AS PERSPECTIVE MATRICES FOR YEAST CELL ENCAPSULATION IN THE SOL-GEL SYNTHESIS

Cite this: *INEOS OPEN*, **2024**, *7 (1–3)*, 37–39
DOI: 10.32931/io2417a

Received 5 May 2024,
Accepted 21 May 2024

http://ineosopen.org

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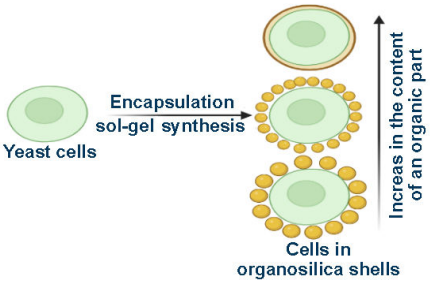
Abstract

This report presents a comparative analysis of the structures and viability of methylotrophic yeast encapsulated into organosilica matrices of various compositions. The encapsulation of methylotrophic yeast is shown to result in the formation of cell-in-shell structures using biocompatible silicon polyethylene glycol and silicon glycerolate. After the encapsulation, the cells retain an intact membrane and are viable, which is important for their further application in biotechnology, ecology, and medicine.



Key words: silicon polyolates, cell encapsulation, sol-gel synthesis, cells in shells.

Introduction

Silicon is one of the most abundant element in the Earth's crust, which is used as a building material and substrate by living organisms [1]. Diatoms synthesize frustules from amorphous silica. Different forms and protective functions of diatom's silica architectures along with their green synthesis conditions (occurs at ambient temperature and pressure, and at neutral pH) inspired the field of biomimetics, aimed at replicating this protective silica constructs for cell encapsulation through soft chemical synthesis [2, 3]. One of the widely used approaches is the sol-gel technology [4]. Usually, alkoxysilanes are used as starting compounds in the sol-gel synthesis, the hydrolysis and polycondensation of which lead to the formation of alcohols, resulting in cell lysis [5, 6]. Furthermore, it contributes to the formation of a hard shell around the cells; this requires the introduction of alkylalkoxysilanes and organic polymers [7–10]. The encapsulation of microorganisms into organosilica matrix of alkylalkoxysilanes, namely, tetraethoxysilane (TEOS) and methyltriethoxysilane (MTES) through the one-step sol-gel synthesis was studied earlier by our research group. Methylotrophic yeasts possessing an effective short-chain alcohol oxidation system were used as a biological part of the biocatalysts. In these conditions, at the TEOS:MTES 15:85 vol % ratio of silane precursors, in the presence of poly(ethylene glycol) (PEG) or poly(vinyl alcohol) (PVA), the yeast cells became the centers of formation "cell in the protective organosilica shell" architectures [11, 12]. This technology is limited by the spectrum of microorganisms in use and, as a consequence, the range of application fields where the encapsulated cells can be used. A solution to this problem may be the use of silicon polyolates, which do not cause denaturation and/or precipitation of biological macromolecules and the organic component provides a favorable microenvironment for whole cells. Silicon polyolates have been used previously in the process of biomimetic mineralization of polysaccharides, proteins and synthetic biopolymers [13–15]. In general, the cell encapsulation into organosilica matrices may provide the development of new classes of biomimetic hybrid materials for the creation of biocatalysts, cell-based sensors, as well as artificial cell development and wastewater treatment systems in the future.

Results and discussion

A comparative analysis of the effectiveness of biocatalysts based on methylotrophic yeast *Ogataea polymorpha* VKM Y-2559 (All-Russian Collection of Microorganisms, Pushchino, Russia) immobilized on organosilica matrices of various compositions was performed.

The cell encapsulation was carried out by one-stage sol-gel synthesis under basic catalytic conditions, and the following reagents were used. The first option: SPEG matrix, which is based on TEOS:MTES 15:85 vol % and PEG-3000; the second option: STPEG silicon poly(ethylene glycol) synthesized from TEOS and PEG-400 [16]; and the third option: STG silicon glycerolate synthesized from TEOS and glycerol [13] (Table 1). The detailed methodology for the cell encapsulation was published earlier [17].

