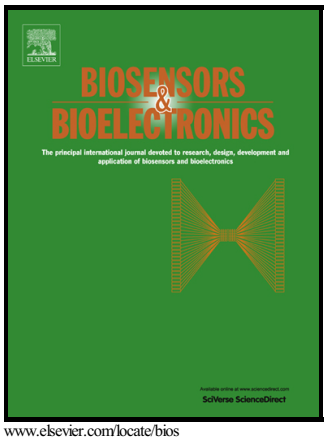


www.elsevier.com/locate/bios

Yeast-based self-organized hybrid bio-silica sol-gels for the design of biosensors

O.N. Ponamoreva, O.A. Kamanina, V.A. Alferov, A.V. Machulin, T.V. Rogova, V.A. Arlyapov, S.V. Alferov, N.E. Suzina, E.P. Ivanova

PII: S0956-5663(14)00633-2
DOI: http://dx.doi.org/10.1016/j.bios.2014.08.045
Reference: BIOS7036

To appear in: *Biosensors and Bioelectronic*

Received date: 22 May 2014
Revised date: 14 August 2014
Accepted date: 18 August 2014

Cite this article as: O.N. Ponamoreva, O.A. Kamanina, V.A. Alferov, A.V. Machulin, T.V. Rogova, V.A. Arlyapov, S.V. Alferov, N.E. Suzina and E.P. Ivanova, Yeast-based self-organized hybrid bio-silica sol-gels for the design of biosensors, *Biosensors and Bioelectronic,* http://dx.doi.org/10.1016/j.bios.2014.08.045

Yeast-based self-organized hybrid bio-silica sol-gels for the design of biosensors

O.N. Ponamoreva[1], O.A. Kamanina[2], V.A. Alferov[2], A.V. Machulin[3], T.V.Rogova[2], V.A. Arlyapov[2], S.V.Alferov[1], N.E. Suzina[3], E.P. Ivanova[4]

[1] *Department of Biotechnology, Tula State University, Pr. Lenina 92, Tula 300012 Russia - olgaponamoreva@mail.ru*

[2] *Department of Chemistry, Tula State University, Pr. Lenina 92, Tula 300012 Russia*

[3] *Laboratory of cytology of microorganisms, G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pr. Nauki 5, Pushchino Moscow reg. 142290 Russia*

[4] *Faculty of Science, Engineering and Technology, Swinburne University of Technology, PO Box 218, Hawthorn, Victoria, 3122 Australia*

Key words: whole-cell biosensor; sol-gel silica; encapsulation of living cells; methanol biosensor; methylotrophic yeast

Abstract

The methylotrophic *Pichia angusta* VKM Y- 2559 and the oleaginous *Cryptococcus curvatus* VKM Y- 3288 yeast cells were immobilized in a bimodal silica-organic sol-gel matrix comprised of tetraethoxysilane (TEOS), the hydrophobic additive methyltriethoxysilane (MTES) and the porogen polyethylene glycol (PEG). Under carefully optimized experimental conditions, employing basic catalysts, yeast cells have become the nucleation centers for a silica-organic capsule assembled around the cells. The dynamic process involved in the formation of the sol-gel matrix has been investigated using optical and scanning electron microscopic techniques. The results demonstrated the influence of the MTES composition on the nature of the encapsulation of the yeast cells, together with the architecture of the three-dimensional (3D) sol-gel biomatrix that forms during the encapsulation process. A silica capsule was found to form around each yeast cell when using 85% (vol) MTES. This capsule was found to protect the microorganisms from the harmful effects that result from exposure to heavy metal ions and UV radiation. The encapsulated *P. angusta* BKM Y- 2559 cells were then employed as a biosensing element for the

detection of methanol. The *P. angusta*-based biosensor is characterized by high reproducibility (Sr, 1%) and operational stability, where the biosensor remains viable for up to 28 days.

Graphical abstract

1. Introduction

Due to their simplicity of use and their ability to provide valuable functional information, living microorganisms are increasingly being employed as biosensing platforms (Hansen and Sorensen 2001) (Daniel et al. 2004) (Pasco et al. 2011). The coupling of cells with physical-chemical transducers allows the development of portable bioanalytical devices such as whole-cell microbial biosensors. These have been broadly used in ecological monitoring applications and biotechnological processes, since the information generated by the living cells is integral in nature, representing the overall response of the whole organism (Rodriguez-Mozaz et al. 2006) (Su et al. 2011) (Xu and Ying 2011) (Wen et al. 2013) (Ponomareva et al. 2011). The key aspect of such biosensing devices is that a whole-cell immobilization occurs, resulting in the formation of a functionally active biomatrix, coupling the biomatrix (bioreceptor) with a transducer (Michelini and Roda 2012).

Many innovative developments in the field of biomaterials have been inspired by nature. For example, in diatoms, one of the most important components of the cell wall is silica. This creates a glass-like protective shell around the cell (Hamm et al. 2003). This nature-created approach has served as a model for the development of technologies for the encapsulation of live cells into a silica matrix (Blondeau and Coradin 2012)(Nassif et al. 2002) (Rajan Premkumar et al. 2002) (Meunier et al. 2010a). It has been shown that silica-based materials have advantages over using organic polymers for the immobilization of cells; these include their ability to retain water with negligible swelling, chemical and biological stability, mechanical stability (Blondeau and Coradin 2012), controlled porosity (Dickson and Ely 2013), optical transparency (Perullini et al. 2014) and, importantly, the ease with which the chemistry of the sol gel can be varied to fabricate matrices that possess different architecture and properties (Milea et al. 2011) (Wang and Guo 2014). Of particular interest is the immobilization of whole-cells into silica-organic matrices (Avnir et al. 2006) (Desimone et al. 2009), which retains not only all of the advantages associated with the silica-based structures described above, but also offers greater flexibility,

thus lowering the pressure of the solid medium on the encapsulated microorganisms, and increasing degrees of porosity, providing a medium in which effective diffusion of substances can occur (Gill and Ballesteros).

