Application of encapsulation into UV curable ORMOCER® in a construction of optical sensors.

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1. Introduction

Biotechnological processes require affordable and rapid on-line sensing techniques. Current sensors use amperometry with an oxygen electrode or hydrogen peroxide electrode, and their usefulness is limited by sensitivity to electromagnetic stirring and oxygen consumption. In addition, electroactive species present in the complex measurement medium may cause interference. Enzyme based fiber optic sensors suitable for in-situ, continuous monitoring of reactants under harsh reaction conditions were developed during European Commission funded project MATINOES .

The Sensor is based on the measurement of oxygen consumption due to oxidation of glucose catalyzed by enzyme glucose oxidase. Ruthenium complex serves as an optical transducer.
Its fluorescence is quenched proportionally with oxygen concentration

The encapsulation into UV curable polymer ORMOCER® was used for fabrication of a sensitive layer of an optical sensor of oxygen and glucose. The enzyme glucose oxidase was protected against harsh immobilization conditions by pre-immobilization on porous carrier.

The sensors, developed within the project MATINOES, were tested in a laboratory bioreactor during yeast fermentation and biodegradation of methyl-*t*-butylether. The tests proved ORMOCER®s durability and stability of the sensor in one batch. A course of bioreactor test can be seen on www.icpf.cas.cz/bio/matinoes.
This paper describes the results of tests with SAFIBRA multichannel instrument and stability of sensitive layers during cultivation in a bioreactor.

Material and methods

Preparation of sensitive films on lenses:
The sensitive films consisted of a mixture of ORMOCER® with Ru-tris(4,7-diphenyl-1,10-phenanthroline)$^{2+}$ complex and glucose oxidase pre-immobilized on a porous support. The mixture was dropped on acrylate or quartz lens and cured under UV lamp. A coated lens – sensitive element – was mounted on the tip of a probe. The probe used in 5L bioreactor Applicon is on Fig. 1.

Multichannel SAFIBRA SD-5:

Optical fibers were used to monitor 6 detection sites. Each sensor consisted of ORMOCER® fluorescent layer interrogated by an optical fiber "Y" coupler carrying the excitation and fluorescent light. Pulsed light from a blue LED was coupled into the fiber bundle. Fluorescent light was directed to a photomultiplier and photons were counted using a discrete preamplifier feeding pulses into a gated counter synchronized to the excitation by an embedded microcontroller. The system was multiplexed by interrogating separate sensors using an array of LEDs, excited sequentially. Light from all the sensors was detected by the same photomultiplier.
The fluorescence lifetime was determined by the measurement of light intensities at four points after the cessation of the excitation.

The tests of sensitive layers:
The basic test for evaluation of coating performance was a response to aerobic-anaerobic (AA) transition and a response to increasing concentration from 0 to 1 mmol glucose/L (GL). The values compared were relative changes of fluorescence lifetimes during aerobic-anaerobic transition and the change of concentration of glucose from 0 to 1 mmol/L. An example of the test in a bioreactor is on Fig. 2. The basic test was used for evaluation of influences of changes in coating composition fabrication and environments of the measurement on sensitivity and stability of layers. We tracked the influences of temperature, pH, film fabrication parameters, composition of ORMOCER®, storage of sensitive elements, sterilization in autoclave and conditions in the bioreactor.
Off-line control of glucose concentration was done with enzymatic kit Sigma P7119.

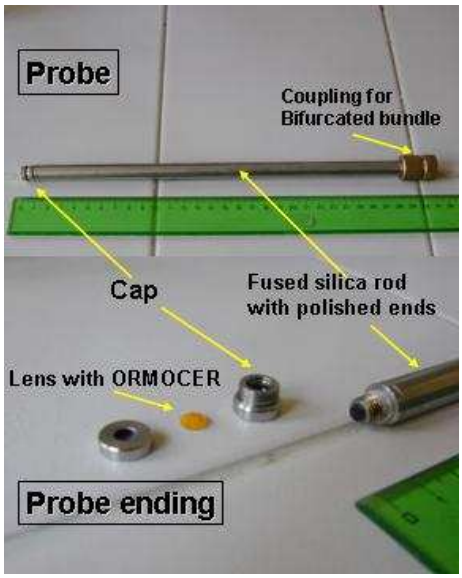


Fig. 1. MATINOES sensor probe.

