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Non-Invasive Online Monitoring of Cell Growth in Disposable Bioreactors with a Planar Coil

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Abstract

To ensure high quality output of biotechnological processes, relevant process parameters need to be monitored. As bioprocesses are increasingly executed in single use bioreactors, there is an increasing demand for new sensors applicable to these processes. In this work, we present a low-cost sensor system for continuous non-invasive cell growth monitoring, especially for single use bioreactor applications. The system consists of a planar coil connected to a low cost network analyzer. The coil is attached to the outside of the polymer foil of the single use bioreactor and an impedance spectrum is measured. To evaluate the sensor, E. coli cultivations are performed in a modified cultivation setup, which enables measurements through the polymer foil of a Sartorius BIOSTAT® CultiBag RM, and additionally allows sampling of culture medium for optical density reference measurements. The resonance peak of the coil in the impedance spectrum, is observed as measure for the optical density. Regardless of the simple sensor construction, we found a good correlation between optical density and the damping ratio of the resonance peak.

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Keywords: cell growth monitoring, single use bioreactor, inductive permittivity measurements, dielectric spectroscopy

1. Introduction

The continuous measurement of all relevant process parameters is the key factor for maximizing stability, reproducibility and efficiency of bioprocesses. Especially in food and pharmaceutical industry, cell cultivation is

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increasingly executed in single use bioreactors (SUB) made of polymer foils. Therefore, new sensors are required, as traditional sensors are commonly designed as reusable devices that are inserted into the bioreactor. However, inserting a sensor is not possible using SUBs as they are a sterile self-contained unit. New sensors for SUBs are usually designed as single-use devices that can endure a gamma radiation sterilization process and are installed within the SUB by the manufacturer [1, 2]. However, to realize a reusable cost effective solution, we explore different non-invasive approaches for monitoring cultivation processes parameters through the SUB polymer foil. In a first approach, we presented cell growth monitoring with a coplanar transmission line [3]. The sensing principle shows good results for a sensor submerged in the culture medium, but the polymer foil of a SUB significantly lowers the electrical field strength in the culture medium, resulting in a very low penetration depth. Thus, accuracy and reproducibility of a cell density measurement from outside the SUB using a coplanar transmission line are limited and not entirely satisfactory. As the foil does not disturb the magnetic flux density, we now investigate an alternative approach based on a coil inducing an electrical field inside the culture medium.

