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ORIGINAL ARTICLE

Application of organosilicate matrix based on methyltriethoxysilane, PVA and bacteria *Paracoccus yeei* to create a highly sensitive BOD

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Abstract
We have studied immobilization of *Paracoccus yeei* VKM B-3302 cells in an organosilica sol–gel matrix consisting of tetraethoxysilane, methyltriethoxysilane and polyvinyl alcohol as a structure-modifying agent. Optical microscopy showed that higher amounts of methyltriethoxysilane make the solid material structure softer. In addition, formation of structures, probably, with bacterial cells inside was spotted. We have analyzed the catalytic power of the immobilized bacteria and discovered that the material's catalytic potential is the highest at 50% of methyltriethoxysilane. Therefore, this seems to be the best ratio of precursors in a material for bacteria to become effectively encapsulated. Analysis of the material structure by low-temperature nitrogen absorption and scanning electron microscopy revealed that in the given conditions the material got crack-like mesopores and spherical particles of about 25 µm in diameter with immobilized bacterial cells on their surface. The study found that the fabricated organosilica material can effectively protect bacterial cells against UV radiation, pH change, high salinity and high heavy metal ion concentration.

Keywords Organic–inorganic hybrid sol–gel · ORMOSIL · Biocatalyst · Amperometric BOD biosensor

Introduction

Materials, which result from an interaction between chemically different components, most often organic and inorganic, shaping a particular spatial structure, are known as functional hybrids. Together with live bacterial cells they form biological materials or biohybrids, as a rule, by way of sol–gel method. Development of cell-immobilizing biocompatible matrices is crucial for better performance of functional hybrids. A new field of materials science related to the development of organic–inorganic matrices used to immobilize different biocomponents (Zheng and Boccaccini 2017) has shown fast progress in the past two decades. The latest achievements in the field of biomaterials, biohybrids and functional nanomaterials have brought to being new materials, which attracted the attention of researchers and industrial companies. Building a hydrogel, sol–gel, polymer or mineral nanofilm shell around a bacterium, produces a so-called "synthetic spore", i.e., particularly stable biostructure protected against negative external factors (Hong et al. 2013).

Immobilization of a biomaterial in an organosilica matrix has several advantages compared to organic and inorganic polymers. A bacterial cell either shelled, or encapsulated in an inorganic matrix is an ideal stable "live material" (Meunier et al. 2010) and can serve as a biocatalyst with high potential, which can be used to create bioreactors (Chen et al. 2018), biosensors (Yang et al. 2009; Depagne et al. 2011), bio-decomposers of toxic compounds (Sukiasyan et al. 2018), implants (Catalano et al. 2012), biofuel cells (Blondeau and Coradin 2012).

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Exposure to a variety of external physical and chemical factors of the media may damage efficiency of the biomaterial used in a bioreceptor element of a biosensor and influence the outcome of the study. Moreover, natural object features are unstable and constantly changing, which also affects bioagent's efficiency. Oxidizing power of a biomaterial depends on pH, temperature, presence of heavy metal ions, UV radiation, and ion power of solution. Immobilization methods, especially sol–gel immobilization, help to receive a more stable, ablation-resistant, high-efficient and highly responsive biomaterial. Compared to free systems, an immobilized biomaterial is often more stable, sustainable, powerful and selective (Es et al. 2015). A sol–gel technology is an easier and simpler way to create electrochemical and optical biosensors that can be used to measure concentration of different solutes in solution samples. In some cases, sol–gel immobilization is employed to enhance heat tolerance, mechanical strength and resistance of a biomaterial to dissolved solids (Weiser 2017).

Immobilization of microbes in tetraethoxysilane and methyltriethoxysilane organosilica matrices makes it possible to encapsulate every single cell and prevent its further division (Kamanina 2016). A firm and hard organosilica matrix plays a role of a safe capsule for an immobilized cell and external solution. An encapsulated cell measures only 2.3–3 μm compared to the size of a yeast cell, which is 2 μm (Ponamoreva et al. 2015).

In the work Lin et al. (2006), researchers created a biocatalyst using *B. licheniformis, D. maris* and *M. marinus* immobilized in sol–gel matrices consisting of tetraethoxysilane (TEOS), dimethyldiethoxysilane (DMDES) and polyvinyl alcohol (PVA) in a process catalyzed by acid. Heterogenic biocatalyst could function at 30 °C and pH 7.9. The use of an acid catalyst demands continuous mixing (often by ultrasound) before bacteria addition. As a result, a monolith matrix is formed, which does not allow separate encapsulation of each bacterial cell.

Earlier, researchers studied immobilization of yeast cells in organosilica sol–gel matrices to find out that the cells do not lose their catalyzing power, are protected from external negative factors (Kamanin et al. 2014) and play a role of biological cleansers (Kamanina 2016). Yeast cells are often chosen as a bioagent for encapsulation in an organosilica sol–gel matrix. The use of bacterial cells for the same purpose has never been studied before, however, we believe the matter is worth studying, because bacterial cells have a different structure and may alter the process of sol–gel matrix formation.