Another significant advantage of using such matrices is that the sol-gel method used for the development of silica and silica-organic based materials does not require expensive equipment, and is economical and safe for the environment (Brinker and Scherer 1990). Sol-gel based polymer materials of different architecture, flexibility and porosity can be developed using a range of synthesis conditions (pH, catalyst) and reagents (silane precursors, organic porogen) (Gupta and Kumar 2008). One of the disadvantages of this sol-gel method, however, is the release of alcohol, which could be toxic for many microorganisms (Avnir et al. 2006) (Desimone et al. 2009) (Trogl et al. 2010). Consequently, the initial immobilization of the microorganisms into the sol-gel matrix was accomplished predominantly using *Saccharomyces cerevisiae* yeast, which is involved in alcohol fermentation processes. Subsequently, although with varying success, the encapsulation of live cells into the sol-gel matrix was expanded to include prokaryotes (bacteria (Nassif et al. 2002) (Muller et al. 2008), cyanobacteria (Dickson and Ely 2013)) and eukaryotes (yeast (Yang et al. 2011)), algae (Perullini et al. 2014), animals (Desimone et al. 2010) and plant cells (Meunier et al. 2010b)). In a number of recently published reviews, the use of sol-gel technology for encapsulation and the immobilization of live cells are described (Dickson and Ely 2013) (Avnir et al. 2006) (Dickson and Ely 2013) (Depagne et al. 2011). Notwithstanding the observed advantages of silica and silicon materials as encapsulation agents (i.e. chemical inertness, good thermal and mechanical stability, the ability to maintain cell viability for an extended period of time, transparency, which enables the registration of biological activity of cells by optical means, controlled porosity and the effective retention of the cells allowing for analysis in a flow) the processes used for the fabrication of biosensitive hybrid sol-gel biomatrices require further development (Depagne et al. 2011).

The aim of this work was to optimize the process by which whole yeast cells can be immobilized into silica-organic matrices, whilst preserving all of the advantages associated with the silicon-based architecture, but exhibit flexibility, thus lowering the solid medium pressure on the encapsulated microorganisms. Furthermore, the aim was to develop an encapsulation medium that is porous so that effective diffusion of materials requiring detection by the biosensor could occur. Finally, the aim of this work was to evaluate the applicability of this

approach for the design of novel biosensing systems. We selected the methylotrophic *Pichia angusta* VKM Y- 2559 and the oleaginous *Cryptococcus curvatus* VKM Y- 3288 yeasts as being suitable candidates for immobilization in the sol-gels as the activity of these yeast cells should not be affected by the presence of alcohol. In fact, the presence of alcohol increases the stability of the bioreceptor. Methylotrophic organisms, along with *Pichi pastoris, Pichia methanolica, Hansenula polymorpha* (or *Pichia angusta)* and *Candida boidinii,* are also frequently used in genetic engineering applications as hosts for recombinant proteins and peptides (Lang and Philp 1998) (Haritash and Kaushik 2009), which are known for their metabolic regulation, including the presence of strong promoters that are induced by the presence of methanol ((Gonchar et al. 1998) (Zaitsev et al. 2012), (Dmytruk et al. 2011) (Arlyapov et al. 2012).

2. Materials and Methods

2.1. Strains and growth conditions

The methylotrophic yeast *Pichia angusta* VKM Y-2559 and the oleaginous yeast *Cryptococcus curvatus* VKM Y-3288 were received from the National Collection of the Institute of Biochemstry and Physiology of Microorganisms (Pushchino, Russia). Growth conditions are in Supplementary (Supplementary Data 1).

2.2. Immobilization of cells using a fiber glass filter

Drop (0.003 cm^3) of biomass suspension (biomass content of 150 mg/cm^3 or $1.3 \pm 0.1 \times 10^9$ CFU/cm^3) was applied onto 3×3 mm section of porous fiber glass filter (Whatman GF/A, Sigma) and air dried for 15 minutes at 20°C.

2.3. Encapsulation of cells by sol-gel method

The silane precursors used were tetraethoxysilane (TEOS, Sigma-Aldrich) and methyltriethoxysilane (MTES, Sigma-Aldrich). A mixture of silane precursors, with a hydrophobic MTES additive content ranging from 0 to 100% (vol) with respect to the total amount of the silane precursor, was used. The porogen (Ferak Berlin), polyethylene glycol 3000 (PEG), and the catalyst, NaF, pH 7.6, were used.

A suspension of yeast cells of volume 0.25 cm^3 ($1.3 \pm 0.1 \times 10^9$ CFU/cm^3) in phosphate buffered saline (PBS) (20 $mmol/dm^3$ pH 7.6) was added to 0.1 cm^3 of a 20% solution of PEG in phosphate buffered saline and stirred for 3 minutes (Elmi CM-70M07). A mixture of tetraethoxysilane (TEOS) and 0.5 cm^3 of methyltriethoxysilane (MTES) was then added and

mixed for 3 minutes. A mixture of silane precursors with a hydrophobic MTES additive content of 85% vol with respect to the total amount of the silane precursor was used. 0.025 cm^3 of the catalyst solution containing 0.2 mol/dm^3 NaF was then added and stirred for 15 min. A volume of 0.005 cm^3 was then withdrawn, spread on a porous glass filter (Whatman GF/A, Sigma) and dried for 15 min at 20°C.

2.4. Electrochemical measurements

A 3×3 mm^2 section of the glass fiber filter containing immobilized cells was then washed prior to use with a phosphate buffer solution for 5 minutes, then placed on a surface of a Clark oxygen electrode and fixed with a nylon mesh. A Clark-type oxygen electrode (Kronas, Russia) was composed of a Pt working electrode (5 mm diameter) and an Ag/AgCl reference/counter electrode. The sensor response was calculated as a maximum rate of current variation in time. All measurements were made in a sodium-potassium phosphate buffer medium (pH 7.6).

