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Low-cost sensor system for non-invasive monitoring of cell growth in disposable bioreactors

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Abstract

To ensure productivity and product quality, the parameters of biotechnological processes need to be monitored. Along temperature or pH, one important parameter is the cell density in the culture medium. In this work, we present a low-cost sensor system for online cell growth monitoring in bioreactors via permittivity measurements based on coplanar transmission lines. To evaluate the sensor, *E. coli* cultivations are performed. We found a good correlation between optical density of the culture medium and the effective permittivity at a frequency of 1 kHz when the sensor is submerged into the culture medium. Measurements at higher frequencies additionally allow monitoring the osmolarity. Furthermore, an improved sensor was successfully used for first non-invasive measurements through the polymer wall of a disposable bioreactor.

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Keywords: cell growth monitoring, single use bioreactor, permittivity measurements, dielectric spectroscopy, coplanar transmission line

1. Introduction

For the control of bioprocesses, a variety of parameters needs to be monitored, e.g. temperature, glucose, pH, pO_2, pCO_2 or cell density. Today, cell cultivation is increasingly performed in single use bioreactors (SUB) made of polymer foil, especially in food and pharmaceutical industry. The major advantage of SUBs compared to traditional bioreactors is that development and optimization are significantly accelerated, because SUBs are purchased

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presterilized and are simply disposed after use. This means, no sterilization and cleaning has to be performed [1]. The sensors for monitoring the bioprocess are typically designed as single-use devices that can endure a gamma ray sterilization, as they need to be placed inside the bioreactor. In this work, a different approach for monitoring cell growth is presented. Here, we explore the use of a high frequency coplanar transmission line as senor for noninvasive dielectric measurements through the polymer wall of the SUB. In a preliminary experiment a sensor system, consisting of a low cost vector network analyzer (VNA) and a coplanar probe, primarily designed for application as a sensor for humidity in human tissue [2], is tested. Therefore, *E. coli* cultivation is first performed in a flask with the sensor submerged to investigate its general performance as a cell growth monitoring device. Subsequently, the design of the sensor is improved to allow measurements through the polymer wall of the SUB.

2. Preliminary Setup

The procedure for monitoring cell growth presented in this paper is based on a transmission measurement of an electromagnetic wave travelling through the coplanar transmission line depicted in Fig. 1(a). This transmission line consists of an inner conductor centered between two ground planes, all assembled on a RO6010 substrate. This substrate has a relative permittivity of $\varepsilon_r = 10.2$. The space between inner conductor and ground plane is s = 1.1 mm and the width of the inner conductor is w = 2.8 mm. These dimensions lead to a matched impedance of $Z_0 = 50\ \Omega$ for the unloaded probe in air ($\varepsilon_r = 1$). With an edge length $l = 20$ mm, the size of the quadratic sensing area is 400 mm^2. Fig. 1(b) shows a *CST Microwave Studio* simulation for the coplanar transmission line submerged into the culture medium. The electromagnetic field is partly inside the substrate and partly inside the culture medium above the transmission line. Thus, the propagation of an electromagnetic wave through the coplanar probe is influenced by the permittivity of the culture medium. The permittivity is a measure for the polarizability of a material and is therefore directly correlated to the cell density in the culture medium [3]. In our experiments, we measure the scattering parameter S_{21}, which describes the transmission of an electromagnetic wave through our sensor. In [4], an algorithm for calculating the permittivity from the S_{21} is presented. Here, the coplanar transmission line poses a special case as the wave travels through two parallel materials with different permittivity. Therefore, the algorithm delivers the so-called effective permittivity, a superposition of the substrate permittivity and the culture medium permittivity.