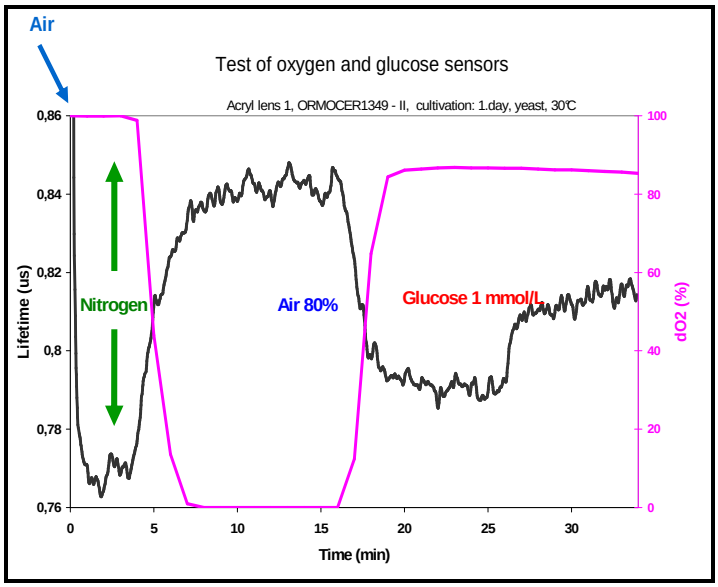


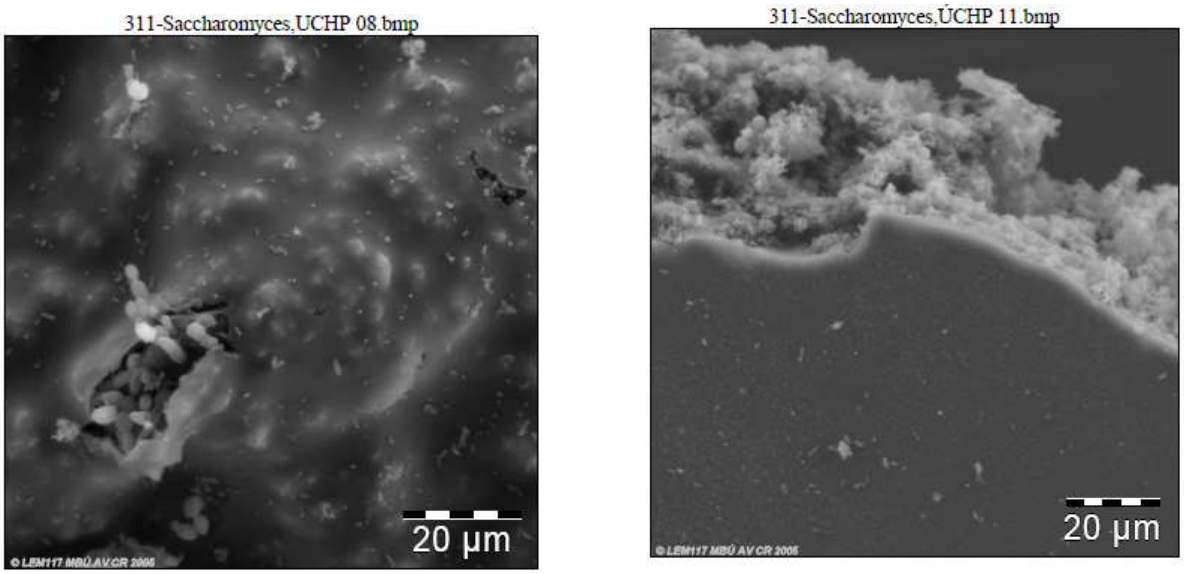
Fig 2. Test of oxygen and glucose sensors.

Results and Discussion
MATINOES glucose sensors in a bioreactor.
The range of applications and stability of sensors is dependent on electrical and optical part on mechanical construction and materials. In case of application in harsh conditions the most critical component is a sensitive film. The parameters of MATINOES sensors tested in a laboratory bioreactor are in Table 1. The concentration range of a sensor was enlarged to 20 mmol/L by changing of a complex and components ratio in a sensitive film.
After sterilization by isopropanol, the glucose sensors were tested during 6 days' yeast cultivations. Each day, a response to AA transition and GL test were conducted, and the samples for off-line

determination of glucose concentrations were taken. The parameters of MATINOES sensor did not change during one cultivation batch. SEM photos (Fig. 5) show that the sensitive layer looks like a sandwich, with Sepabeads with GOX being caught between two ORMOCER® sheets. The ORMOCER® parts were not contaminated. Yeast cells started to grow in the ORMOCER®'s ruptures and proliferate inside the layer on the Sepabeads' surface.