We extracted and described *Paraccocus yeei* VKM V-3302 from an activated sludge sample. *Paraccocus yeei* VKM V-3302 can oxidize a wide range of substrates, which is essential for development of biosensors used to determine integral properties like biochemical oxygen demand—the main water pollution level indicator. Our suggestion was that like yeast studied before, bacteria immobilized in sol–gel matrices with different composition will be encapsulated separately, while stay functional, able to oxidize a wide range of substrates, and also protected from negative external factors. Mechanism of sol–gel capsule formation around bacteria, pore size and protective power of matrix are topics for further study. Organosilica sol–gel encapsulation of *P. yeei* enables development of heterogenic biocatalysts that in the future can be used both to measure biochemical oxygen demand and for wastewater cleansing. Hence, the objective of this work is to develop a heterogenic biocatalysts based on *Paracoccus yeei* VKM V-3302 cells encapsulated in an organosilica sol–gel matrix made using methyltriethoxysilane and tetraethoxysilane and study formation process and the structure of the material resulting from bacterial cell immobilization. We deem it relevant to consider employment of such a heterogenic biocatalyst as a bioreceptor element for BOD (biochemical oxygen demand) assessment in water.

Materials and methods

Cultivation of microbial cells

We used bacterial cells *Paracoccus yeei* BKM B-3302 isolated from activated sludge (Kharkova et al. 2019) and grown on a rich mineral medium (liquid glucose–peptone nutrient medium). The cell grown medium was sterilized by autoclaving at a pressure of 1.15 atm. within 45 min. The cells were grown aerobically for 18–20 h in 750 cm^3 shaker flasks at a temperature of 29 °C. Then the resulting biomass was centrifuged at room temperature for 10 min (8000 rpm). Next, the centrifuged was washed with 20 mM phosphate buffer, pH 6,8. The settled cells were transferred into fresh portions of the buffer, distributed in portions, and pelleted in «Eppendorf» (1.5 ml) centrifuge (Germany) for 5 min at 8000 rpm. The washed biomass was weighed and stored in microtubes at a temperature of minus 25 °C.

Encapsulation of cells by sol–gel method

The silane precursors used were tetraethoxysilane (TEOS) and methyltriethoxysilane (MTES). A mixture of silane precursors with hydrophobic additives MTES content from 0 to 100% (vol.) with respect to the total amount of the silane precursor was used. A suspension of cells of $1.2 \pm 0.1 \times 10^9$ CFU/cm^3 in phosphate buffered saline (pH 6.8) was added to 0.02 cm^3 of a 5% solution of PVA (Ferak Berlin) in phosphate buffered saline and stirred for 5 min (Elmi CM-70M07), then a mixture of tetraethoxysilane (TEOS) (Sigma) and methyltriethoxysilane (MTES) (Sigma) of volume 0.1 cm^3 was added and mixed again for 3 min.

Then, 0.005 cm^3 of the catalyst solution of 0.2 mol/dm^3 NaF was added and stirred for 15 min, then placed on a surface of Clark oxygen electrode (Econix-Expert Ltd., Russia) and fixed with a nylon mesh.

Sample pore structure study by low-temperature nitrogen absorption method (BET method)

The samples were successively washed by swinging in distilled water and ethyl alcohol for 15 min each time at the rate of 150 swings per minute to bring them to a single standard. The wash liquid was removed by decantation. The samples were then dried in a heat chamber for 120 min at 200 °C. A 0.1–0.3 g standardized subsample was put in a pre-weighed quartz cuvette and into a sample preparation device Beckman Coulter M SA-PREPTM (Beckman Coulter, USA), where it was dried using ultrapure nitrogen (99.999%, GOST 9293–74) for 60 min at a temperature of up to 60 °C. Prepared sample was placed in a gas removal port of Beckman Coulter M SA 3100TM (Beckman Coulter, USA). All gas was removed within 60 min under pressure of 0.001 mm Hg at 60 °C. After that, the sample in a cuvette was cooled and weighed to determine its precise weight (± 0.0005 g). Then it was put into an analysis port of SA 3100TM devise to measure its surface properties (specific surface area, pore structure) based on nitrogen absorption at a constant temperature. Pore structure (micro-, meso- or macroporous) was studied under relative pressure ranging from 0 to 0.995 mm Hg.

UV resistance of encapsulated cells

Microbes encapsulated in a sol–gel matrix were subject to UV (Vilber Lourmat, Russian) 254 nm light exposure at 680 µW/cm^2 for 5 h. Then fragment containing immobilized cells (3 × 3 mm) was placed on the surface of a Clark-type oxygen electrode, fixed with a nylon net and washed with a buffer phosphate solution for 5 min before use.

Impact of pH, heavy metal ions and salinity of medium on respiratory activity of encapsulated bacteria

Respiratory activity of encapsulated bacteria was assessed using amperometric biosensor. The system was washed with potassium and sodium phosphate buffer solution (20 mmol/dm^3, pH 7.6) immediately before assessment. The impact of metal ions was measured using metal ion solutions at the maximum allowable concentration for water sources or 10-, 25-, 50-, and 100-fold above it. pH impact was measured using a citrate–phosphate buffer with pH ranging from 2 to 12 as a base solution. Salinity impact was measured using 0 to 20% sodium chloride solution in a cuvette.

Results and discussion

Optimizing component ratio to create high-efficient organosilica matrix

Good surface contact is important for successful immobilization of bacteria in a sol–gel matrix. An inorganic sol–gel matrix can be modified with bioorganic and organic compounds (Ismail 2016), including polymers (Babiarczuk et al. 2020), for a better environment around bacterial cells, higher stability of the matrix structure and to regulate its rheological properties and structure formation process. To modify a matrix, polyvinyl alcohol was used, because it is chemically and microbiologically stable, nontoxic and biocompatible (Szczesna-Antczak et al. 2004; Wang et al. 2020). The nature of silane precursors is another important factor affecting the sol–gel structure and properties. Tetraethoxysilane is the most popular of them (Vollet et al. 2008; Bernands et al. 1991; Kamanin et al. 2014) To modify an organosilica matrix structure, it can be used in combination with an organically modified alkoxysilane with a general formula $R'_nSi(OR)_{4-n}$, in which R' represents an alkyl (a hydrophobous agent). A covalent Si–R' bond insensitive to hydrolysis restricts formation of Si–O–Si bonds; therefore, $Si(OR)_4$ and $R'_nSi(OR)_{4-n}$ ratio determines the properties of a final material. Without a hydrophobous agent, solid structures with higher mechanical restriction for cells are formed. Too much hydrophobous agent results in a soft gel structure. This must be taken into account, when you immobilize cells.

