MONITORING OF YEAST CELL CONCENTRATION USING A MICROMACHINED IMPEDANCE SENSOR

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ABSTRACT

This paper describes the design, modelling and experimental characterization of a micromachined impedance sensor for on-line monitoring of the viable yeast cell concentration (biomass) in a miniaturized cell assay. Measurements in Saccharomyces Cerevisiae cell culture show that the permittivity of the cell suspension depends linearly on the biomass concentration within the range of 0 to 9 g/l/. In order to compensate the measurements for changes in the dielectric properties of the background electrolyte, the use of a three-electrode configuration in combination with a semi-permeable pHEMA membrane was explored. Measurements showed that the impedance of the hydrated pHEMA varies with only the background electrolyte conductivity, and not with the concentration of cells, indicating that pHEMA is suitable for this purpose. The optimal pHEMA membrane thickness was determined using finite element modelling.

Keywords: Dielectric spectroscopy, permittivity, cell concentration, microporous membrane, pHEMA

INTRODUCTION

There is a growing interest in the miniaturization of cell cultivation systems, both for single-cell[1] analysis and for fermentation studies[2,3]. For the latter, monitoring of biomass with time is important for the assessment of the influence of fermentor conditions.

Dielectric spectroscopy is a convenient method for the determination of the viable cell concentration. In this method, the impedance of an electrochemical cell that contains the cell suspension is measured. The design of the electrochemical cell requires only two metal electrodes with a defined spacing. Such a device can be fabricated using standard thin-film processes and is cheap, small, scalable, steam-sterilizable and suitable for integration with other microfabricated electrochemical devices. The electrical equivalent circuit of the electrochemical cell and a typical impedance curve are shown in Fig. 1.

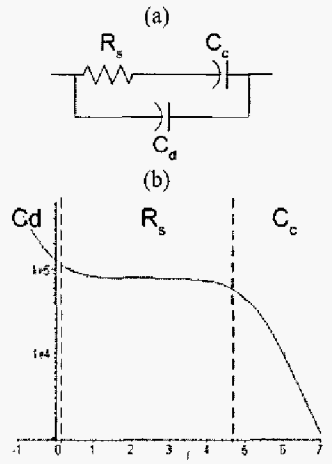

Fig. 1. (a)Electrical equivalent circuit and (b) typical impedance curve. Cd is the electrode double layer capacitance.

The value of the electrolyte resistance (R_s) and the electrochemical cell capacitance (C_c) depend on the sensor dimensions and the conductivity and permittivity of the electrolyte, respectively.

Asami et al.[4] have described theoretically how the concentration of living cells affects the conductivity and permittivity of the cell suspension within the frequency range in which dielectric dispersion occurs. Hauttmann et al.[5] showed that dielectric dispersion has a maximum at 1.6 MHz for S. *cerevisiae* cells. Our work relates this theory to experimental results obtained with a micromachined impedance sensor that was designed for the on-line monitoring of biomass in a miniaturized yeast assay.

Furthermore, we have explored a method that compensates for changes in the dielectric properties of the background electrolyte by differential measurement using a three-electrode configuration[6] in which the impedance measured between two closely spaced electrodes is made insensitive to biomass by covering it with a porous membrane (Fig. 2).

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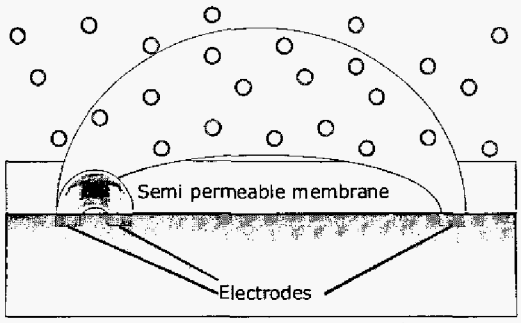


Fig. 2. Schematical representation of the three-electrode configuration.

The dielectric properties of a microporous poly(2-hydroxyethyl methacrylate) (pHEMA) membrane were characterized for this purpose. The advantages of pHEMA as membrane material are that it can easily be photostructured using standard photolithographic processes and that it withstands autoclavation.

FABRICATION

Planar Ta/Pt electrodes were fabricated on a silicon substrate using sputtering and lift-off photolithography. The processed electrode configuration consists of two 2200 μm × 100 μm electrodes, spaced by 60 μm (Fig. 3).