2.5. The effect of heavy metal ions and UV exposure on the respiratory activity of immobilized microorganisms

Before introducing an analyte solution into a measuring dish, salts of heavy metals were added to the working solution as described in Table S1 (Supplementary Data). The biosensor response was measured as described in subsection 2.4.

Microorganisms encapsulated into the sol-gel matrix according to subsection 2.3 were exposed to UV radiation at a wavelength of 254 nm and an irradiation intensity of 680 $\mu W/cm^2$ for 5 h. After irradiation respiratory activity of immobilized microorganisms was measured as described in subsection 2.4.

2.6. Microscopy

To monitor the process of encapsulation of yeast cells into silica sol-gel matrix, an optical light microscope Nikon Eclipse Ci (Nikon, Japan) equipped with a camera ProgRes SpeedXT core 5 (Jenoptik, Germany) was used. The observation was carried out in the phase contrast mode.

Samples of the yeast cells and *C. curvatus* and *P. angusta* to be analysed using scanning electron microscopy (SEM) were fixed in 1.5 % glutaraldehyde solution in 0.05 M cacodylate buffer pH 7.2 for 1 hour at 4°C and then post-fixed in a 2% solution of osmium tetraoxide in the same buffer for 4 hours at 20°C. They were further dried using a freeze drying device JFD- 320 (JEOL, Japan) according to the manufacturer's instructions. The samples of cells and the silica

sol-gel matrix were then applied to the surface of the metal plate used for analysis and coated with a platinum-carbon mixture in a vacuum sputtering equipment JEE-4X (JEOL, Japan). The scanning electron microscopic analysis was then performed using a JSM-6510 LV (JEOL, Japan) microscope.

2.7. GC analysis

The ethanol content of the samples was measured using a Crystal 5000.2 gas chromatograph (Chromatec, Russia) equipped with a flame ionization detector and a capillary column DB-FFAP (50 m × 0,32 m × 0,50 mm) (Agilent, USA). The analysis conditions were as follows: thermostat oven temperature 70° C, evaporator temperature 200° C, detector temperature 250° C and helium carrier gas flow rate of 0.10 dm^3/hr.

3. Results and Discussion

3.1. The dynamics of encapsulating yeast cells under basic catalysis conditions

Silane precursors were added to a suspension of microorganisms in an aqueous solution of PEG at a pH of 7.6 in the presence of NaF, then hydrolyzed and condensed to form a sol-gel polymer structure. The real time formation of a capsule around each yeast cell was observed using optical microscopy. Spherical particles of 1 to 5 microns in diameter could be clearly observed within the structure of the silica sol-gel matrix(Supplementary, Fig. S1). Since the cells of methylotrophic *P. angusta* VKM Y-2559 yeasts are spherical in shape and 2-3 μm in diameter (Supplementary, Fig. S2), interpretation of the nature of the sol-gel matrix formation may be obscure. Therefore, the cells of the yeast *C. curvatus* VKM Y-3288 were also used, since these cells are larger and rod-shaped (Supplementary, Fig. S3).

After a period of 1 hour had elapsed, single spherical particles of about 0.3 μm in diameter were observed to appear on the surface of the cells, forming a sol (Fig. S3). After 3 hours had elapsed, the number of the sol-gel particles was seen to have increased, forming a single-layer chains around each individual cell (Fig. S3, 3h). After 5 hours, all yeast cells were seen to be encapsulated (Fig. S3, 5h) (Supplementary, movie). The silica biomatrix formed around the cells retained its structure for the entire 24 h period of observation (Fig. S3, 24h). It appeared that the microorganisms had become the nucleation centres of the self-organized bio-silica-hybrid capsules. Previously, it was shown that under certain conditions, the formation of a silica capsule around microorganisms was possible (Nassif et al. 2002). This protects each cell but does not prevent the rapid diffusion of small molecules through the pores of the capsule

(Dickson et al . 2012). The dynamics of capsule formation is investigated for the first time in this study.

3.2. The effect of TEOS / MTES ratio of the silane precursors on the architecture of the bio-silica-hybrid capsule

TEOS is the most commonly used silane precursor. In addition, an organically modified alkoxide of general formula $Et_nSi(OEt)_{4-n}$, where Et stands for ethyl (a hydrophobic additive) can also be used. The formation of the loose gels is due to an increased concentration of the hydrophobic additive, a factor that was taken into account during the immobilisation of the cells (Supplementary Data 2).

Of particular interest is the architecture of the bio-silica matrix that resulted from the formation of the capsule using an 85/15 silane precursor ratio (Fig. 1). In this case, a capsule is formed around each cell, with the encapsulated cells forming the common structure associated with a hybrid biomaterial. We suggest that PEG plays an important role in this process, where it is likely to interact with the wall of the yeast cells.

Figure 1

Formation of a similar 3D biomatrix structure to that obtained using the methylotrophic yeast *P. angusta* (Supplementary, Fig. S4) confirms that different yeasts species can adopt similar mechanisms for encapsulation. In both cases, each yeast cell acts as the centre of a self-assembled hybrid silica-organic capsule, with the architecture of the emerging structure appearing to depend only on the ratio of silane precursors used during the encapsulation process. Completion of the 3D hybrid architecture requires up to 5 hours.

3.3. The effect of the TEOS/MTES ratio of silane precursors on of the performance of the bioreceptor

The effect of the architecture of the biomatrix on the physiological activity of the yeast after immobilization and during storage was evaluated by registering the changes in respiration of the yeasts in the presence of the natural substrate, methanol. A biosensor was constructed, which was based on the Clark type oxygen electrode, in which the surface was modified using

yeast cells immobilized into a silica-organic matrix. The operating principle of such a microbial sensor was based on measuring oxidation of methanol by the immobilized microorganisms due to their respiratory activity, which results in the decrease of oxygen content within the electrode space, detected as a change in electric current over time (Fig. 2, inset).