We created 7 bioreceptors based on *Paracoccus yeei* immobilized in an organosilica matrix using TEOS and MTES in different proportions and PVA as a structure-modifying agent. Morphology of organosilica matrices with different ratios of TEOS and MTES was visualized by optical microscopy (Fig. 1).

It showed that higher concentration of a hydrophobous agent decreases density of the formed matrix with *P. yeei* immobilized in it. The matrix structure density is the highest without a hydrophobous agent. Spherical particles of up to 25 µm in size appear at a higher MTES ratio. TEOS/MTES ratios of 25/75 or 0/100 result in a soft matrix, from which bacterial ablation can occur. Encapsulated bacterial cells seem to be visible on the surface of the spherical particles in a matrix with TEOS/MTES ratios of 50/50 or 40/60. These we believe to be the best ratios for bacterial immobilization.

Sol–gel matrix formation process was studied by IR spectroscopy, with IR spectra registered every 5 min for 30 min (Fig. 2). They are typical for gels synthesized using PVA as a porogen in the process of an alkali-catalyzed reaction.

IR spectra feature intensive absorption bands at 1070 and 770–790 cm^{-1} corresponding to Si–O stretches

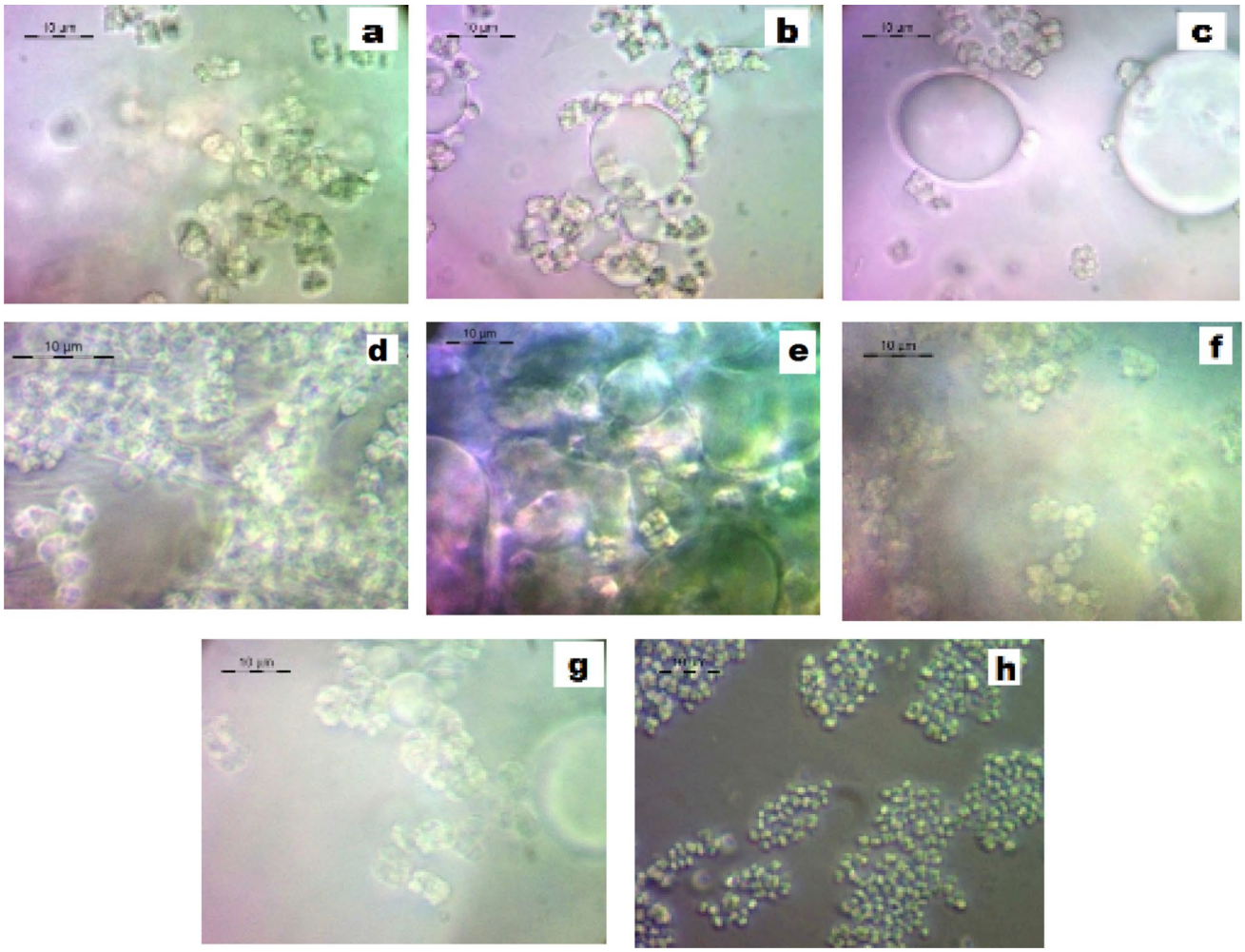

Fig. 1 Photographs of bacteria *Paracoccus yeei* immobilized in a sol–gel matrix of various compositions, obtained using optical microscopy. TEOS/MTES rations: **a**—100/0, **b**—75/25, **c**—60/40, **d**—50/50, **e**—40/60, **f**—25/75, **g**—0/100, **h**—pure culture of bacteria *Paracoccus yeei* VKM B-3302