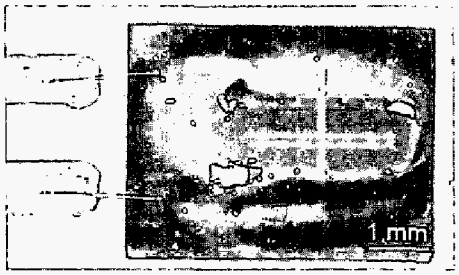


Fig. 3. Photograph of fabricated planar electrode structure.

One pair of electrodes was covered by a pHEMA membrane. The HEMA mixture consists of 2.426ml HEMA, 0.092 ml crosslinker (TEGDMA), 0.1538 gr. UV initiator (2,2dimetoxy-2-phenylacetophenone, DMPAP) and 1.169 ml solvent (ethyleneglycol). The UV initiator is added at the end, in order to prevent the mixture from polymerizing too early.

The prepared solution is pipetted on the sensor, covered with a piece of Mylar foil and subsequently exposed to 366 nm UV light for 2 minutes. The membrane thickness of 300 μm was high enough to assure that the electric field between the electrodes is concentrated within the hydrated pHEMA layer.

EXPERIMENT

The theoretical response of the capacitance to varying cell concentrations was calculated, based on the single-shell model for characterizing the suspended cells, the impedance model shown in Fig. 1 (a), the conformal mapping technique[7] and the actual suspension parameters.

The relation between the electrochemical cell capacitance and the viable S. *cerevisiae* cell concentration was also measured in the frequency range of 1-2 MHz in a standard yeast buffer solution, consisting of 10 g/l ammonium sulphate, 6 g/l potassium phosphate monobasic and 1 g/l magnesium sulphate heptahydrate. The pH of the buffer solution was adjusted to 5.0 using concentrated sodium hydroxide. The applied voltage was kept below 100 mV_{rms} in order to prevent redox reactions to take place.

The electrolyte conductivity was measured in solutions containing known concentrations of sodium chloride using the pHEMA-covered pair of electrodes. The measurements were compared to the theoretical electrolyte conductivity values in order in order to determine whether the measured conductivity is a good measure for the electrolyte conductivity. The sensor response is also measured at different viable cell concentrations in order to verify whether the membrane is permeable to yeast cells.

RESULTS AND DISCUSSION

The measured capacitance shows a linear response to concentration and matches the calculations quite well for typical fermentor concentrations of yeast cells (Fig. 2) in a standard yeast buffer solution. The sensor performance depends to a large extent on the match between the sensor dimensions and the dielectric properties of the yeast suspension. The measurements show that a detection limit below 1 g/l can be achieved once the sensor dimensions meet the conductivity of the liquid.

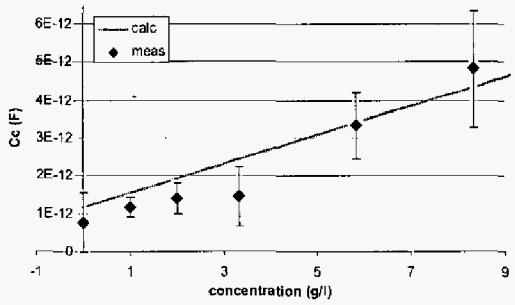


Fig. 4. Theoretical and measured response in yeast in buffer.

After placement in solution, the electrolyte resistance measured with the pHEMA covered electrode pair keeps decreasing for 40 minutes and then reaches a stable value. This can be seen in Fig. 5. It is assumed that this is due to hydration of the membrane. In future configurations, the electrodes will be much more closely spaced and the membrane thickness required to assure the electrical field between the electrodes concentrates within the hydrated membrane layer will be much lower. This will decrease the time required for membrane hydration. The time can be reduced even further by adding solvent to the HEMA mixture, resulting in an opener structure.

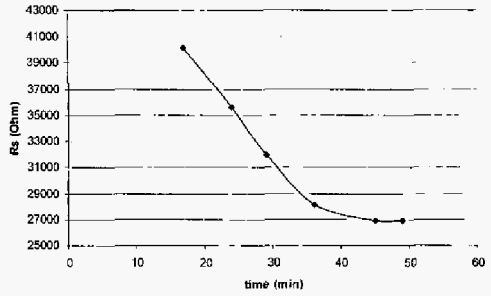
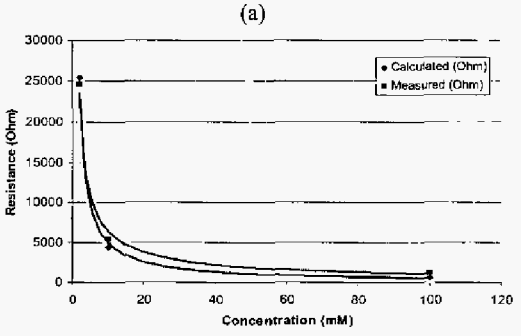


Fig. 5. Electrolyte resistance versus time.