The viability of the yeast cells encapsulated into the STPEG- and STG-based matrices was estimated by fluorescence microscopy using a dye system for identification of living and

Table 1. Mass ratio of the silica and polymer components in the organosilica matrices in use

Matrices	Mass ratio of the reagents in the sol-gel synthesis
SPEG: TEOS/MTES:PEG-3000	4:1 (~20% of PEG)
STPEG: TEOS:PEG-400	0.125:1 (~90% of PEG)
STG: TEOS:glycerol	0.25:1 (~75% of glycerol)

dead cells (Live/Dead Yeast Viability Kit); the structural features of the encapsulated yeast cells were studied by scanning electron microscopy (Fig. 1).

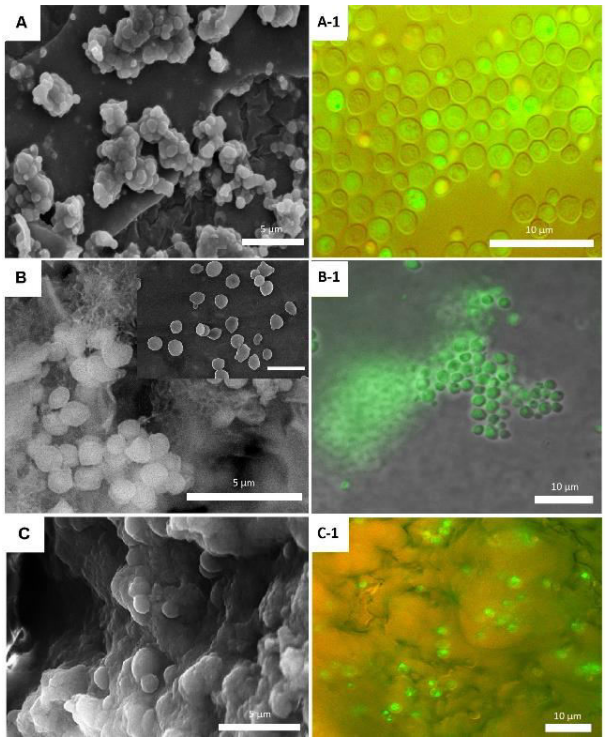


Figure 1. SEM micrographs and fluorescence microscopy images: *Ogataea polymorpha* VKM Y-2559 cells encapsulated into the SPEG-matrix (**A**, **A-1**), into the STPEG-matrix (**B**, **B-1**), and into the STG-matrix (**C**, **C-1**); free *Ogataea polymorpha* VKM Y-2559 cells (an inset in Fig. **B**; the bar represents a 5 μm scale).

The separate cells packed into spherical particles with the sizes ranging from 0.7 to 2 μm can be seen in the biohybrid material based on methylotrophic yeast encapsulated into the SPEG matrix (Fig. 1A) [11]. The PEG hydrogels were arranged around the cell surface as three-dimensional networks with silica particles. The encapsulation of yeast cells into the STPEG matrix led to the formation of tighter film-like shells around the cells, which could be explained by the lower water content in the system, as was shown earlier (Fig. 1B). More information about the STPEG matrix can be found in our previous report [17]. Such an architecture is explained by the application of low-molecular-weight PEG, which forms linear structures in aqueous solutions. The formation of a shell of small particles forming a single system was observed when the third STG matrix was used. The sizes of the shell particles depended on the organic content of the matrix, an increase in which led to a decrease in the sizes of the individual particles. Based on green fluorescence of methylotrophic yeast (Figs. 1A-1, B-1, C-1), it can be concluded that all the cells retained an intact membrane and were viable.

Conclusions

The encapsulation of methylotrophic yeast leads to the formation of cell-in-shell structures using biocompatible silicon polyolate compounds. This approach can be further successfully applied for the cells that are unstable to alcohols, which will significantly expand the application scope of encapsulated microorganisms in biotechnology, ecology, and medicine.

Acknowledgements

The authors are grateful to T. G. Khonina and E. V. Shadrina (I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia) for the synthesis of silicon polyethylene glycolates and silicon glycerolates and the possibility to use them in the work.

This work was supported by the Ministry of Science and Higher Education of the Russian Federation as part of state assignment no. FEWG-2024-0003.

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