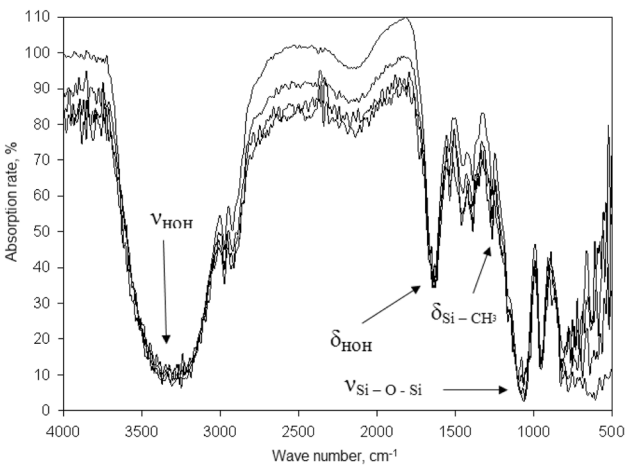

Fig. 2 IR-spectrum of the organosilicate sol–gel matrix of the sample using the structure-controlling agent PVA

(asymmetric and symmetric). Bands in the 2970–2980 cm $^{-1}$ and 1380–1390 cm $^{-1}$ ranges correspond to ν_{C-H} and δ_{C-H} CH_3 group stretches, respectively, while those at 2880–2890 cm^{-1}—to ν_{C-H} CH_2 group stretch. There are also bands at 1110–1000 cm^{-1}, corresponding with Si–O–C stretches, those at 1310–1000 cm^{-1} corresponding with C–O–C stretches and at 1270 cm^{-1} corresponding with Si–CH_3 group stretches. Bands at 3650–3590 cm^{-1}, 3400 cm^{-1} and 1640 cm^{-1} are associated with OH group stretch. A 1150–1085 cm^{-1} band is characteristic of CH_2–O–CH_2 stretch. All the results received are congruent with those described in scientific literature (Pereira et al. 2000).

The impact of the biomatrix architecture on the physiologic activity of immobilized bacteria was assessed by the change of their respiration in the presence of glucose and glutamate solution usually used to measure biochemical oxygen demand. For this purpose, a Clark-type oxygen

biosensor modified with cells immobilized in an organosilica matrix was applied. The bacterial sensor registers a change of current rate in time, when oxygen level in a pre-electrode space falls as a result of increasing respiratory activity of substrates oxidized by immobilized bacteria. Bacterial oxygen uptake rate depends on the substrate concentration in the sensor cell. Whole-cell bacterial biosensor is catalytic, i.e., biologic response in such a system is provided by fermentation reactions that occur in bacteria. Calibration curves in Fig. 3 are quite well approximated by Michaelis–Menten equation:

$R = \frac{R_{\max}[S]}{K_M + [S]}$.

Here, $R_{\max}$ represents maximum oxygen uptake rate of immobilized bacteria happening at $[S] \rightarrow \infty$. K_M represent the Michaelis constant, i.e., substrate concentration at which $R = R_{\max}/2$.

IUPAC's Analytical Chemistry Division and Physical and Biophysical Chemistry Division adopted recommendations

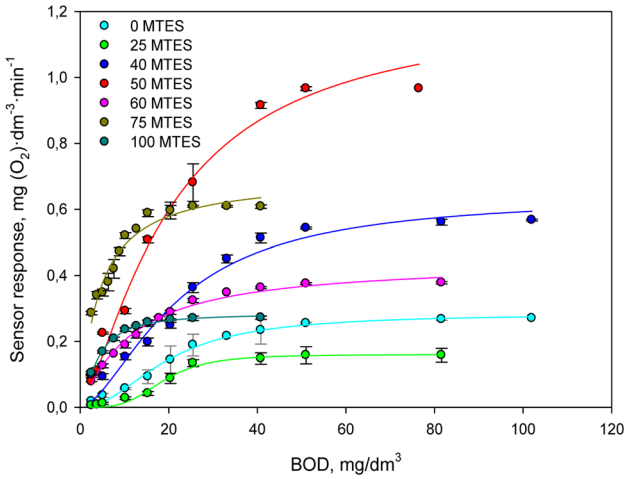

Fig. 3 Calibration dependences of the responses of the developed biosensors on the BOD

on the definition, classification and nomenclature describing responses and efficiency of electrochemical biosensors (Mehrotra 2016). According to these recommendations, basic features of biosensors are related to calibration—sensitivity, range, and detection limit. In addition, operational and long-term biosensor stability was measured to Table 1. Main characteristics of bioreceptor elements based on microorganisms *Paracoccus yeei.*

Analysis of the data received showed that a biosensor based on *Paracoccus yeei* immobilized in a 50/50 TEOS/MTES matrix is more sensitive and stable than bioreceptors with a different ratio of silane precursors, probably, due to a higher ability of the matrix with 50% of MTES hydrophobous agent to encapsulate bacteria. This, however, requires further investigation by BET and SEM methods. It is noteworthy that *P. yeei* immobilization in an organosilica sol–gel matrix allowed fabrication of a more sensitive biosensor element with a lower detection limit and smaller relative standard deviation compared to immobilization of the same bacteria in an N-vinylpyrrolidone-modified PVA hydrogel (Arlyapov et al. 2020) or in a biocompatible redox-active hydrogel with nanotubes (Arlyapov et al. 2021) likely thanks to a better ability of sol–gel to keep *P. yeei* immobilized, while also vital and preserving their physiological and biochemical properties. The developed biosensor can be used to measure biochemical oxygen demand in undiluted samples of relatively pure river water and purified wastewater and in diluted samples of polluted river water and wastewater.