The results in Fig. 6 show that the resistivity measured with a pHEMA covered conductivity sensor in 1-100 mM NaCl solutions is higher than the theoretical and experimentally obtained response of an uncovered sensor with a constant factor, indicating that the conductance measured between the electrodes indeed arises from the electrolyte conductance, but is lowered by the insulating membrane.

The measured cell capacitance in the range of 1-2 MHz shows no dependence on the viable yeast cell concentration, indicating that the membrane is indeed impermeable to yeast cells.



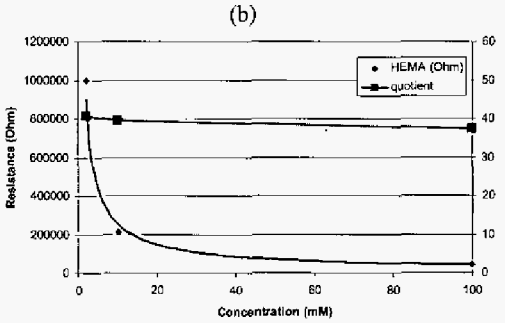


Fig. 6. Theoretical electrolyte resistance and measurements with (a) a planar conductance sensor and (b) a planar conductance sensor covered with a pHEMA membrane.

To find the optimal pHEMA thickness, the electrical field distribution in this system was simulated using finite-element modelling. The width of all electrodes was defined as 200 µm, the length of the outer electrodes is set to 200 µm and the spacing between them to 400 µm. The length of the inner electrode is set to 2 µm and the spacing between the two most closely spaced electrodes is set to 2 µm as well. The electrodes are covered by a layer of varying thickness, which is assigned the observed dielectric properties of the hydrated pHEMA membrane. The volume outside this layer is assigned the dielectric properties of a cell suspension.

The current through the electrodes decreases with increasing membrane thickness (Fig. 7), as electrolyte conductivity decreases. The current through the inner electrodes reaches a steady value at a thickness of 2 micron, indicating that for this thickness the electrical field lines do not penetrate the cell-containing solution so that the measured signal does not depend on the cell concentration.

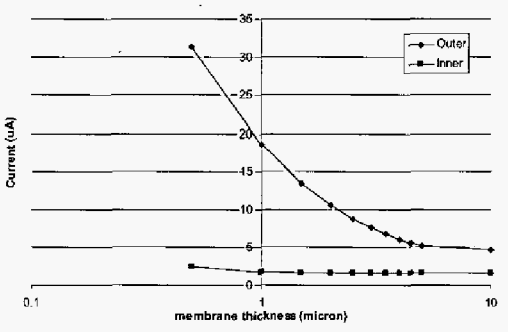


Fig. 7. Simulated relation between electrode current and membrane thickness.

Future sensor designs will be based on the optimal pHEMA thickness resulting from these finite-element simulations.

CONCLUSION

The measured capacitance shows a linear response to concentration and matches the calculations quite well for typical fermentor concentrations of yeast cells (Fig. 2) in a standard yeast buffer solution, confirming the relevance of the applied theory on microreactor scale.

The measurements with the pHEMA covered electrode pair show that the measured resistance does depend linearly on the actual electrolyte conductivity and that the measured cell capacitance is independent of the viable yeast cell concentration. This confirms that the pHEMA membrane gets hydrated, but is impermeable to yeast cells. This makes the proposed 3-electrode configuration suitable for determining the background electrolyte conductivity independent of the viable cell concentration. This signal can thus be used for compensating the measured impedance spectrum for changes in the background electrolyte.

The optimal hydrated membrane thickness arises from the observed dielectric properties of the membrane and finite element modelling and is found to equal 2 micron for the electrode configuration under study. The dielectric and transient properties of the membrane can be optimized by altering the physical membrane properties.

FUTURE PLANS

Integrated sensor designs, which integrate the proposed conductance sensor with other electrochemical sensors required for the online monitoring of fermentor conditions, are being processed at the cleanroom facilities of MESA+. The sensors will be tested in a fed-batch process and finally be integrated in a microscale fed-batch fermentor. There will be a focus on optimizing and processing the pHEMA membrane in a reproducible way.

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