Technical Note

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Mode of operation of optical sensors for dissolved oxygen and pH value

Introduction

Reasonable and smart bioprocess development begins at microliter scale. Medium- to high-throughput and comparatively low effort in time and money make the use of microtiter plates very attractive. However, the major objective is how to gain significant process data for biomass characterization and scale-up. Relevant process parameters have to be accessed. m2p-labs offers the BioLector ® technology, which is tailored for this purpose. Biomass development is monitored online in 48 microbioreactors in parallel. Moreover, important bioprocess parameters are available in real-time and noninvasive. The online detection of pH value and dissolved oxygen is permitted *via* optical sensors (optodes).

Operating principle

Excitation light is beamed through the transparent bottom of the microtiter plate and reflected at the optodes immobilized at the well bottom. Depending on the environmental conditions the response of an optode is modified. The emitting signal is analyzed (Figure 1).

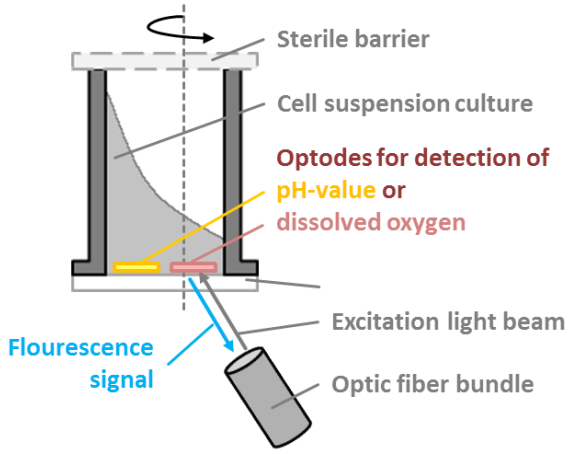

Figure 1: Operating principle of optodes.

Environmental response of optodes

Optodes consist of special dyes that respond to environmental conditions. Instead of the intensity of the dyes, the fluorescence or luminescence lifetime is analyzed. The lifetime is more robust

against extrinsic influences, like variance in distance, coat thickness or brightness of the excitation light beam. Nevertheless, some influence factors still have to be considered, e. g. temperature, special media components or fluorescent proteins. Measured bioprocess parameters are pH value (Figure 2) and dissolved oxygen (Figure 3).

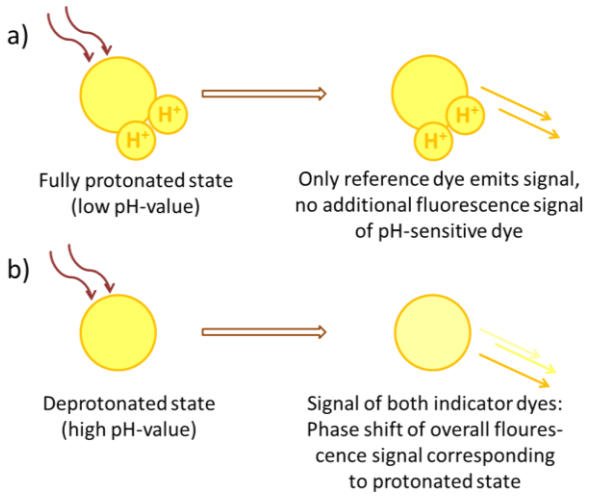

Figure 2: Principle of response of pH-sensitive dye.

The fluorescent pH dye consists of a hydronium sensitive dye and a reference dye. The reference dye is required to receive a stable and reliable signal. The entire fluorescence signal of the pH dye depends on its protonation degree: The higher the concentration of hydronium ions in solution (low pH value), the lower will be the fluorescence of the pH sensitive dye. The combination of reference and pH sensitive dye results in a measurable phase shift of the responding signal.

The oxygen sensitive dye is luminescent. Its fluorescence decay time is longer (µs range) than the fluorescence of pH-detection. Therefore, no second reference dye is needed here.

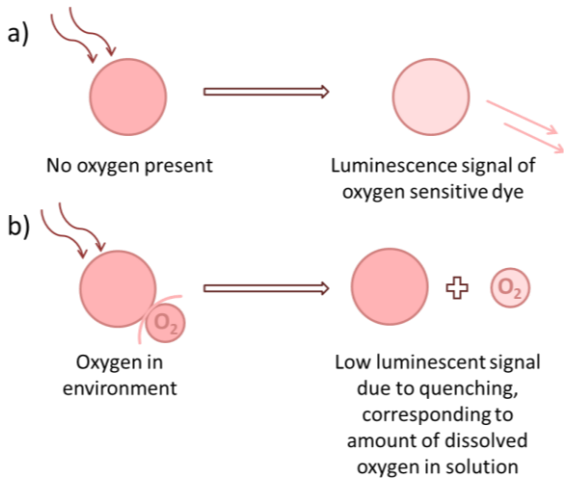

Figure 3: Principle of response of oxygen detecting

Oxygen in solution is detected through quenching. Energy absorbed by the dye is transferred to present oxygen. The phase shift of the resulting lifetime signal is analyzed and corresponds to the concentration of dissolved oxygen.

The measurable pH-range is about 5.00 to 8.50 with a resolution of up to 0.02 pH. Dissolved oxygen can be monitored from 0 to 100 % air saturation with a resolution of ± 5 % absolute. Definite values depend on the Lot. No. and calibration range.

Please visit the website for further information: www.m2p-labs.com, www.beckman.com

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HEADQUARTERS
m2p-labs GmbH
Arnold-Sommerfeld-Ring 2
52499 Baesweiler, Germany

Phone +49 - 2401 805 330
Fax +49 - 2401 805 333
info@m2p-labs.com

SUPPORT
EUROPE
Phone +49-2401-805-335
support@m2p-labs.com

N. & S. AMERICAS
Phone +1 631 501 1878
supportUS@m2p-labs.com

ASIA PACIFIC
Phone +852 9207 6841
supportAsia@m2p-labs.com