Figure 2

The rate at which the methylotrophic yeast cells consume oxygen depends on the concentration of methanol present in the measuring cell. The change in current over time was taken as the biosensor response. The enzymatic activity of the encapsulated yeast cells was found to follow that predicted by the Michaelis-Menten law (Fig. 2).

The Chemistry Analytical Division and The Physical and Biophysical Chemistry Division of the International Union of Pure and Applied Chemistry (IUPAC) has adopted principles and definitions related to biosensor responses that determine the effectiveness of their operation (Thevenot et al. 2001). According to these guidelines, sensitivity, working or linear concentration ranges, detection limit and quantitative determination limit, are regarded as biosensor calibration characteristics.

The sensitivity of these biosensors to the presence of methanol was calculated as the slope of the regression line. The limit of methanol detection using microbial biosensors was determined by the formula: $C_{min\ P} = 3S_0/S$, where S is the sensitivity factor and S_0 is the standard deviation of the analytical background signal (Table 1). The quantitative determination limit was determined according to the statistical criteria: the relative standard deviation of concentration being equal to 0.33 (Table 1).

Table 1.

Notably, a biosensing element fabricated using a silane precursor ratio of 15/85 was also found to demonstrate the optimal analytical characteristics (Table 1, Fig. 3).

Figure 3

Biosensors based on methylotrophic yeast cells encapsulated in bimodal silica sol-gel matrix containing different amounts hydrophobic additives are characterized by exhibiting similar values of relative standard deviation (Sr within 1%) (Table 1).

Depending upon the ratio of silane precursors during biosensor operation, the newly fabricated biosensing system described here was found to be functional between 8 and 26 days (Table 1), indicating an excellent metabolic activity and the viability of yeast cells encapsulated in silica matrices. It should be noted that cells coated with a silica shell are not only firmly held at the surface of the electrode, but are also protected from any microbial contamination that may be present in the operating environment.

3.4. Benefits of sol-gel encapsulation to protect cells from harmful effects

3.4.1. Protection against the toxic effect of heavy metal ions

The ability for the silica capsule to effectively protect the immobilized yeast cells was evaluated according to the ability of the capsule to reduce the toxic effects of heavy metal ions present towards the yeast cells (Fig. 4).

Figure 4

It can be seen from the data presented in Figure 4 that the biosensor response values are largely unaffected by the presence of all metal ions, with the exception of Cu^{2+} and Cd^{2+}, presumably because of the high toxicity of these metal ions to microorganisms. When exceeding the threshold limit value (TLV) by a factor of 100, it can be seen that the reduction in the biosensor response is no more than 20%, whilst the activity of yeast cells not protected by the silica capsule was seen to decrease by 90% (Fig. 4). Lin et al. and Hong et al. (Lin et al. 2006, Hong et al. 2013) reported similar results in their studies of microorganisms co-immobilized in an ORMOSILS matrix. These authors found that the biochemical oxygen demand (BOD) responses of these biosensors are practically independent of the presence of most metal ions, with the exception of Ag^{+}, Cd^{2+} and Pb^{2+}. Arlyapov (2013) investigated the effect of heavy metal ions on the response of a BOD biosensor based on the yeast *Debaryomyces hansenii* immobilized in a modified polyvinyl alcohol (PVA). When exceeding the TLV value by a factor of 100, it was found that the response reduction was less than 60%. Thus the results obtained in this study are in agreement with results reported previously, and clearly indicate the protective characteristics of the silica capsule described here.

3.4.2. Protection from UV irradiation

UV radiation is often used in microbiology, biotechnology, and in medical sterilization equipment. As a result, it is important to understand to what extent the silica capsules protect the yeast cells from exposure to irradiation. The 15/85 silane precursor encapsulated yeast cells were irradiated using short wave UV radiation (wavelength λ = 254 nm) for 5 hours, after which time they were assessed for the activity as biosensors as described previously. It is found that the analytical and metrological characteristics of these bioreceptors were virtually no different to those that had not been exposed to the UV radiation (Table 1). .

These results demonstrate the excellent protective function afforded to the yeast cells by the silica matrix under the experimental conditions used in this study. It is therefore suggested that UV irradiation could be applied for the periodic sterilization of a biosensor system based on encapsulated yeast cells.

3.4.3. Analysis of real samples

In order to test and calibrate the receptor element of the biosensor developed in this study, we measured the methanol content in process waste obtained from a chemical plant located in the Tula region of Russia. For reference, the methanol content was determined independently using gas chromatography (GC). The results obtained are shown in Table 2.

Table 2

A statistical analysis of the methanol concentration results obtained by both the GC and biosensor methods revealed that the two methods of determination are heterogeneous in their reproducibility, and were not significantly different. Unlike the chromatographic method, however, the biosensor systems allowed for the rapid analysis of methanol concentration without the need for extensive sample preparation. As a result, it can be seen that the used of biosensors based on methylotrophic yeast encapsulated in a silica matrix represents a promising tool for monitoring the concentration of methanol in effluent streams.

CONCLUSIONS

The results obtained by the use of sol-gel technology for the encapsulation of living cells extend the range of microbial immobilization approaches that allow forming a silica shell around each cell. Yeast cells became nucleation centers, initiating a dynamic assembly of silicate-organic capsules of less than 1 μm in diameter around the yeast cells, with complete cell coverage being achieved in 5 hours. These yeast cells clearly participated in the formation of a self-organized-hybrid 3D-architecture. This research contributes to the understanding of the interaction mechanisms that take place between living cells and silicates, and is of a particular significance for the development of hybrid bio silicate-organic materials, including whole-cell biosensors. It was shown that methylotrophic yeast cells of *P. angusta* BKM Y-2559 encapsulated in a bimodal silica matrix do not lose their activity in the presence of the alcohol formed as a result of sol-gel process. The cells function efficiently under conditions of stress (in the presence of heavy metals or exposure to UV radiation), which allows them to be applied in the development of amperometric biosensors for the determination of methanol in, for example, sewage systems. These biosensors also have the ability to remain active after being exposed to UV irradiation, which occurs during the periodic sterilization of bioanalytical systems.

