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Differential Inductive Sensor for Continuous Non-Invasive Cell Growth Monitoring in Disposable Bioreactors †

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Abstract: In this work, we present a low-cost sensor system for continuous non-invasive cell growth monitoring, especially for single use bioreactor (SUB) applications. The sensor system is based on a differential transformer. Using this differential setup, the influence of the primary magnetic flux is eliminated from the measuring signal, enabling highly sensitive non-invasive detection of permittivity changes in the culture medium. To evaluate the sensor, *E. coli* cultivations are performed and the cell density is measured through the polymer foil of a SUB. We found a linear dependency with low data scattering between measuring signal and cell density.

Keywords: cell growth monitoring; single use bioreactor; inductive permittivity measurements; dielectric spectroscopy; differential transformer

1. Introduction

For high quality output of biotechnological cultivations, it is necessary to monitor and control bioreactor processes. Thus, sensitive sensor systems that allow continuous and preferably non-invasive monitoring of relevant parameters during the cell cultivation are required. The continuous measurement of biomass is one of the most important parameters, which have to be determined. It gives an overview over the process performance, the process state and condition of the used cells. Today, cell cultivation is increasingly performed in single use bioreactors (SUB) made of polymer foil, especially in food and pharmaceutical industry. Since SUBs are a sterile self-contained unit, biomass 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, this is very expensive and elaborate. Thus, in this work, an innovative approach for continuous non-invasive cell growth monitoring through the SUB polymer foil is investigated to realize a reusable cost effective solution. Therefore, the permittivity of the cell culture is used as a measure for the biomass.

In recent years, we explored different non-invasive approaches for cell growth monitoring. For example, good results have been achieved for a sensor submerged in the culture medium using a coplanar transmission line [3]. However, 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 polymer foil does not disturb the magnetic flux density, an alternative approach is to use a coil inducing an electrical field inside the culture medium [4]. The measuring effect is the cell density dependent repercussion of the secondary magnetic flux. The limiting factor when using only a single coil is that the measuring signal is dominated by the influence of the primary flux of the coil and thus the weak secondary flux from the medium is hardly detectable. Therefore, the employment of a differential transformer for non-invasive cell growth monitoring seems to be the most promising approach [5]. In this differential setup, the influence of the primary flux through the measuring coil is eliminated from the measuring signal. Preliminary measurements show a good correlation between the biomass concentration in the culture medium and the measuring signal [5]. However, there is no information about the influence of other parameters, like measuring frequency or filling level, on the measuring signal. In order to design a system achieving accurate and reproducible results, the knowledge about the impact of these parameters on the measurement is important. Hence, in this work, measurements are presented characterizing the differential transformer and leading to an optimized setup to further increase the sensitivity, accuracy and reproducibility.

2. Experimental Setup

A schematic of the setup employed in this work is depicted in Figure 1. It consists of three coils, all assembled on a ferrite core (8 mm diameter, $\mu_r = 300$, Frequency 0.1–3 MHz). The coils are wound with an insulated copper wire with a diameter of 1.8 mm. The receiver coils L_{s1} and L_{s2} have a similar inductance of $L_s = 10$ mH (2 mm high, 33 mm diameter). In order to reach an increased sensitivity, a high turn ratio is realized by choosing a 100-fold lower inductance for the transmitter coil $L_p = 0.1$ mH (10 mm high, 15 mm diameter). Applying an excitation voltage U_p (5 V_{pp}) to the transmitter coil L_p, an identical flux through both receiver coils L_{s1} and L_{s2} is produced. As the windings of L_{s1} and L_{s2} are in opposite directions, the induced voltages by the primary flux cancel out from the measuring voltage U_s. Thus, the measuring voltage is $U_s = 0$ for an unloaded sensor. When the sensor is attached to the SUB, the primary flux induces eddy and displacement currents in the medium. The secondary flux from these currents only induces a voltage in L_{s2} as it is placed much closer to the medium than L_{s1}. The induced voltage in L_{s1} can be neglected. Therefore, the measuring voltage U_s equals the induced voltage in L_{s2}. In [5], it is shown that the real part of the measuring voltage U_s' depends on the polarizability of the medium (ε_r') and the imaginary part of the measuring voltage U_s'' depends on the dielectric losses (ε_r'') and the conductivity (κ).