2. Introduction

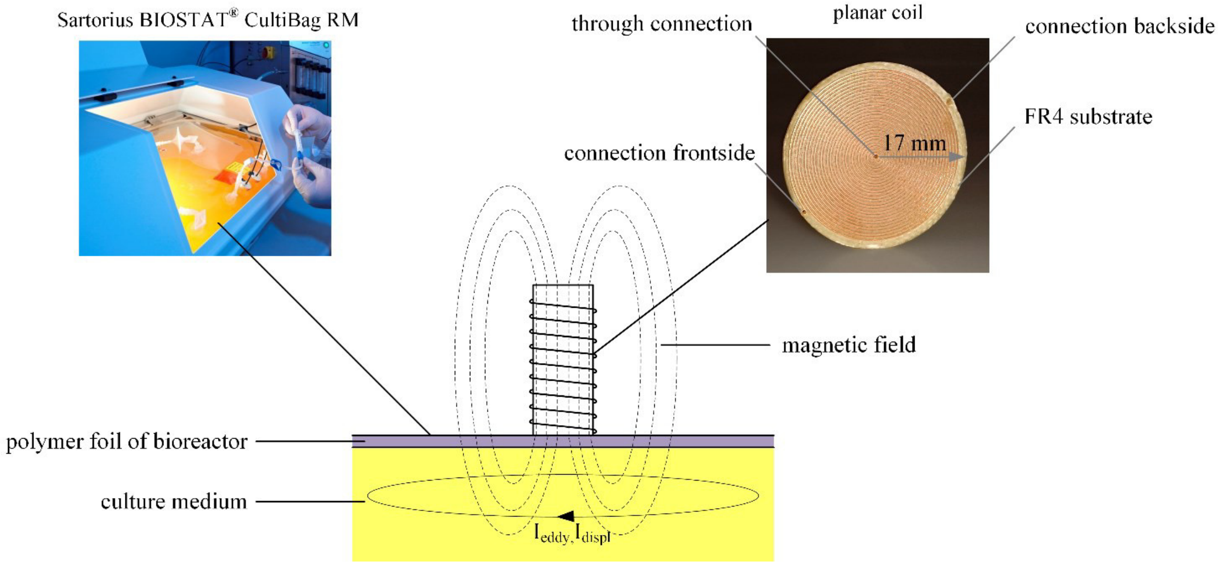

Fig. 1. Schematic of the setup for non-invasive cell growth monitoring

Fig.1 depicts a schematic of the setup for non-invasive cell growth monitoring. The setup consists of a low-cost network analyzer (VWNA3, SDR-Kits, approx. 500 €) and a two-sided planar coil on a standard FR4 substrate with an inductivity of approx. 30 μH. The coil is attached to the outside of the polymer foil of a Sartorius BIOSTAT® CultiBag RM. The magnetic field of the coil induces an electrical field in the culture medium. This electrical field produces eddy currents in the medium, depending on the electrical conductivity, and displacement currents depending on the relative permittivity of the cell culture. The measuring effect is the cell density dependent repercussion of these currents on the magnetic field of the coil. This effect is principally shown in [4, 5] using a commercially available inductive probe (HP E5050A Colloid Dielectric Probe, Hewlett-Packard) for dielectric measurements, based on two magnetically coupled coils for application in traditional bioreactors. However, it is mandatory to submerge the E5050A into the culture medium and therefore it is not applicable to SUBs.

In Fig. 2 the impedance spectrum of the planar coil attached to the SUB polymer foil is shown. Here the foil is not in contact with culture medium. Combined with the intrinsic capacity, the coil behaves like a parallel resonant circuit, with a resonance frequency of $f_{res} = 11.47$ MHz. With the 3 dB bandwidth BW_{3dB}, the damping ratio of the unloaded resonator can be determined to $D = BW_{3dB} / 2f_{res} = 0.019$.

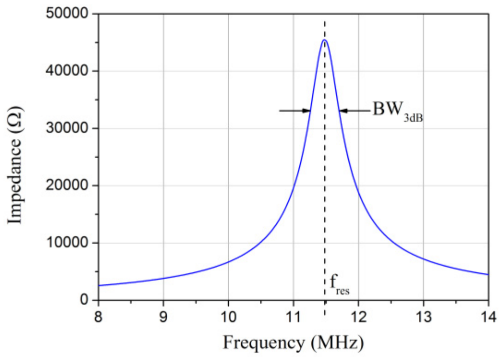

Fig. 2. Impedance spectrum of the unloaded planar coil

In the next step, the sensor response is investigated via measurements of saline as model for the culture medium. Therefore, the NaCl concentration is gradually increased from 0 wt.% to saturation concentration of 30 wt.%. The measured impedance spectra are depicted in Fig. 3 (a). Increasing the NaCl concentration leads to an increase of losses, as conductivity and polarization losses of the aqueous solution are increasing. It can be seen, that the senor is sensitive to losses of the medium in contact with the polymer foil: The amplitude of the resonance Peak lowers and the bandwidth increases when increasing NaCl concentration. Calculating the damping ratio D shows a linear dependency between D and the NaCl concentration, as depicted in Fig 3 (b).

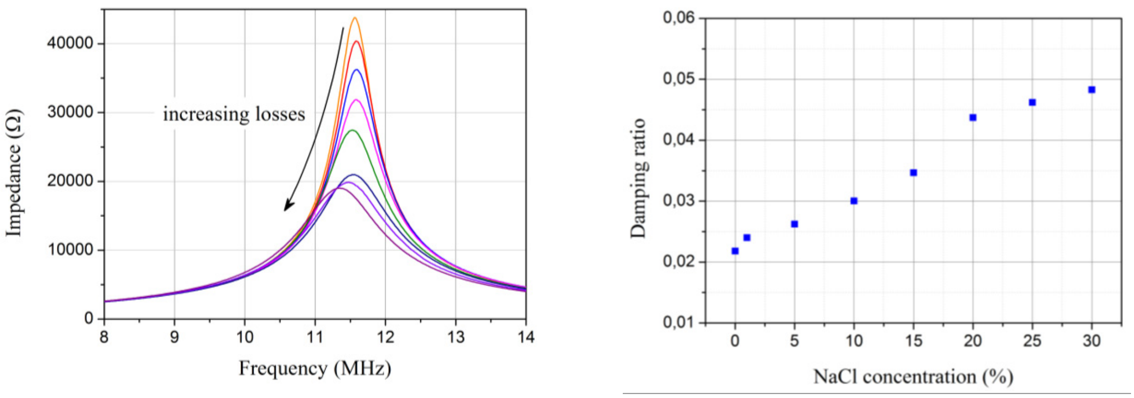

Fig. 3 (a) Impedance spectra for increasing NaCl concentration; (b) Damping ratio in dependence of NaCl concentration.

