Contactless Investigations of Yeast Cell Cultivation in the 7 GHz and 240 GHz Ranges

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Abstract. Using a microfluidic system based on PTFE tubes, experimental results of contactless and label-free characterization techniques of yeast cell cultivation are presented. The PTFE tube has an inner diameter of 0.5 mm resulting in a sample volume of 2 µl for 1 cm sample length. Two approaches (at frequencies around 7 GHz and 240 GHz) are presented and compared in terms of sensitivity and applicability. These frequency bands are particularly interesting to gain information on the permittivity of yeast cells in Glucose solution. Measurements from 240 GHz to 300 GHz were conducted with a continuous wave spectrometer from Toptica. At 7 GHz band, measurements have been performed using a rat-race based characterizing system realized on a printed circuit board. The conducted experiments demonstrate that by selecting the phase as characterization parameter, the presented contactless and label-free techniques are suitable for cell cultivation monitoring in a PTFE pipe based microfluidic system.

1. Introduction

The continuing trend in microbiology to conduct cell cultivation with highly parallel techniques and decreasing culture volumes creates an urgent need for efficient and reliable tools to provide automated cell cultivation monitoring. The segmented flow architecture presented in [1] allows for performing high throughput cultivation in pipe based bioreactors. In addition, cultivation methods based on parallelism are more commonly applied, which increases the need for effective contactless and label-free techniques to characterize the cultivation progress. These techniques mainly use the fact that the increase of number of cells within a single compartment evokes a change in the average permittivity, which is detectable using microwave spectroscopy. The permittivity of disperse substances has been actively studied and detailed models for disperse media can be found in [2]. This work demonstrates two techniques being well suited for contactless and label-free characterization of cell cultivation. Other approaches to investigate the relative permittivity of liquids often require the liquid under test to be in direct contact with the sensing coplanar waveguide structure. Considering the described pipe based microfluidic system, a much higher sensitivity is demanded, since the electric field needs to penetrate the wall thickness of the PTFE channel. Therefore, a differential method, presented in [3], was modified and put into operation, resulting in a phase sensitive biosensor working at 7 GHz. Measurements beyond 200 GHz were conducted with a continuous wave (CW) Terahertz spectrometer.
to investigate the possibilities and limitations of monitoring yeast cultivation in the sub-terahertz range. Sec. 2 presents the function and experimental setup of the 7 GHz Biosensor followed by a discussion of the simulated results. Sec. 3 introduces the continuous wave Terahertz spectrometer and describes the conducted experiments. The measurement results are presented in Sec. 4 and finally, Sec. 5 discusses both methods.

2. 7 GHz Biosensor
To investigate cell cultivation at comparatively low frequencies, a biosensor structure working in the 7 GHz range has been designed and put into operation.

2.1. Concept of the 7 GHz Biosensor
The 7 GHz Biosensor consists of passive microwave structures and follows a differential principal. The operation concept is shown in Fig 1. The input signal is fed to a Wilkinson power divider. The divider splits the signal up into two ideally identical signals to a reference path and a sensing path, each consisting of microstrip lines. These lines are crossed by a PTFE tube building the microfluidic channel. The PTFE tube contains the sample that is to be investigated with the Biosensor. The microstrip line used as the reference path ($S_{21}^{(ref)}$ in fig. 1), is covered by a section of the tube, containing tetradecane, which is used to confine the liquid solution to be examined. Moreover, tetradecane serves as a reference fluid. The sensing path is covered by a section of the PTFE tube containing the sample of interest, in this case a glucose yeast mixture. To compare the propagation characteristics of the two paths, the signals labeled $S_{21}^{(S)}$ and $S_{21}^{(REF)}$ are fed to a ring hybrid. This coupler provides the interference of $S_{21}^{(S)}$ and $S_{21}^{(REF)}$ with 180 ° phase shift for the designated centre frequency at its output. A change in the permittivity of the sample alters the signal $S_{21}^{(S)}$. This variation can be observed at the hybrids output. Theoretical investigations on the transfer function of the designed biosensor show that the shift in frequency of the observed notch in fig. 2 and thus the level shift of the signal at a fixed frequency are mainly caused by a phase variation in the sensing path ($S_{21}^{(S)}$ in Fig. 1). Fig. 2 presents the numerically simulated transmission ($S_{21}^{(S)}$ in Fig. 1) of the biosensor. Attenuation and phase shift have been artificially introduced in the sensing path: the phase shift in the propagation path of $S_{21}^{(S)}$ is swept from 0 to 10 ° and the attenuation from 0 to 10 dB. One can see that the main parameter that changes the frequency of the observed notch is obviously the phase shift.

2.2. Experimental Setup
For the conducted experiments, the described sensor structure has been realized on a Rogers 3003 printed circuit board using SMA connectors for measurements. The PTFE tube, being part of a microfluidic system, is pressed on top by PVC jaws.

3. CW Spectrometer system
To investigate the effects at frequencies beyond 200 GHz, a continuous wave Terahertz spectrometer from Toptica [4] was used.
3.1. Principle of the CW System

The continuous wave spectrometer makes use of GaAs-photomixers to convert the beat signal of two near-infrared lasers into tunable terahertz radiation. The resulting signal is then guided by an interferometer, propagates through the sample under test (SUT) and is subsequently detected by a second photomixer. The measured parameter is the photo current in the receiver photomixer, which is a measure of the electric field of the terahertz wave. It is

\[ I_{PH} \propto E_{THz} \cdot \cos \Delta \phi = E_{THz} \cdot \cos 2\pi \Delta f / c, \]

where \( E_{THz} \) is the amplitude of the terahertz electric field, \( f \) the terahertz frequency, \( c \) the speed of light in vacuum and \( \Delta L \) basically the path length difference between transmitted and received signal of the spectrometer, resulting in a phase shift \( \Delta \phi \). [5] gives a detailed description. As the equation shows, the signal amplitude can directly be derived from the measured photo current. Using measurements with a reference sample, for example a yeast glucose mixture that has been in cultivation for a certain time, a relative phase can be extracted from the gathered data.