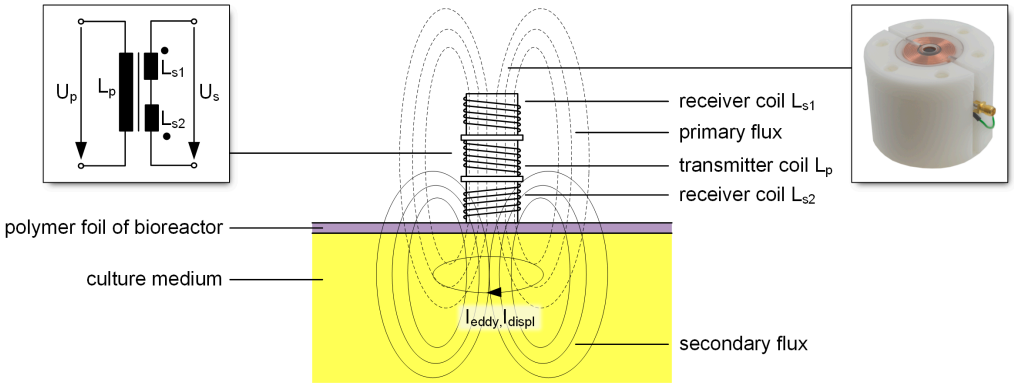

Figure 1. Schematic of the custom made differential transformer for non-invasive cell growth monitoring in single use bioreactors.

3. Sensor Characterization

In the following, the behavior of the differential transformer is characterized via measurements of isopropanol water mixtures. The corresponding theoretical permittivity values were calculated using [6]. In a first step, the dependency of the real part of the measuring voltage U_s' on the measuring frequency is investigated for a 1:4 isopropanol water mixture ($\varepsilon_r' = 65.72$). Since the setup is not

entirely symmetrical, the measuring voltage U_s' is not equal to zero. As given in Figure 2, U_s' shows two extremes in the frequency range 105–110 kHz. This might be due to a resonant inductive coupling between the coils leading to an increased primary magnetic flux in the culture medium. Accordingly, the sensitivity of the differential transformer is also optimal in this frequency range, considering the difference between U_s' for pure isopropanol ($\varepsilon_r' = 20.18$) and U_s' for a 1:4 isopropanol water mixture ($\varepsilon_r' = 65.72$) in Figure 3. Thus, the following measurements will be performed at the optimal measuring frequency of 108.4 kHz.

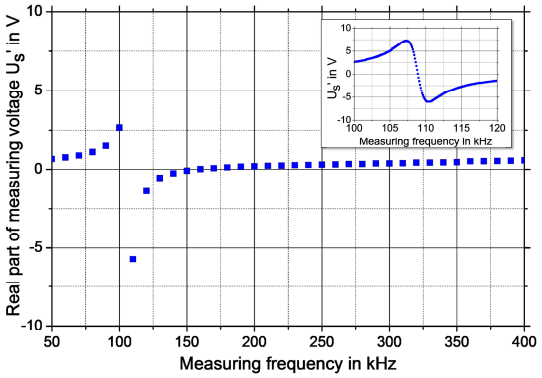

Figure 2. Real part of the measuring voltage U_s' in dependency on the measuring frequency for a 1:4 isopropanol water mixture.

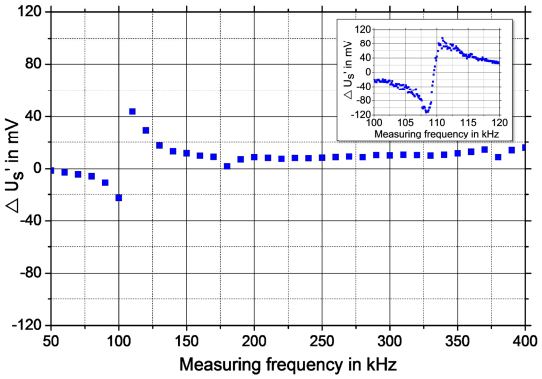

Figure 3. Difference between U_s' for pure isopropanol and U_s' for a 1:4 isopropanol water mixture in dependency on the measuring frequency.

At this frequency, a linear correlation between U_s' and the polarizability of the medium ε_r' can be observed using measurements of isopropanol water mixtures (see Figure 4). The measured sensitivity is 20 mV/10 ε_r'. By averaging every measuring point 16 times, leading to a measuring time of approximately 20 s, a three-fold standard deviation of 7 mV is determined.