ACKNOWLEDGEMENTS

The work was supported by Ministry of Education and Science of Russian Federation (State Task project code 1764) and the Federal Targeted Programme for Research and Development in Priority Areas of Development of the Russian Scientific and Technological Complex for 2014-2020 (project No. 14.574.21.0062).

References

Arlyapov, V., Kamanin, S., Ponamoreva, O., Reshetilov, A., 2012. Enzyme and Microbial Technology 50(4-5), 215-220.

Avnir, D., Coradin, T., Lev, O., Livage, J., 2006. Journal of Materials Chemistry 16(11), 1013-1030.

Blondeau, M., Coradin, T., 2012. Journal of Materials Chemistry 22(42), 22335.

Brinker, C.J., Scherer, G.W., 1990. Sol-Gel Science. The Physics and Chemistry of Sol-Gel Process. San Diego New York Boston London Sydney Tokyo Toronto.

Daniel, M., Sharpe, A., Driver, J., Knight, A.W., Keenan, P.O., Walmsley, R.M., Robinson, A., Zhang, T., Rawson, D., 2004. Journal of Environmental Monitoring : JEM 6(11), 855-865.

Depagne, C., Roux, C., Coradin, T., 2011. Analytical and Bioanalytical Chemistry 400(4), 965-976.

Desimone, M.F., Alvarez, G.S., Foglia, M.L., Diaz, L.E., 2009. Recent Patents on Biotechnology 3(1), 55-60.

Desimone, M.F., Hélary, C., Mosser, G., Giraud-Guille, M.-M., Livage, J., Coradin, T., 2010. Journal of Materials Chemistry 20(4), 666.

Dickson, D.J., Ely, R.L., 2013. Applied Microbiology and Biotechnology 97(5), 1809-1819.

Dmytruk, K.V., Smutok, O.V., Dmytruk, O.V., Schuhmann, W., Sibirny, A.A., 2011. BMC Biotechnology 11, 58.

Gill, I., Ballesteros, A., 2000. Bioencapsulation within synthetic polymers (Part 1): sol–gel encapsulated biologicals. Trends in Biotechnology 18(7), 282-296.

Gonchar, M.V., Maidan, M.M., Moroz, O.M., Woodward, J.R., Sibirny, A.A., 1998. Biosensors & Bioelectronics 13(9), 945-952.

Gupta, R., Kumar, A., 2008. Biotechnology Advances 26(6), 533-547.

Hamm, C.E., Merkel, R., Springer, O., Jurkojc, P., Maier, C., Prechtel, K., Smetacek, V., 2003. Nature 421(6925), 841-843.

Hansen, L.H., Sorensen, S.J., 2001. Microbial Ecology 42(4), 483-494.

Haritash, A.K., Kaushik, C.P., 2009. Journal of Hazardous Materials 169(1–3), 1-15.

Inama, L., Diré, S., Carturan, G., Cavazza, A., 1993. J Biotechnol. 30(2), 197-200.

Lang, S., Philp, J., 1998. Antonie van Leeuwenhoek 74(1-3), 59-70.

Meunier, C.F., Dandoy, P., Su, B.L., 2010a. Journal of Colloid and Interface Science 342(2), 211-224.

Meunier, C.F., Rooke, J.C., Leonard, A., Xie, H., Su, B.-L., 2010b. Chemical Communications 46(22), 3843-3859.

Michelini, E., Roda, A., 2012. Analytical and Bioanalytical Chemistry 402(5), 1785-1797.

Milea, C.A., Bogatu, C., Duta, A., 2011. Bulletin of the Transilvania University of Braşov. Series I: Engineering Sciences 4 (53)(1), 59-66.

Muller, W.E., Engel, S., Wang, X., Wolf, S.E., Tremel, W., Thakur, N.L., Krasko, A., Divekar, M., Schroder, H.C., 2008. Biomaterials 29(7), 771-779.

Nassif, N., Bouvet, O., Noelle Rager, M., Roux, C., Coradin, T., Livage, J., 2002. Nature Materials 1(1), 42-44.

Pasco, N.F., Weld, R.J., Hay, J.M., Gooneratne, R., 2011. Analytical and Bioanalytical Chemistry 400(4), 931-945.

Perullini, M., Ferro, Y., Durrieu, C., Jobbágy, M., Bilmes, S.A., 2014. Journal of Biotechnology 179(0), 65-70.

Ponomareva, O.N., Arlyapov, V.A., Alferov, V.A., Reshetilov, A.N., 2011. Applied Biochemistry and Microbiology 47(1), 1-11.

Rajan Premkumar, J., Rosen, R., Belkin, S., Lev, O., 2002. Analytica Chimica Acta 462(1), 11-23.

Rodriguez-Mozaz, S., Lopez de Alda, M.J., Barcelo, D., 2006. Analytical and Bioanalytical Chemistry 386(4), 1025-1041.

Su, L., Jia, W., Hou, C., Lei, Y., 2011. Biosensors & Bioelectronics 26(5), 1788-1799.

Trogl, J., Kuncova, G., Kuran, P., 2010. Folia Microbiologica 55(6), 569-575.

Wang, S., Guo, Z., 2014. Biointerfaces 113(1), 483-500.

Wen, G., Li, Z., Choi, M.M.F., 2013. Journal of Food Engineering 118(1), 56-61.

Xu, X., Ying, Y., 2011. Food Reviews International 27(3), 300-329.