3.2. Experimental Setup

To investigate the sample of interest with the CW spectrometer, it needs to be exposed to the THz beam, what is accomplished by a sample holder fixing the PTFE tube. Since the thickness of the tube (0.55 mm in fig. 5) is almost equal to tube interior diameter (0.5 mm in fig. 5), a considerable amount of the radiated power is transmitted through the PTFE walls, which reduces measurement sensitivity.

To investigate the propagation behavior of the liquid exclusively, additional measurements have been performed using an epoxy resin aperture, which covers the sidewalls of the tube. The setup is sketched in Fig. 5. Since all the THz radiation, which is detected at the second photo mixer propagated exclusively through the sample, the phase shift due to cell cultivation is expected to be higher than in the case without the epoxy resin aperture.

4. Measurement Results

4.1. 7 GHZ Measurements

To experimentally determine the transfer characteristic of the microwave biosensor, scattering parameters were measured with a standard network analyzer. From the measured transmission, the shift in the notch frequency (in fig. 6) over time was derived. The result is depicted in Fig. 6. As it is shown, the biosensor exhibits a 125 MHz frequency shift of the transmission notch after 20 hours of cultivation. After that time, considering a cell doubling time of 90 min [6], one can assume no considerable increase in number of cells for the considered sample volumes. Comparing the measured results and the simulated ones from Sec. 2.1, one can say that the observed behavior in the measured transmission is mainly caused by a change in phase in the sensing paths transmission \( S_{21}(\text{S}) \). According to the performed ADS simulations, the frequency shift of 125 MHz corresponds to a phase change in \( S_{21}(\text{S}) \) of 1.2 °. Thanks to the rat-race based characterization procedure, this change is emphasized in order to achieve relevant measurement sensitivity.

4.2. CW Measurements

In [7] it has been shown that the evaluation of the amplitude of the photo current is not sufficient to distinguish between yeast samples at different cultivation times. Here the phase needs to be exploited...
Fig. 7 Change in phase versus cultivation time at 240 GHz, (a) without epoxy aperture, (b) with epoxy aperture
for proper monitoring of the cultivation state. Fig. 7 (a) displays the change in phase over cultivation duration for a Glucose yeast solution at 240 GHz. As the figure depicts, a change of 46° within 16 h is observed. As already mentioned in sec. 3.2, since a part of the radiated power is transmitted through the sidewalls of the tube, which decreases the effect on the phase of the signal, the sensitivity of the measurement can be emphasized by using the aperture described in Sec. 3.2 assuring that all radiation that is reaching the receiving photo mixer was propagating through the sample under test exclusively and not through the walls of the tube. For this modified configuration, the relative phase change over time is depicted in Fig. 7 (b). It is demonstrated that a much higher change in phase is achieved by using the aperture depicted in Fig. 5. Within 7 h, a change of 120° has been observed.

5. Conclusion
Investigations on the feasibility of characterization techniques of yeast cell cultivation at 7 GHz and 240 GHz have been performed. The amplitude information can be used to differentiate between samples exhibiting large difference in relative permittivity. However, to monitor yeast cell cultivation, one has to be able to differentiate between glucose with and without yeast. Thus it is necessary to detect comparatively small variations in permittivity. For this case, it is mandatory to exploit the phase information. This leads to the conclusion that mainly the change of the relative permittivity of the mixture evoked by progress of a yeast culture can be detected by the demonstrated techniques and hence be used for monitoring cell cultivation. Owing to its differential principle, the presented 7 GHz biosensor exhibits a high sensitivity of $\Delta f_m/t_c=125$ MHz/20h, enabling characterization of cell cultivation in pipe based microfluidic systems. The high sensitivity at 240 GHz of $\Delta \phi/t_c=2.9°/h$ along with the advantage of potentially compact sensor structures renders an exploitation of this frequency range extremely attractive for biosensing applications. Further experiments revealed that this sensitivity can be increased significantly to $\Delta \phi/t_c=17°/h$ by using the demonstrated aperture.

References
[1] K. Martin et al., “Generation of larger numbers of separated microbial populations by cultivation in segmented-flow microdevices,” Lab Chip, 2003, 3, pp. 202–207.
[2] C. Persch, “Messung von Dielektrizitätskonstanten im Bereich von 0.2 GHz bis 6 GHz und deren Bedeutung für die Mikrowellenerwärmung von Lebensmitteln,” Dissertation, Universität Fridericiana Karlsruhe, 1997.
[3] C. Song and P. Wang, “A radio frequency device for measurement of minute dielectric property changes in microfluidic channels,” Appl. Phys. Lett. 94, 023901 (2009).
[4] http://www.toptica.com/products/terahertz_generation/lasers_and_photomixers_for_cw_terahertz
z_generation.html, “cw Packages: Lasers and Photomixers for Frequency-Domain Terahertz Spectroscopy,” TOPTICA Photonics AG, 1998-2012, called september 9, 2012
[5] A. Roggenbuck et al., “Coherent broadband continuous-wave terahertzspectroscopy on solid-state samples,” New Journal of Physics,12, April 2010, pp 043017 (13pp)
[6] R. Lohaus, “Simulation der Hefezelle(Saccharomyces cerevisiae) und ihres Lebenszyklus,” mbi Technical Report, 2002
[7] J. Wessel et al., “Contactless Characterization of Yeast Cell Cultivation at 7 GHz and 240 GHz,” Biowireless 2013, Austin, USA, pp 70-72