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Monitoring of yeast cell concentration using a micromachined impedance sensor

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Abstract

This paper describes the design, modeling 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 characteristic frequency describing the β-dispersion of *S. cerevisiae* cells is around 2.8 MHz. The permittivity change of the cell suspension was measured for the concentration range 0–9 g/l and depends linearly on the biomass concentration. In order to compensate the measurements for the electric properties of the background electrolyte, which increases the sensitivity and allows measurements in different media, the use of a three-electrode configuration in combination with a semi-permeable poly(2-hydroxyethyl methacrylate) (pHEMA) membrane was explored. Measurements show that the impedance of hydrated pHEMA varies with the background electrolyte conductivity only, 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 and was found to be 1 μm for the electrode configuration under study.

Keywords: Dielectric spectroscopy; Permittivity; Cell concentration; Microporous membrane; pHEMA

1. Introduction

There is a growing interest in the miniaturization of cell cultivation systems, both for single-cell analysis [1] 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.

This paper will demonstrate that dielectric spectroscopy is a convenient method for the determination of biomass. In this method, the impedance of an electrochemical cell that contains the cell suspension is measured. The method is minimally invasive and selective for viable biomass only, as dead cells with leaky membranes do not affect the measurement. 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. 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.

The dielectric spectra of biological cells show three distinct dispersions in the frequency range of 1 Hz to 1 GHz, termed α-, β- and γ-dispersion [4]. β-Dispersion originates from interfacial polarization, that is the accumulation of charges at the boundaries between the membrane and aqueous phases of different electrical properties. This polarization is frequency dependent and shows relaxation [5]. The dispersive frequency range is represented by a characteristic frequency, which depends strongly on the exact morphology of the cells and the electric properties of the cells and the background electrolyte [6]. Previous studies showed characteristic frequencies in the

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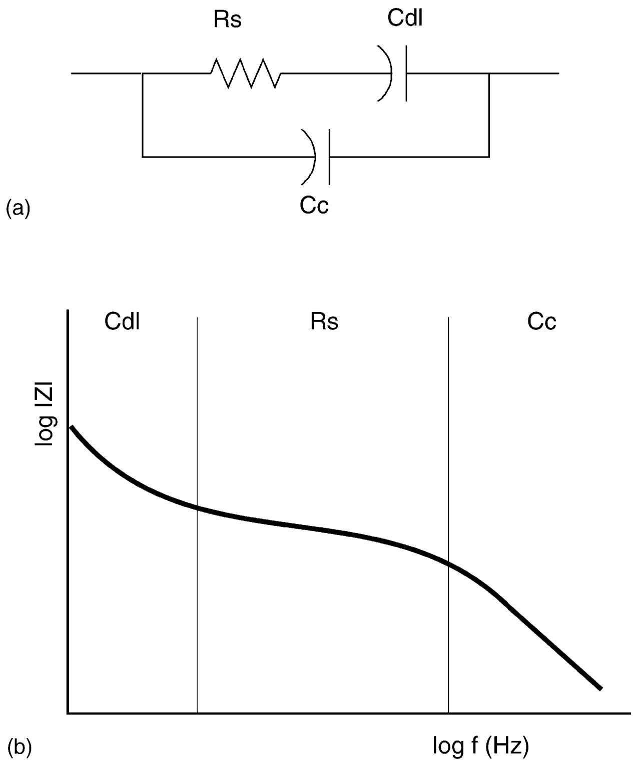

Fig. 1. (a) Electrical equivalent circuit and (b) typical impedance curve. Cd is the electrode double layer capacitance.

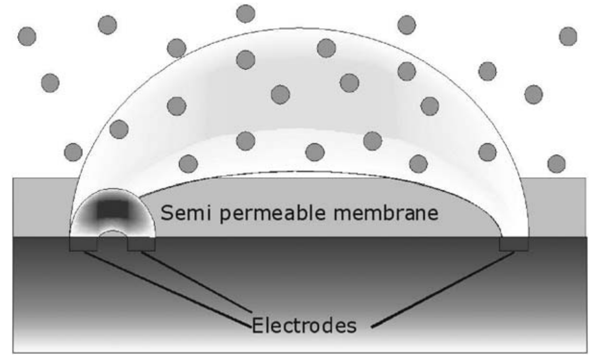

Fig. 2. Schematical representation of the three-electrode configuration.

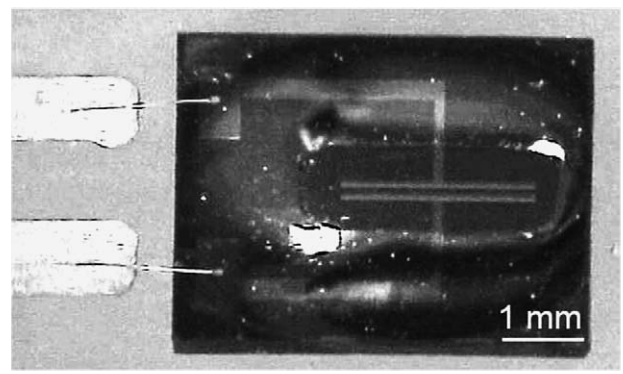

Fig. 3. Photograph of fabricated planar electrode structure.

order of a few MHz for viable *Saccharomyces cerevisiae* cells [6,7]. Asami and Yonezawa [7] have described theoretically how β-dispersion affects the conductivity and permittivity of the cell suspension and how the cell concentration can be estimated from these data. In this work, the viable yeast cells are modeled as single-shell spherical 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. The sensor is also suitable for use in larger (up to industrial scale) fermentation systems.

The main reason that dielectric spectroscopy is still not a widely used method for biomass monitoring, is that the measured signals suffer from interference of the electric properties of the background electrolyte. The ionic concentration of the background electrolyte affects its conductivity. The measured permittivity is affected as well, as it determines the rate of electrode polarization. This effect is frequency dependent and cannot reliably be subtracted out, as the ionic content of the medium changes during the fermentation process [8]. Therefore, we have explored a method that compensates for changes in the electric properties of the background electrolyte by differential measurement using a three-electrode configuration [9] in which the impedance measured between two closely spaced electrodes is made insensitive to biomass by covering it with a porous membrane that prevents cells to approach the electrodes (Fig. 2).

The electric 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 standard sterilization by autoclavation.

2. Device fabrication

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

One pair of electrodes was covered by a pHEMA membrane. The HEMA mixture consists of 2.426 ml HEMA, 0.092 ml crosslinker (TEGDMA), 0.1538 gr. UV initiator (2,2-dimetoxy-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 min. The non-optimized membrane thickness of 300 μm was high enough to assure that the electric field between the electrodes is concentrated within the hydrated pHEMA layer.

3. Experimental

The characteristic frequency for dielectric dispersion typically is around a few MHz for *S. cerevisiae* cells, but the exact frequency value depends on the morphology of the cells and the electrical properties of the medium. Therefore, the medium conductance in a standard yeast buffer in which 7 g/l DW of