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Ratiometric optical sensing of temperature and oxygen based on luminescence quenching

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Abstract

In cultivation processes monitoring of different parameters is indispensable to be able to analyze and control the process. A simply applicable, non-invasive method for on-line monitoring is optical measuring.
One measuring principle used for optical sensing is based on luminescence quenching by the analyte. Therefore a luminescent dye is excited by light of a certain wavelength and the emitted light is detected by a photomultiplier. Either lifetime or intensity of the luminescence can be used to collect the analyte concentration. The dye is attached to the inside wall of the reactor. Excitation can be effected through a transparent reactor wall. Since the risk of infection is minimized thereby, optical sensors are excellently capable for biotechnological applications.
A problem of such sensors is, that the intensity of the luminescence decreases by photobleaching. To get a stable signal fast lifetime measurements are necessary. A new approach in optical sensing is the detection of an internal reference beside the measurement signal. The reference signal has to be independent on the analyte or has to show a different dependence than the measurement signal. The analyte concentration is collected by the ratio of the luminescence intensity of the two signals. Ratiometric sensing is independent on the absolute intensity of the luminescence. Due to this, the signal is not changed by photobleaching, no fast lifetime measurements are necessary. Also mechanical disturbances have less effect on the signal.

Indicator

The dye used in this work is a mixture of two Octaethyleporphyrine(OEP)-complexes, ZnOEP and PtOEP (Fig. 1). ZnOEP shows a luminescence signal at Ex = 410 nm/ Em =580 nm, PtOEP at Ex =500 nm/ Em = 650 nm. The luminescence of PtOEP shows higher dependency on both, oxygen and temperature (Fig. 2).

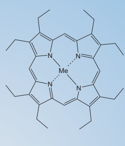

Fig. 1: Metal-OEP

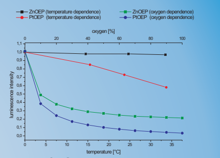

Fig. 2: Oxygen and temperature depence of ZnOEP and PtOEP

Immobilization

1. Oxygen sensing
To enable the detection of oxygen, the dye has to be immobilized in an oxygen-permeable matrix. Therefore, the dye and polysulfone are solved in chloroform. To enhance porosity and to get short response times, ethanol is added and the solution is given onto a porous polypropylene/polyethylene membrane

2. Temperature sensing
To exclude quenching effects caused by oxygen, it is necessary to immobilize the dye in an oxygen-impermeable matrix. Therefore the dye is solved in chloroform. The solution is added to an two-component-epoxy resin. When the resin is cured, the sensor-patch can be used.

It is not possible to exclude the temperature effect from the oxygen-measurements, but since the temperature is measured simultaniously, it is possible to measure oxygen at varying temperature without new calibration.

Optical set-up

To accomplish ratiomeric measurements with a single detector, it is taken advantage of the fact, that the two luminescence signals are excited at different wavelengths. Via alternating excitation at those wavelengths the signals can be temporally seperated.
As shown in figure 3, two LEDs (blue and green) were used as light sources, whose emission was filtered by interference filters of 10 nm half-power bandwith. The excitation light is reflected by dichroic mirrors. The emission light passes the dichroic mirrors and is detected by a photomultiplier (PMT), in front of the PMT another filter is arranged to detain scattered or refelected excitation light.