Yang, S.H., Ko, E.H., Jung, Y.H., Choi, I.S., 2011. Angewandte Chemie International Edition 50(27), 6115-6118.

Zaitsev, M.G., Arlyapov, V.A., A., A.V., Reshetilov, A.N., 2012. Applied Biochemistry and Microbiology 48(5), 570-576.

Highlights:

- Novel approach for methylotrophic yeasts sol-gel encapsulation for methanol biosensors design.
- The yeast cells are the nucleation centres for a dynamic assembly of sol-gel silicate-organic capsules
- The MTES ratio influence the yeast cells encapsulation and the 3-D structure of the sol-gel biomatrix.

Table Captions:

Table 1. Metrological and analytical characteristics of the biosensor based on the methylotrophic yeast *Pichia angusta* BKM Y-2559 immobilized in a silica matrix with different ratios of silane precursors (the measured substance is methanol).

MTES content, %	**Sensitivity, nA·dm^3·min^{-1}·mmol^{-1}**	**Quantitative determination limit, C_l, µmol/dm^3**	**Detection limit, µmol/dm^3**	**Reproducibility[a], Sr, %**	**Operational stability, days**
0	87±5	9	3	1.1	9
10	96±4	7	2.2	1.0	8
33	100±5	6	2	1.0	8
67	110±8	3	1.5	1.0	26
83	140±10	3	1	1.1	14
85	198±5	2	0.7	1.0	17
90	99±7	3	1	1.0	10
100	90±5	4	1.5	1.2	8

[a]Operational stability was calculated as the relative standard deviation for 15 replicate measurements at P = 0.95.

Table 2. Methanol content in the process effluent chemical plant

Wastewater sample	**Methanol concentration, determined by different methods, µmol/dm^3**	
	Using biosensor	Gas chromatography
1	58.0±3.0	55.6±0.2
2	62.0±4.0	58.7±0.7
3	55.0±2.0	53.4±0.5

Figure Captions:

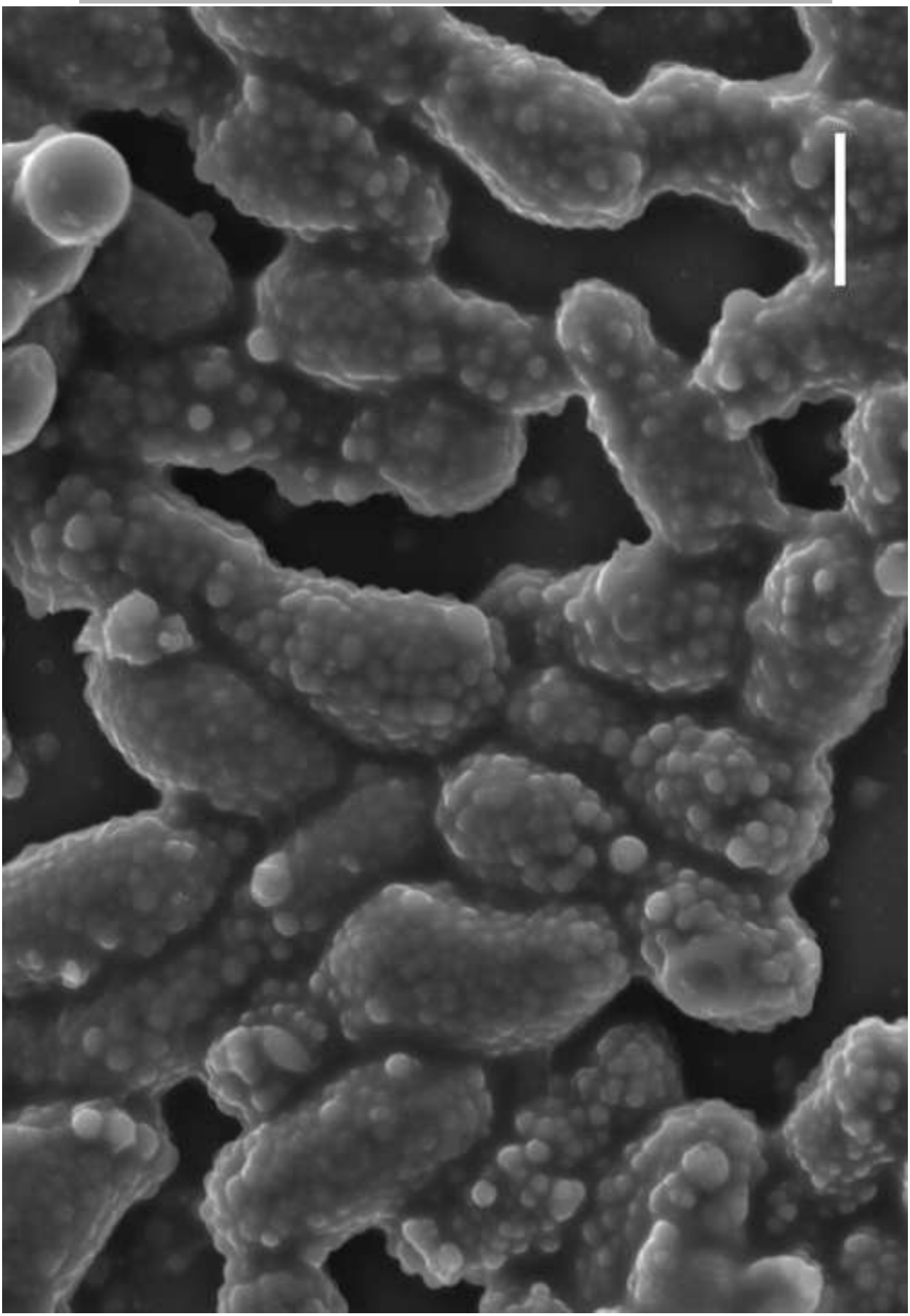
Fig.1. *Cryptococcus curvatus* VKM Y-3288 encapsulation in silica sol-gel. SEM micrographs showing formation of 3D sol-gel biomatrix architecture when the ratio between the silane precursors (TEOS and MTES) (% vol) was 85:15. Scale bar 5 µm.