Table 1: Parameters of MATINOES sensors.

	Oxygen sensor	**Glucose sensor**
concentration range	0–100 % DO	0-3 mmol/L
sensitivity	2%	0.2 mmol/L (at 80 -100%DO)
stability in the bioreactor	> 2 weeks, pH independent	6 days
storage stability	> month	> 2 weeks
sterilization	autoclavable	ethanol, isopropanol, UV
response time (one channel SOMS)	~20 s	~ 20 s



a) b)

Fig. 3. SEM of a glucose sensor (Acrylate lens 1349II+Ru complex+GOX seeding Sepabeads) after 6 days in the bioreactor (yeast cultivation).
a) surface of a sensitive layer in the bioreactor (cell growth in the ORMOCER® rupture)
b) a sensitive layer on the side of acrylate lens

Off-line determination of glucose concentration with multichannel SAFIBRA SD-5

The tests confirmed that individual channels of SAFIBRA SD-5 are identical. The calibration was simultaneously done with four channels (Fig 4) shows differences within experimental error.
Multichannel device was used for determination of glucose concentrations in ciders, mashes and beers. In samples with glucose content 0.5-2 mmol/L, glucose concentrations determined with SD-5 differed ≤ 1% from values found with enzymatic kit Sigma P7119. In presence of compounds lowering oxygen solubility, sensor responses to glucose concentrations were decreased. Fig 5

presents shift in calibration, due to fructose (0.5 g/L) in a solution. The same lifetime drop was observed with raffinose, maltose and sucrose.

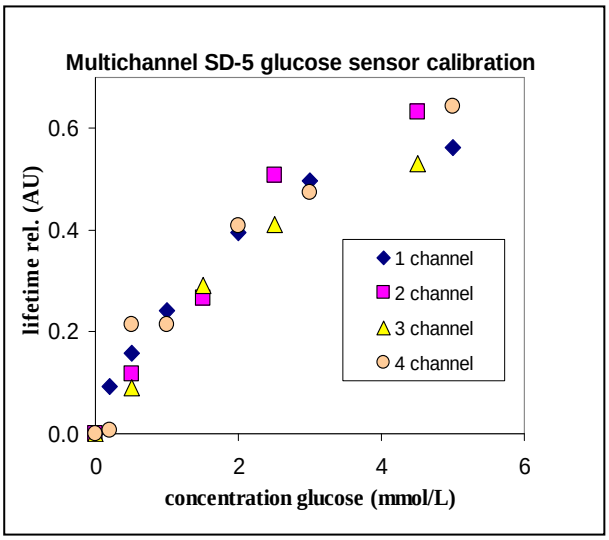


Fig. 4: Calibration of multichannel sensor

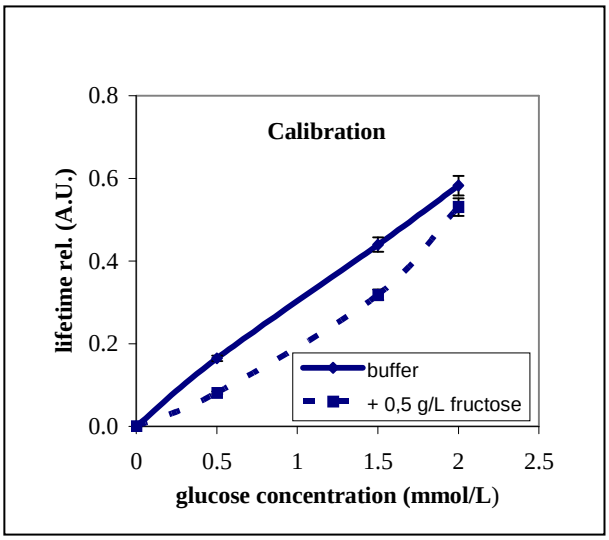


Fig. 5: The influence of fructose on sensor calibration

Conclusions

Encapsulation of pre-immobilized enzyme in UV cured ORMOCER® with an oxygen sensitive complex is a novel technique of a formation of sensitive elements of optical sensors. The advantages of the films are operational, mechanical, chemical stability and resistance to biofouling. The technique is not restricted to glucose oxidase. The ruggedness of a sensor is limited only by stability of pre-immobilized enzyme.

References

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Acknowledgements

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