Organisilica matrix structure analysis

Pore structure and specific surface area of the fabricated biocatalyst based on bacteria immobilized in a 50/50 TEOS/MTES matrix was analyzed by low-temperature nitrogen absorption method. Analysis results are shown on absorption–desorption isotherms in Fig. 4.

The determined isotherms refer to type IV, which is characteristic of absorption in mesoporous bodies and inorganic

Table 1 Main characteristics of bioreceptor elements based on microorganisms *Paracoccus yeei*

	Paracoccus yeei immobilized in sol–gel matrix with a ratio TEOS/MTES, vol. %							*P. yeei,* in modified PVA [*]
	100/0	75/25	60/40	**50/50**	40/60	25/75	0/100	
Sensitivity, 10^{-3}·min^{-1}	7 ± 1	4,2 ± 0, 5	12 ± 1	**30 ± 2**	11,9 ± 0,7	24 ± 4	20 ± 2	5,8 ± 0,3
Concentration range, mg/dm^{-3}	0,1–21	0,08– 20	0,06–24	**0,01–20**	0,03–12	0,2–4,3	0,02–3,8	0,05–5,0
Detection limit, µg/dm^3	41	32	29	**8**	11	13	20	–
Reproducibility, Sr. %	7	14	5	**3**	7	3	15	7
Operational stability, days	11	10	13	**31**	10	16	28	45
Duration of a single measurement	4–6							4–6

[*] Arlyapov et al. (2020)

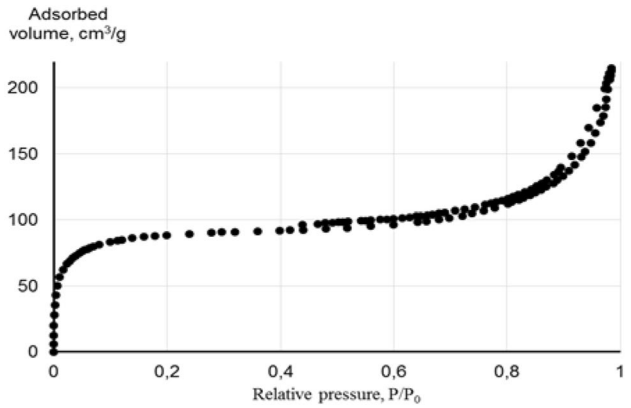

Fig. 4 Adsorption–desorption isotherm for a matrix containing silane precursors TEOS/MTES = 50/50

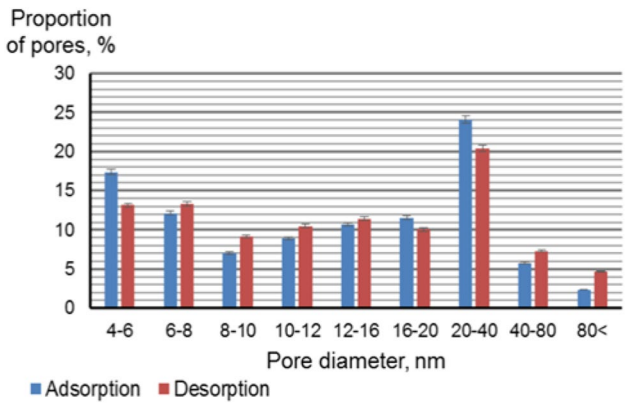

Fig. 5 Plots of pore distribution in a sample containing silane precursors TEOS/MTES = 50/50 based on adsorption/desorption data

oxides (Sing et al. 1985). The shape of a hysteresis loop is diagnostic of pore shape and type. The received hysteresis loop may be referred to type B according to de Boer's classification. Hysteresis loop type in a certain way corresponds to the adsorbent pore structure shape. Pores in the B-type adsorbent are crack-like. The curve features H3-type hysteresis loop (according to IUPAC's classification) (IUPAC 1972) between the absorption and desorption isotherms, meaning that the sample is likely to have crack-like pores.

Pore size distribution for a biocatalyst based on bacteria immobilized in a 50/50 TEOS/MTES matrix was determined by Barret–Joiner–Halenda (BJH) analysis named after the scientists, who first suggested this method (Barrett et al. 1951) (Fig. 5). It can be observed that the distribution curves feature sharp peaks with a base, which means that pores are almost equal in size and hence the system is of a monodisperse type.

Based on the analysis of the data in Fig. 5, it can be concluded that the sample contains mainly pores ranging from 4 to 40 nm in diameter, or mesopores (according to IUPAC's classification) (IUPAC 1972). The shape of the hysteresis loop on isotherms is the evidence of capillary condensation in mesopores, which means that microbes are firmly fixed in the sol–gel matrix and cannot be ablated, while their metabolic byproducts and substrates diffuse freely through these pores. The received results are congruent with the findings of other research teams. TEOS/MTES sol–gel matrix structure was studied in the work (Putz et al. 2017). Type IV isotherms were determined, mesopores were of elongated cylindrical shape. (Chua et al. 2015) discovered that 2-bis(triethoxysilyl)ethane matrix modified with Pluronic F127 consisted of mesopores ranging from 2 to 12.9 nm in diameter. A typical isotherm was determined for the type IV mesoporous materials with cylindrical pores.