Fig. 2. The calibration curve of biosensor response on methanol concentration for biosensor based on sol-gel encapsulated methylotrophic yeast *Pichia angusta* VKM Y-2559 (containing MTES 85%). Inset: The biosensor response on methanol.

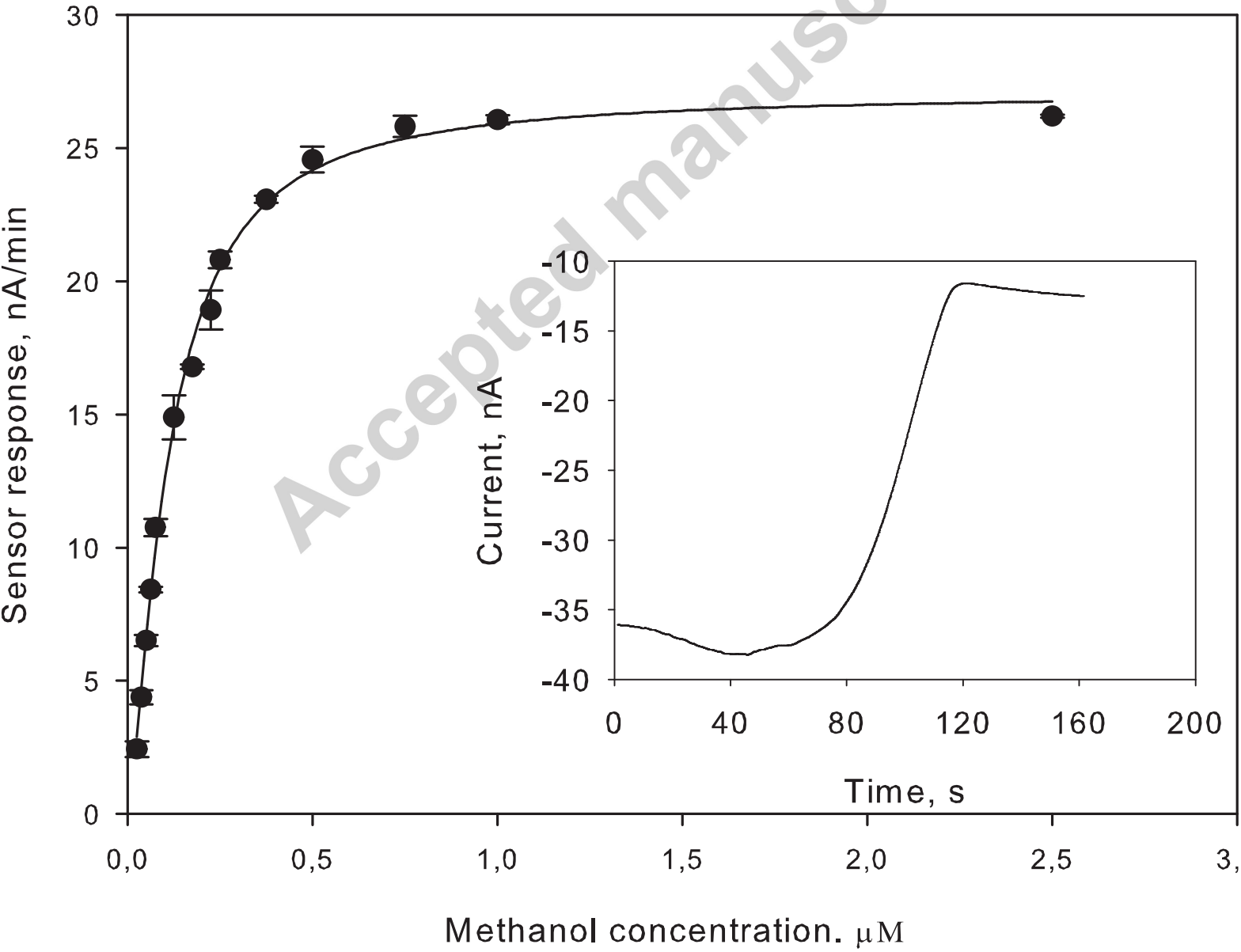
Fig. 3. The sensitivity and detection limit functions on hydrophobic additive content in the sol-gel matrix of bioreceptor.

Fig. 4. The effect of heavy metals on the oxidation activity of yeast cells; a – encapsulated in a sol-gel matrix, b – immobilized by adsorption on glass fiber filters.