Subsequently, we performed two *E. coli* cell cultivations (cell culture 1 & 2) in our experimental setup, which allows both measurements through the polymer foil of the SUB, and sampling of the cell culture for optical density (OD) reference measurements with a *Multiskan GO* at a wavelength of 600 nm. Fig. 4 (a) depicts the bacterial growth curve of *E. coli* determined with the *Multiskan GO* at equidistant 30 min time steps. The growth curve shows an exponential behavior and cell density almost doubles in the interval of 30 minutes. After 210 minutes growth time, the biomass reaches its maximum with an OD of 3.7. In parallel to the OD determination, non-invasive impedance measurements are performed. Increased biomass leads to increased dielectric losses [6] and therefore the measured damping ratio increases analog to the measurements with saline.

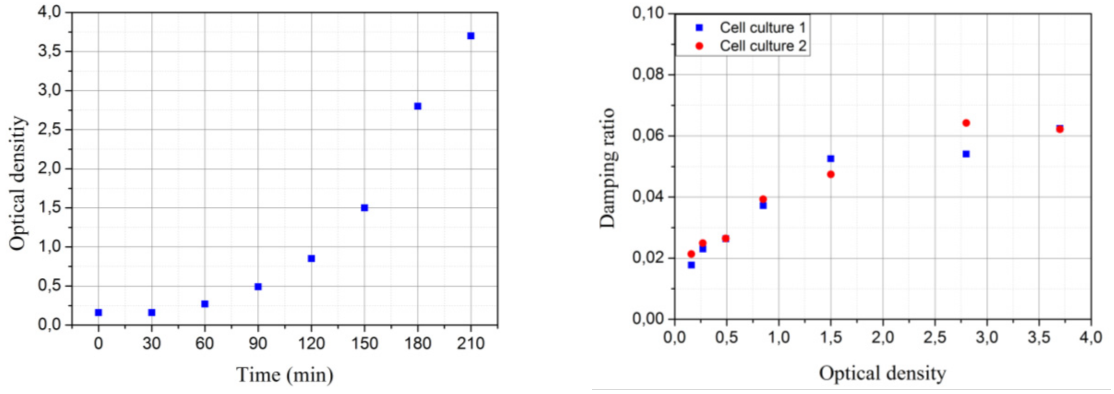

Fig. 4. (a) Bacterial growth curve of cell culture 1; (b) Damping ratio in dependence of the optical density measured at 600 nm.

Fig. 4 (b) shows the dependency of the damping ratio from the OD of the two different cell cultivation processes. The measured damping ratio values of both cultivation processes show only small deviations at low optical densities and a linear dependency between OD and D can be assumed. Towards higher ODs, a saturation of the sensor signal can be observed. However, this simple sensor enables monitoring of cell growth up to an optical density of OD ≈ 3.

3. Conclusion

In this work, we presented an approach for continuous non-invasive cell growth monitoring during cell cultivation in single use bioreactors using a very simple sensor and solely low cost measuring equipment. The setup consists of a double-sided planar coil on a standard substrate attached from the outside to the polymer wall of a Sartorius BIOSTAT® CultiBag RM and a low-cost network analyzer for measuring the impedance spectrum of the coil. In a first step, the sensor performance is investigated via measurements of saline. It could be shown that the sensor responds to increasing conductivity and increasing polarization losses of the medium inside the bioreactor. Subsequently cell cultivations of *E. coli* where performed and impedance spectra of the coil where measured during the cultivation processes. In parallel reference optical density measurements with a *Multiskan GO* where carried out. The damping ratio of the resonance peak of the resonance circuit, consisting of inductivity and intrinsic capacity of the coil increases with increasing optical density. Although the sensor signal saturates towards higher optical density, monitoring of cell growth up to an optical density of OD ≈ 3 is possible.

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