SEM analysis of the fabricated hybrid material based on *Paracoccus yeei* VKM V-3302 immobilized in a 50/50 TEOS/MTES sol–gel matrix is shown in Fig. 6.

50% of MTES in a matrix produce spheres of up to 25 µm in diameter (Fig. 6a) with P*aracoccus yeei* cells on their surface. There are, however, concave spheres signaling about high resilience of the fabricated matrix. This is the first time ever that such fractural structures were received using *Paracoccus yeei* as a biocomponent in the process of an alkali-catalyzed reaction. Although their formation process is to be investigated, it is clear that the bacteria are covered with

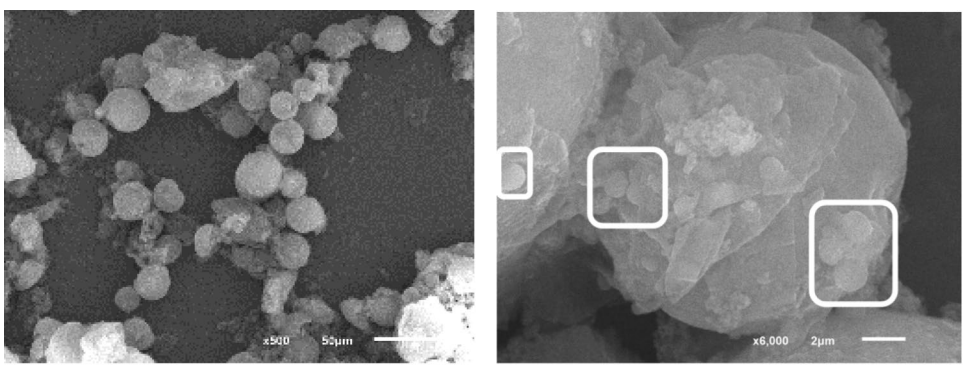

Fig. 6 SEM images of *Paracoccus yeei* VKM B-3302 in a sol–gel matrix with a TEOS/MTES silane precursor ratio of 50/50 (encapsulated cells are framed)

a sol–gel matrix shell, which seems to protect them against negative external factors. This is the subject we consider below.

Protective properties of fabricated organosilica matrix

Earlier studies (Kamanin et al. 2014) showed that a sol–gel matrix is capable to protect immobilized yeast cells from a few external factors like UV light, heavy metal ions, pH change. But its protective properties for immobilized bacteria have not been studied before.

This work is the first to assess protective function of an organosilica sol–gel matrix forming around bacterial cells in an unfriendly environment. To study the impact of salinity, heavy metal ions and pH on the catalytic power of immobilized bacteria, biosensor responses were registered under the influence of a negative factor and the results were compared with the ones demonstrated by free bacteria.

Dependense of the biosensor response on pH of medium can be observed on the curve in Fig. 7a and suggests that cells are well protected.

As the curve shows, the sol–gel matrix protects them within pH range 4 to 11. Here, the relative biosensor response was above 50%. The cells are best protected within pH range 5 to 11, with biosensor response being the highest (at least 80%). Immobilization in an organosilica material helps to expand the optimal pH range, at which bacteria retain high catalytic power (see Fig. 7a). It is noteworthy that the catalytic power of sol–gel immobilized bacteria gradually falls compared to that of their free peers as pH of medium changes from neutral to alkaline.

Figure 7b shows that the fabricated sol–gel matrix efficiently shields the cells from salinity within the studied concentration range (up to 20%). The relative response of the biosensor based on such a matrix weakened only slightly as salinity level increased, while free *P. yeei* demonstrated sharp decline of catalytic power even at the slightest increase in salinity.

A curve in Fig. 8 illustrates high protective value of the fabricated sol–gel matrix against heavy metal ions.

The biosensor response at a 100-fold exceeded maximum allowable concentration level of any heavy metal ion does not fall below half of that received in absence of a toxic agent, unlike a free *P. yeei* biosensor response that fell below this 50% mark in the same conditions. The deepest bacterial catalytic power decline was registered for Cu (II) and Ni (II) ions, probably because of their high toxicity.

Our suggestion is that absorption capacity of the received organosilica sol–gel matrix is the major protective factor for bacterial cells against heavy metal poisoning. Absorbing ions, the matrix surface does not let them into the fermentative systems of the cells (Irani et al. 2012).

To assess the protective capacity of the material against UV radiation, metrological performance of biosensor before and after UV exposure was compared. The results are presented in Table 2.

The organosilica sol–gel matrix can protect from UV radiation. This is evident from retained vitality and catalytic power of the immobilized cells after UV exposure. To compare, cell-rich fluid did not show signs of life in the same conditions. The material, however, does not guarantee 100% UV protection, which becomes obvious from the change of metrological performance of the bioreceptor element exposed to UV.