During cell cultivations, the filling level of the bioreactor varies due to the shaking or stirring of the culture medium and the associated foam forming. To estimate the influence of this process, the dependency of the filling level on U_s' is investigated for a pure water sample. The result is given in Figure 5. It can be seen, that U_s' does not significantly change for a filling level exceeding 20 mm. Hence, the penetration depth of the primary magnetic flux is nearly in this dimension. As a result, the effect of foam forming can be neglected during cell cultivation measurements.

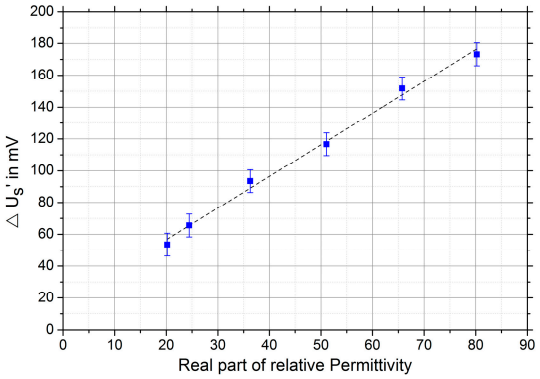

Figure 4. Characterization of the differential transformer with water isopropanol mixtures, f = 108.4 kHz, the vertical error bars indicate ± the three-fold standard deviation.

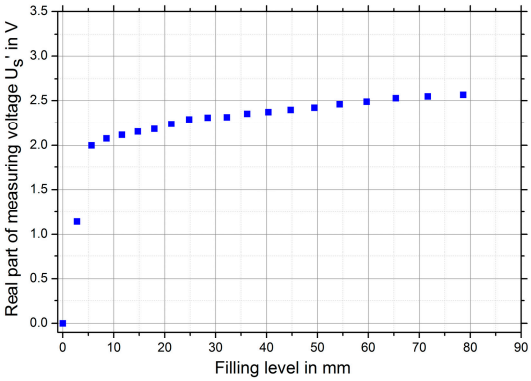

Figure 5. Real part of the measuring voltage U_s' versus the filling level of the bioreactor, f = 108.4 kHz.

4. Results

With this setup, we perform cultivation of *E. coli* and measure the cell density through the polymer foil with the differential transformer and in parallel the optical density with the Multiskan Go as reference. Due to the low measuring frequency of 108.4 kHz, cell polarization can follow the alternating electrical field. This leads to a huge influence of the cell density on the real part of the relative permittivity of the culture medium [7]. Figure 6 shows a linear dependency with a coefficient of determination $R^2 = 0.96$ between U_s' and the optical density of the culture medium up to an optical density of OD = 4. Compared to the results shown in [5], the measured sensitivity is significantly increased using the optimal measuring frequency of 108.4 kHz.

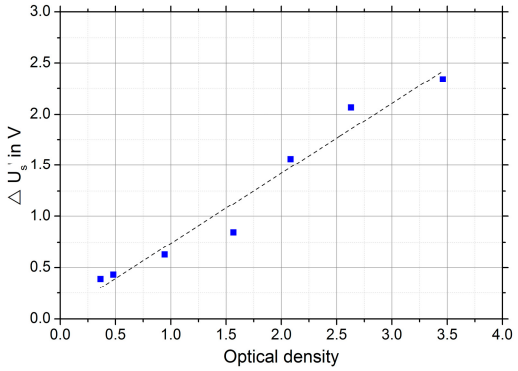

Figure 6. Change in the real part of the measuring voltage U_s' versus optical density during cell cultivation, f = 108.4 kHz.

5. Conclusions

In this work, we presented an approach for continuous non-invasive cell growth monitoring during cell cultivation in single use bioreactors (SUBs) using a simple differential transformer. The setup consists of three coils, all assembled on a ferrite core and attached from the outside to the polymer foil of a SUB. In a first step, the sensor performance was investigated via measurements of isopropanol water mixtures. At the optimal measuring frequency of 108.4 kHz, a linear correlation between U_s' and the polarizability of the medium ε_r' could be observed. Subsequently, we performed *E. coli* cell cultivations. In these experiments, a good correlation between the optical density of the culture medium and U_s' is found. Thus, we conclude that this approach is promising to realize a measuring system for accurate, continuous and non-invasive monitoring of cell growth in disposable bioreactors.

Conflicts of Interest: The authors declare no conflict of interest.

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