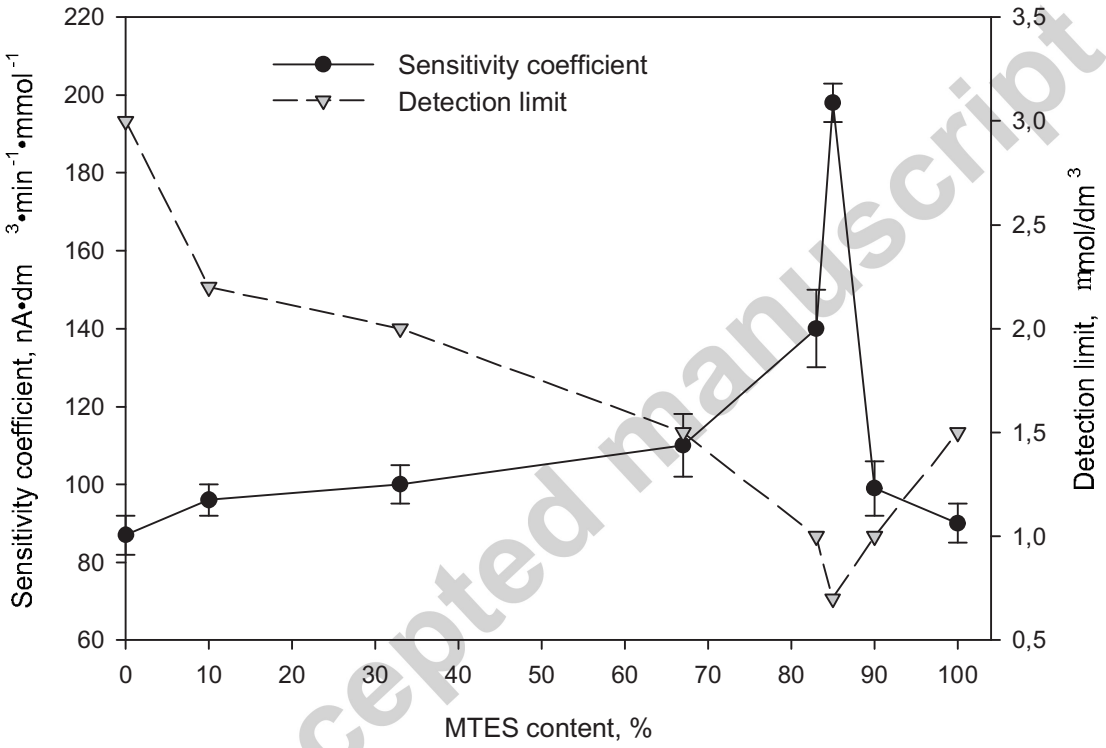
* TLV values are used in accordance with GN- spell out 2.1.5.1315-03 (Russia, Supplementary Data, Table S1.)



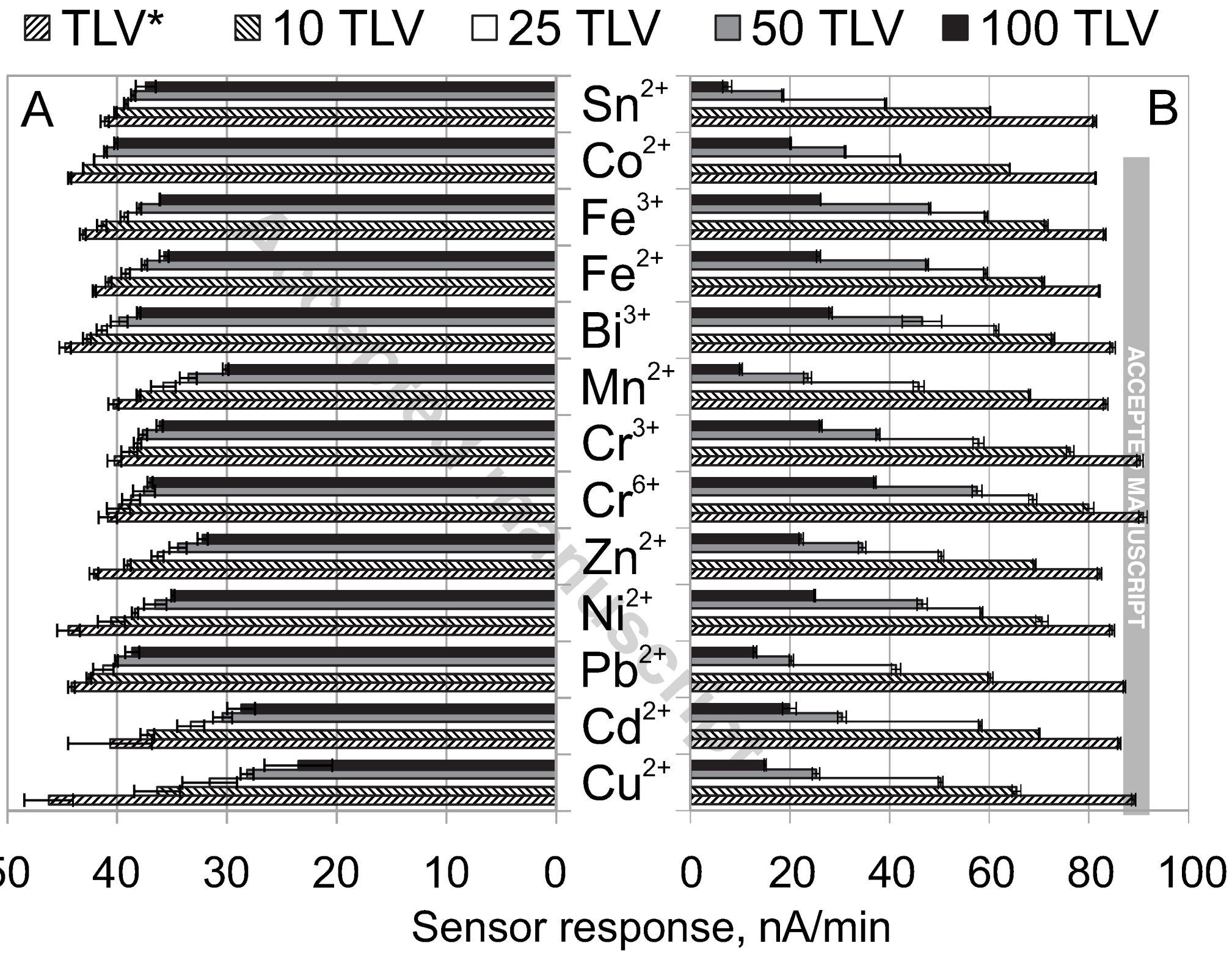
Figure(s)



Figure(s)



Figure(s)


TLV*
10 TLV
25 TLV
50 TLV
100 TLV
A
B
Sn2+
Co2+
Fe3+
Fe2+
Bi3+
Mn2+
Cr3+
Cr6+
Zn2+
Ni2+
Pb2+
Cd2+
Cu2+
50 40 30 20 10 0
0 20 40 60 80 100
Sensor response, nA/min
ACCEPTED MANUSCRIPT

Figure(s)