The fabricated organosilica sol–gel matrix can protect immobilized bacterial cells from a few negative factors studied in this research. This means that the heterogenic

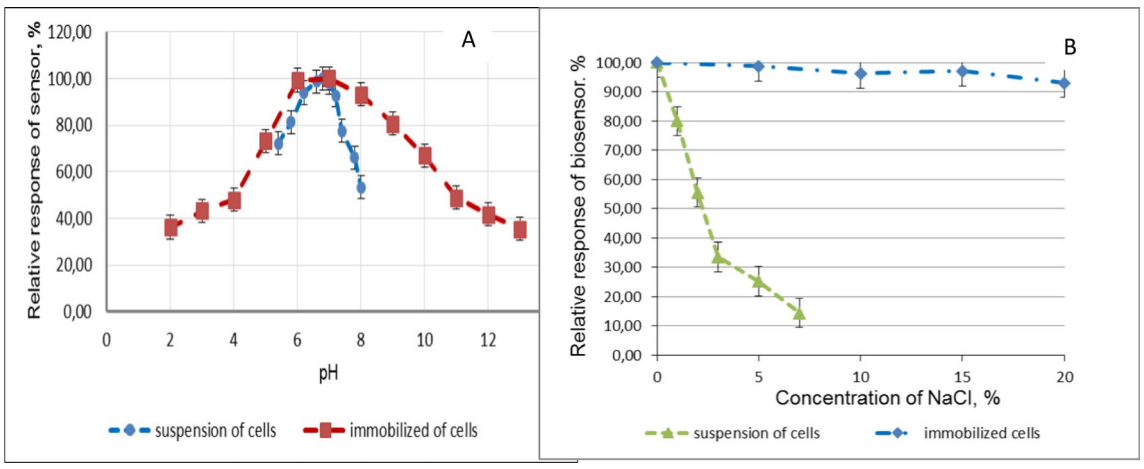

Fig. 7 Protective properties of the developed organosilicate matrix: **a**—dependence of the relative response of the sensor on the pH medium for a suspension of cells and cells in sol–gel matrix; **b**—dependence of the relative response of a biosensor on the salt concentration for a suspension of cells and cells of a sol–gel matrix

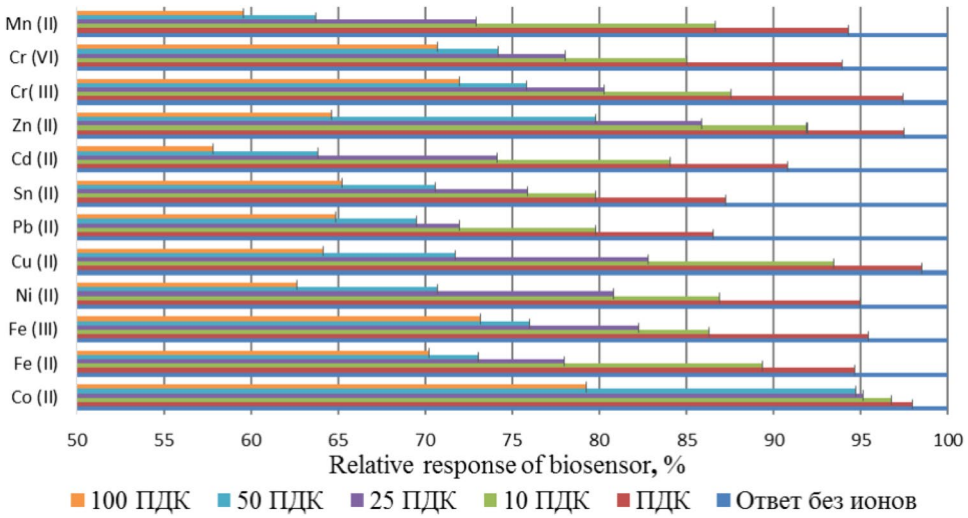

Fig. 8 Magnitude of the relative response of the sensor depending on the concentration of the heavy metal ion

Table 2 Checking the protection of cells from UV radiation by the obtained material

Characteristic	The upper limit of the determined concetracions, mg/dm^3	Sensitivity,10^{-3}· min^{-1}	The lower limit of the determined concetracions, mg/dm^3
Before UV-irradiation	20	30 ± 2	100
After UV-irradiation	9,0	32 ± 4	19

biocatalyst will not lose catalytic power and hence remain effective in conditions unfavorable for bacterial cells, which is crucial when it comes to measurement of biochemical oxygen demand in industrial wastewater samples.

Approbation of BOD biosensor based on fabricated organosilica matrix

Substrate specificity and bacterial immobilization method used to fabricate a bioreceptor element define biosensor selectivity. A broad substrate specificity is an advantage leading to a more precise BOD determination. This research studies substrate specificity of *P. yeei* immobilized in an organosilica matrix for 25 substrates belonging to different classes of organic compounds. Mainly easy-to-oxidize organic substances, which significantly decrease the level of diluted oxygen in water, where they occur, and cause subsequent eutrophication, were chosen as substrates (Fig. 9).

As sol–gel immobilized bacteria are most sensitive to glucose, biosensor response to it is taken for 100%. Bacteria oxidize substances of all the classes of organic compounds, including alcohols, carbohydrates, carbonic acids, amino acids and nitrophenols that potentially occur in waste and surface waters. The study showed that BOD_5 (the BOD value is most commonly expressed in milligrams of oxygen consumed per litre of sample during 5 days of incubation at 20 ºC) measured in water samples of different origin (industrial, household, synthetic waste water) by the fabricated biosensor seem to be highly correlated with the results received by the standard method.

Samples of natural surface water and industrial wastewater underwent analysis by the fabricated biosensor and by the standard method. Water samples were taken and BOD_5 measured by the standard method in accordance with the Federative Environmental Regulations of the Russian Federation. BOD_5 results received by the *P. yeei* biosensor and by the standard dilution method are compared in Fig. 10. Their coincidence falls within a confidence interval.

