Scientific Paper:

Biotechnology and Bioengineering Vol. 90, No. 3, 271-280, 2005

Gas Sensing in Microplates with Optodes: Influence of Oxygen Exchange Between Sample, Air and Plate Material

Sarina Arain[1], Svenja Weiss[2], Elmar Heinzle[2], Gernot T. John[3]
Christian Krause[3], Ingo Klimant[4]

[1]Institute of Analytical Chemistry, Chemo- and Biosensors, University of Regensburg, Regensburg, Germany
[2]Department of Biochemical Engineering, Saarland University, Saarbrücken, Germany
[3]PreSens GmbH, Regensburg, Germany
[4]Institute of Analytical Chemistry, Micro- and Radiochemistry, University of Technology of Graz, Technikerstrasse 4, A-8010 Graz, Austria; telephone: 0043-316-873-4328; fax: 0043-316-873-8304; e-mail: klimant@analytchem.tu-graz.ac.at

Abstract:

Microplates with integrated optical oxygen sensors are a new tool to study metabolic rates and enzyme activities. Precise measurements are possible only if oxygen exchange between the sample and the environment is known. In this study we quantify gas exchange plastic microplates. Dissolved oxygen was detected using either an oxygen-sensitive film fixed at the bottom of each well or a needle-type sensor. The diffusion of oxygen into wells sealed with different foils, paraffin oil, and paraffin wax, respectively, was quantified. Although foil covers showed the lowest oxygen permeability, they include an inevitable gas phase between sample and sealing and are difficult to manage. The use of oil was found to be critical due to the extensive shaking caused by movement of the plates during measurements in microplate readers. Thus, paraffin wax was the choice material because it avoids convection of the sample and is easy to handle. Furthermore, without shaking, significant gradients in pO_2 levels within a single well of a polystyrene microplate covered with paraffin oil were detected with the needle-type sensor. Higher pO_2 levels were obtained near the surface of the sample as well as near the wall of the well. Significant diffusion of oxygen through the plastic plate material was found using plates based on polystyrene. Thus, the location of a sensor element within the well has an effect on the measured pO_2 level. Using a sensor film fixed on the bottom of a well or using a dissolved pO_2-sensitive indicator results in pO_2 offset and in apparently lower respiration rates or enzyme activities. Oxygen diffusion through a polystyrene microplate was simulated for measurements without convection—that is, for samples without oxygen diffusion through the cover and for unshaken measurements using permeable sealings. This mathematical model allows for calculation of the correct kinetic parameters.

Key-words: Optical oxygen sensor, oxygen transfer, optode, microplate sealing