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THE APPLICATION OF SCANNING ELECTROCHEMICAL MICROSCOPY TO EVELUATE ALCOHOL BIOSENSOR BASED ON YEAST CELLS

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Baker's yeast (*Saccharomyces cerevisiae*) is one of the simplest single-cell fungal organisms. *S. cerevisiae* is cheap, widely used in the industry and in the household. Moreover, it is used as a cell model system by researchers because of its similarities to plant and eucaryotic cell catalytic pathways [1]. In order to develop accessible and low-cost tools for monitoring environmental issues a biosensor with living *S. cerevisiae* cells can be used [2]. In the current paper the toxicological effects of alcohols on living yeast cells are investigated by electrochemical methods. Alcohols are used in everyday life as a base in pharmaceutical tinctures and for cleaning products or sanitizers. For this study ethanol and isopropanol as extensively used alcohols were chosen for evaluating the influence to the viability of *S. cerevisiae* cells. Scanning electrochemical microscopy (SECM) is a device, which measures electrochemical characteristics locally [3]. It was used in this research due to its possibility to work in the microscale and quickly observe steady-state current. SECM consisted of a three-electrode system where the ultramicroelectrode (UME) is connected to a positioner that can move in three axes. The electrochemical signal is measured by scanning the surface of the sample. In previous our research, the toxicity of 9,10-phenantrenequinone was investigated [4].

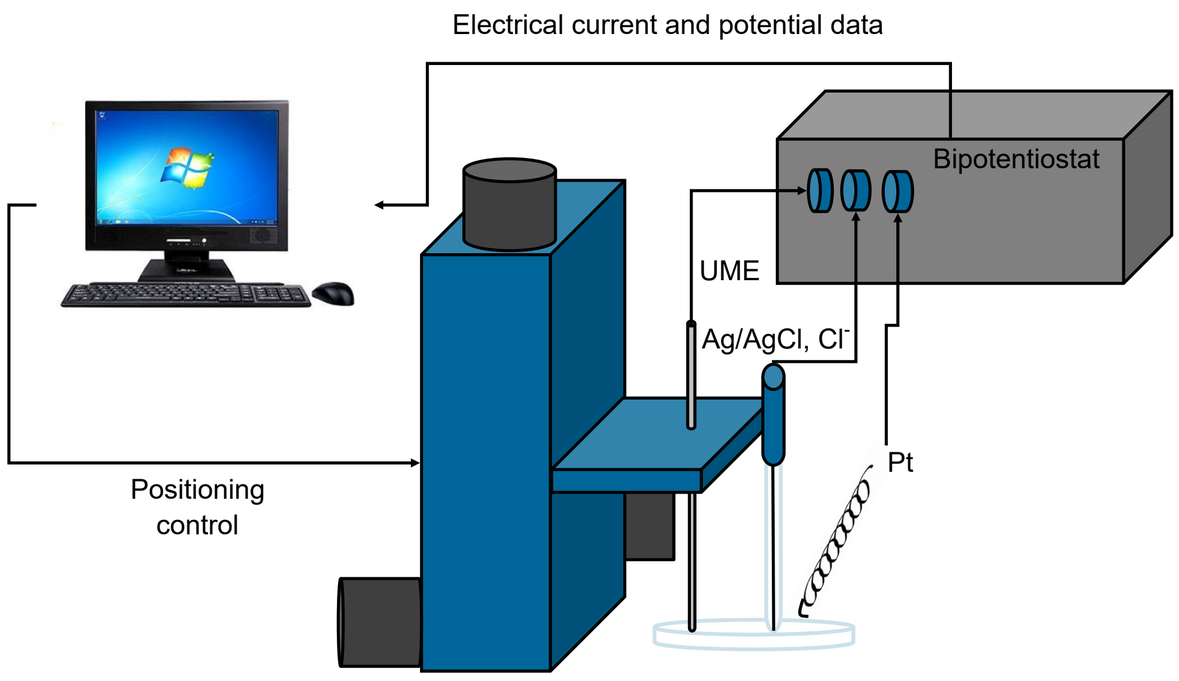

Fig.1 Scanning electrochemical microscopy scheme.

It was found that toxic materials can be used for the development of biosensors if they are applied in low concentrations. Good results were obtained by using toxic materials in biofuel cells, especially if they are immobilized on the electrode rather than adding them to the solution. In the current research, the yeast were used for the detection of ethanol and isopropanol in electrochemical cell. It was found that SECM can be used for the detection of ethanol/ isopropanol concentrations up to 4 mM.