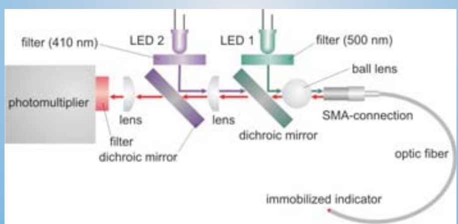

Fig.3: Optical set-up of the sensor

Oxygen sensing

The oxygen-dependence of the two luminescence signals of dyes with different ratios of PtOEP to ZnOEP was tested. The measurements were carried out in the gas phase. The results for the measurements with a dye containing 10 % ZnOEP are shown in figure 4. The measurement signal (blue curve) decreases with increasing oxygen amount. The reference signal (green curve) decreases with increasing oxygen amount at low oxygen concentrations (up to 10 %) and stays constant at higher concentrations. The course is similar for other ratios of ZnOEP and PtOEP. The results differ concerning the oxygen sensitivity, which is represented by the change of the signal-ratio to change of oxygen. At low oxygen concentrations, dyes with high amount of ZnOEP are more sensitive, at high oxygen concentrations dyes with high amount of PtOEP are more sensitive. The dye with a ratio of PtOEP:ZnOEP= 7:3 shows a good sensitivity for all oxygen concentrations (Fig. 5).

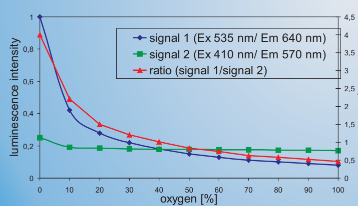

Fig. 4: Oxygen dependence of luminescence signals and Ratio, PtOEP:ZnOEP = 9:1

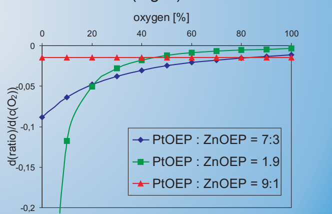

Fig. 5: Oxygen sensitivity of different mixtures of PtOEP and ZnOEP

Temperature sensing

Also the temperature-dependence of the two luminescence signals of dyes with different ratios of PtOEP to ZnOEP was tested in the gas-phase. The luminescence signals of the different dyes show similar dependences, examplarily the results for the measurements with a dye containing 10 % PtOEP are shown in figure 6. While the reference signal (blue curve) is nearly independent on changings of temperature, the measuring signal decreases with increasing temperature. In figure 7 the change of the signal-ratio to change of temperature is plotted against the temperature. For all tested dyes the sensitivity increases with increasing temperature. The best sensitivity is shown by the dye, that contains 60 % PtOEP.

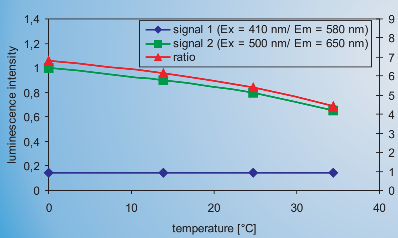

Fig. 6: Temperature dependence of luminescence signals and ratio, PtOEP:ZnOEP = 1:9

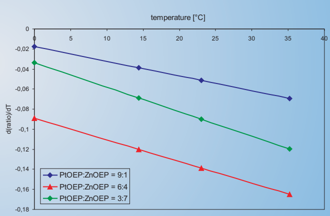

Fig. 7: Temperature sensitivity of different mixtures of PtOEP and ZnOEP

Summary

A ratiometric optical sensor was tested, different mixtrues of ZnOEP and PtOEP were used as indicator. Two temporally seperated luminescence signals were detected (Ex = 410 nm/ Em = 580 nm and Ex = 500 nm/ Em = 650 nm). Since reference and measurement signals of the indicator show different dependence on both, oxygen and temperature, ratiometric sensing of both is possible with the same optical system, only the immobilization of the indicator has to be changed. Due to that, temperature and oxygen can be measured simultaniously and oxygen measurements at varying temperatures are possible.
Variation of the composition of the dye results in change of sensitivity. For oxygen sensing, the dye containing 30% ZnOEP shows good sensitivity for all oxygen concentrations. For measurements at low oxygen concentrations, a higher amount of PtOEP results in higher sensitivity, while a higher amount of ZnOEP decreases the sensitivity high oxygen concentrations.
Temperature sensing is the more sensitive the higher the temperature is. The most sensitive dye is the dye with the ratio ZnOEP: PtOEP = 4:6.