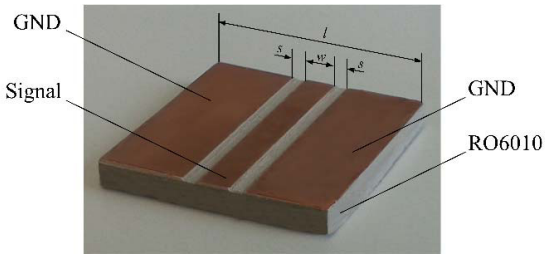
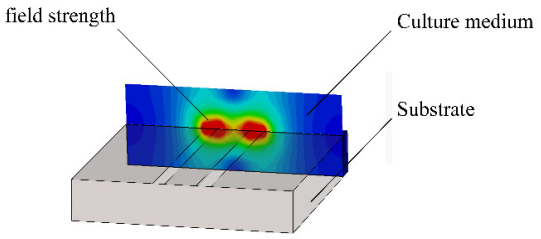


Fig. 1 (a) Photo of the coplanar line



(b) CST simulation of the sensor

In a first step, cell cultivation is performed in a flask with the described sensor submerged in the culture medium. The S_{21} is measured using a low-cost VNA, the VWNA3 by SDR kits [5]. With this configuration, we found a linear correlation between the real part of the effective permittivity at a frequency of 1 kHz and the optical density at 600 nm measured with a Multiskan GO. The results depicted in Fig 2 (a) show that cell growth monitoring is possible with our setup. Furthermore, one major advantage of the coplanar transmission line is its large bandwidth, allowing simultaneous monitoring of cell growth and the culture medium's osmolarity with one sensor. This is demonstrated via measurements of different salinities at a higher frequency of 500 MHz. At this frequency, the polarization of cells cannot follow the alternating electrical field. Therefore, the results depicted in Fig. 2(b) are independent of the cell density in the culture medium. There is a good linear correlation between salinity and real part of the effective permittivity, making precise determination of salinity from a permittivity measurement possible.

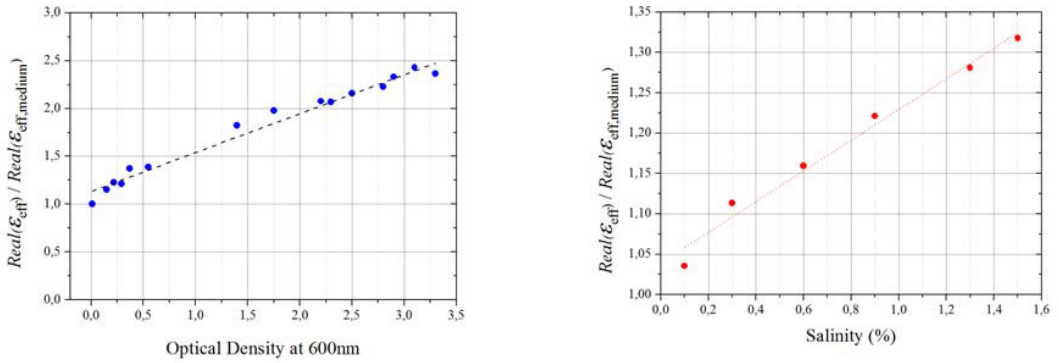


Fig. 2 (a) Correlation between the real part of the effective permittivity at a frequency of 1 kHz and the optical density (b) Measurement of saline at 500 MHz to demonstrate the sensors ability to monitor osmolarity.

However, the goal of this work is monitoring cell growth non-invasively through the polymer wall of a SUB. Simulations show that the polymer foil decreases the electromagnetic field in the culture medium as the field is concentrated in the polymer foil due to its low permittivity. Therefore, the penetration depth of the currently used sensor is dramatically decreased as shown in Fig. 3. This reduces the sensitivity to virtually zero when measuring through the foil. Therefore, the subsequent step is to improve the sensor design in order to increase the penetration depth.

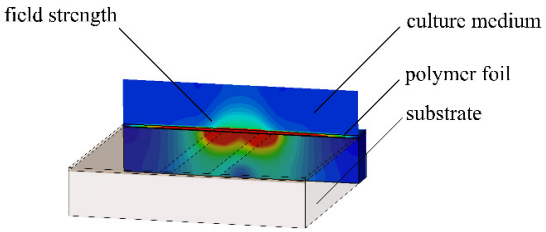


Fig. 3. CST simulation of a measurement through a polymer wall: The electromagnetic field is concentrated in the polymer wall of the SUB

3. Non-invasive measurements

To gain a higher penetration depth, the structure of the sensor is modified as depicted in Fig. 4.