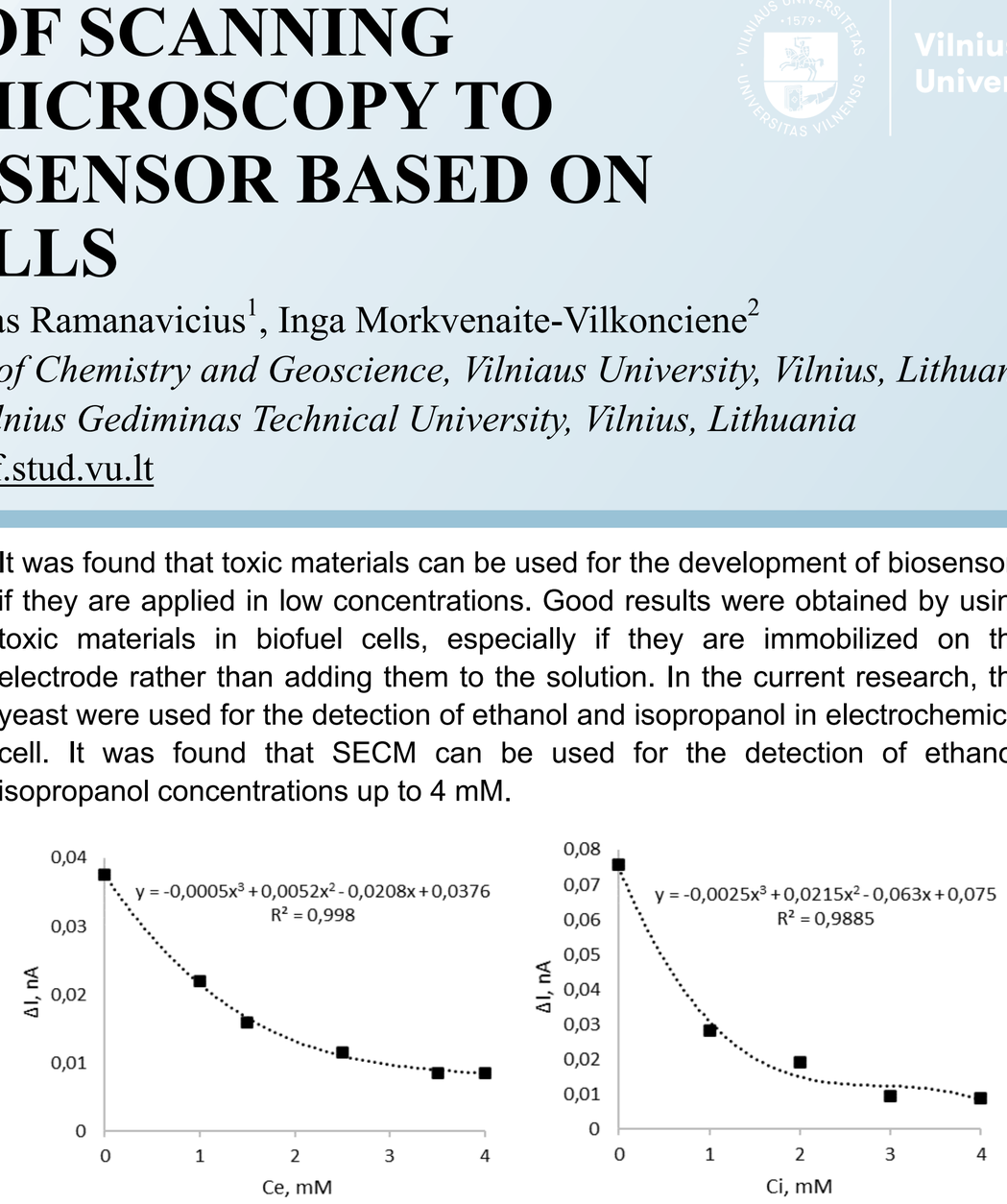

Fig. 2 Dependence of current on concentrations of Ethanol (Ce) and Izopropanol (Ci) from horizontal scans with Scanning electrochemical microscopy.

From the obtained horizontal scans data we can state that the results in both cases are similar, the detection limit in both cases is up to about 4 mM alcohol concentration.