The correlation between the results received by a biosensor based on *P. yeei* immobilized in a 50/50 TEOS/MTES sol–gel matrix and by the standard method is $R = 0.9983$, which is higher compared to the analogues described earlier (Arlyapov et al. 2013, 2020; Jouanneau 2014). Thus, a biosensor based on *P. yeei* extracted from an activated sludge and immobilized in an organosilica 50/50 TEOS/MTES matrix using PVA as a structure-modifying agent is an advanced wastewater pollution monitoring tool.

Conclusion

In this work, we have studied immobilization of *Paracoccus yeei* VKM V-3302 cells in an organosilica sol–gel matrix consisting of tetraethoxysilane, methyltriethoxysilane and polyvinyl alcohol as a structure-modifying agent. Optical microscopy showed that higher amounts of methyltriethoxysilane make the solid material structure softer. In addition, formation of structures, probably, with bacterial cells inside was spotted. We have analyzed the

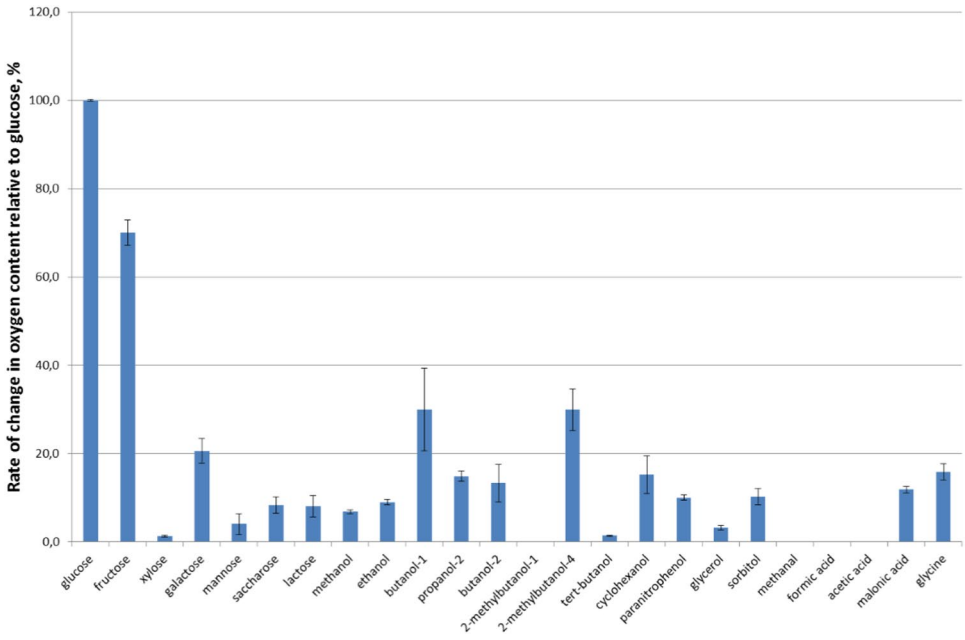

Fig. 9 Substrate specificity of bacteria *Paracoccus yeei* immobilized in a sol–gel matrix with a TEOS/MTES silane precursor ratio 50/50 (substrate concentration 0.04 mol/dm^3)

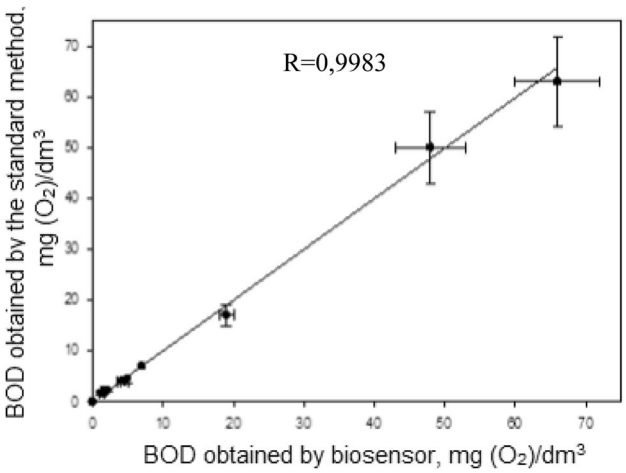

Fig. 10 Correlation of data obtained by the standard method and the using a biosensor based on bacteria *Paracoccus yeei* immobilized in a sol–gel matrix with a ratio of silane precursors TEOS/MTES 50/50

catalytic power of the immobilized bacteria and discovered that the material's catalytic potential is the highest at 50% of methyltriethoxysilane. Therefore, this seems to be the best ratio of precursors in a material for bacteria to become effectively encapsulated. Analysis of the material structure by low-temperature nitrogen absorption and scanning electron microscopy revealed that in the given conditions the material got crack-like mesopores and spherical particles of about 25 μm in diameter with immobilized bacterial cells on their surface. The study found that the fabricated organosilica material can effectively protect bacterial cells against UV radiation, pH change, high salinity and high heavy metal ion concentration.

Since the fabricated material features high protective properties and bacterial cells can oxidize a wide range of substrates even when encapsulated in an organosilica matrix, development of bioreceptor elements for BOD biosensors effective in unfavourable conditions, in which bacterial cells cannot normally function, has become possible.

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Authors' contributions KO, AV, PO conceptualization; KO, LD, RP, PE conducted the experiments; KO, AV, PO data analysis.

Availability of data and materials The authors confirm that the data supporting the findings of this study are available within the article.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

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