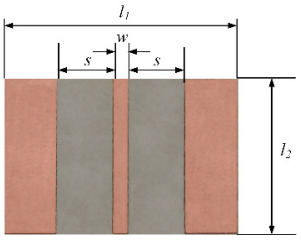


Fig. 4. Coplanar probe with increased spacing between inner conductor and ground planes

The lengths are l_1 = 30 mm and l_2 = 20 mm. As for the previous design, the used Substrate is RO6010, but the spacing between inner conductor and ground plane is increased to s = 7.15 mm and the width of the inner conductor is decreased to w = 2 mm. These dimensions cause an impedance different from 50 Ω for the probe in air. This needs to be accounted for when using the algorithm described in [4] for calculating the effective permittivity from the S_{21}. To evaluate the sensor, cell growth of *E. coli* is monitored through the polymer wall of a SUB. The results are depicted in Fig. 5, indicating good correlation between the real part of the effective permittivity and the optical density at 600 nm. Although the sensor signal seems to saturate towards high optical densities, it is possible to monitor the complete cultivation process up to an optical density of 3.6.

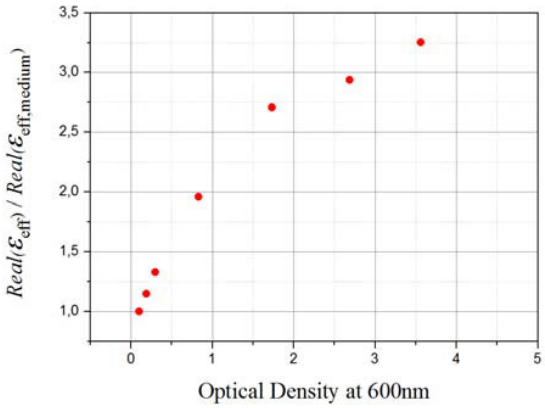


Fig. 5. Non-invasive monitoring of cell growth: Correlation between the real part of the effective permittivity at a frequency of 1 kHz and the optical density

4. Conclusion

In this work, we presented a novel approach for continuous non-invasive cell growth monitoring in bioreactors via permittivity measurements based on coplanar transmission lines. We performed *E. coli* cultivations and found good correlation between the optical density of the culture medium and the effective permittivity at a frequency of 1 kHz when the sensor is submerged in the culture medium. Measurements at higher frequencies additionally allow monitoring the osmolarity. Subsequently, the sensor design was adapted to enable measurements through the polymer wall of a SUB. With this sensor, an *E. coli* cultivation could be monitored up to an optical density of 3.6.

References

[1] Glindkamp A., Riechers D., Rehbock C., Hitzmann B., Scheper T. ,Reardon K.F., Sensors in disposable bioreactors status and trends. Advances in Biochemical Engineering/Biotechnology, vol. 115, pp. 145–169, 2009.

[2] Reinecke T., Hagemeier L., Ahrens S., Doroschenko Y., Klintschar M., Zimmermann S., A novel coplanar probe design for fast scanning of edema in human brain tissue via dielectric measurements, Sensors and Actuators B: Chemical, vol. 220, pp. 522-527, 2015.

[3] Markx G. H., Davey C. L., The dielectric properties of biological cells at radiodfrequencies: Applications in biotechnology, Enzyme Microbial Technol., vol. 25, pp. 161–171, 1999.

[4] Baker-Jarvis, J.; Vanzura, E.J.; Kissick, W.A., Improved technique for determining complex permittivity with the transmission/reflection method, Microwave Theory and Techniques, IEEE Transactions on, vol. 38, no. 8, pp. 1096-1103, 1990

[5] Bayer T., A Small, Simple, USB-Powered Vector Network Analyzer Covering 1 kHz to 1.3 GHz. QEX, vol. 1, pp. 32, 36, 2009.