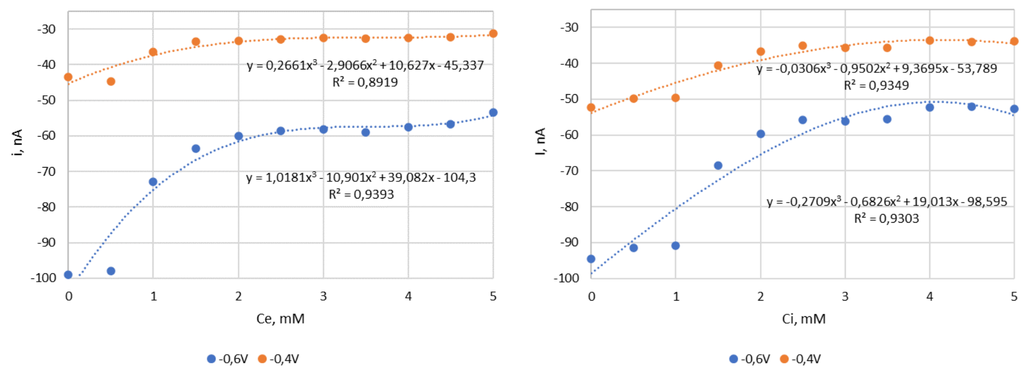

Fig. 3 Graphs of current dependence on Ethanol (Ce) and Izopropanol (Ci) concentration at -0.4 and -0.6V potentials from cyclic voltammetry data.

The obtained plots of current versus alcohol concentration showed that the yeast responded to slight differences in alcohol concentration in solution. It responds mostly up to ~2 mM, and later current changes are less noticeable.

Atomic force microscopy (AFM) allows the imaging of high-resolution surface topography. Similar to SECM, the atomic force microscope is based on the principle of operation of probe microscopy. AFM measures the force between the probe and the sample.

Atomic force microscope data was obtained at 0 and 4 mM ethanol concentrations (Fig. 4). The study was performed to see if morphological changes were observed at the highest concentration of the developed biological sensor, the yeast cells were still alive.

From the AFM data obtained, we found that small amounts of ethanol concentration (up to 4 mM) did not affect the topography of the yeast and morphological changes were not observed.

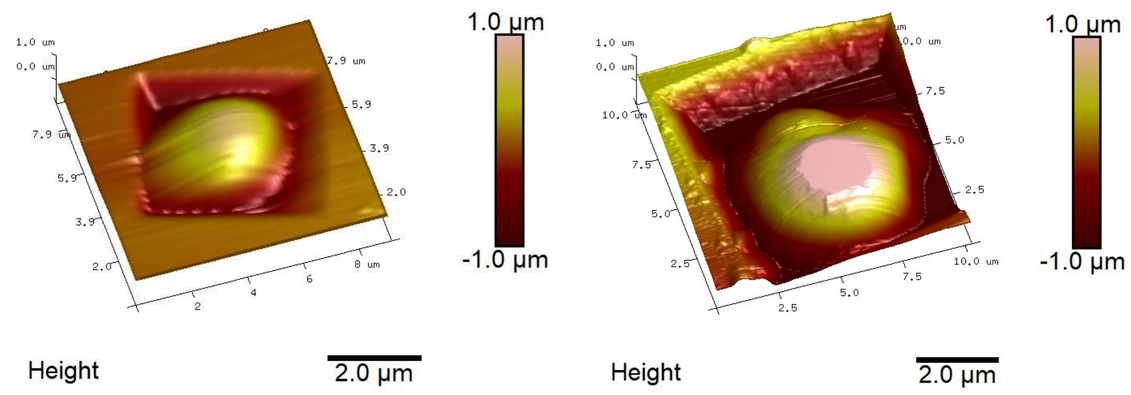

Fig.4 AFM yeast cell scan data. On the left is a control cell scan. On the right cell which was treated with 4 mM ethanol. Conditions: NP-D needles, scanning freqency 0.45 Hz.

References

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Conclusions

1) The results recorded by the SECM method in the presence of ethanol and isopropanol are similar, the detection limits of both alcohols are ~4 mM.
2) Using the cyclic voltammetry method, it was found that this method can detect small changes in alcohol concentration up to 4 mM.
3) Atomic force microscopy scans shows that 4 mM of ethanol did not affect the topography of the yeast or morphological changes.

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