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RF Characterization of intracellular microalgae lipids

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Abstract

This work presents an original approach to sense the lipid intracellular content of microalgae, by the Radio Frequency (RF) dielectric spectral characterization of these unicellular organisms. To do so, a “homemade” open-ended coaxial sensor was associated to a Vector Network Analyzer (VNA), which allowed a broadband characterization in the frequency range from 30 MHz to 3 GHz. The sensor combines a coaxial line with a modified SMA connector. This study shows that the lipid quantification can be determined with the complex dielectric spectra properties extracted from the reflection coefficient (\underline{S}_{11}) measurement. A calibration of the open-ended coaxial probe is performed by using three different conditions (vacuum, water, methanol) from which the complex dielectric behavior is mastered. A model inversion is finally achieved to estimate the complex permittivity spectrum of the biological samples in a large frequency range, which is related to the microalgae lipid content.

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Keywords: RF characterization; Broadband; RF sensors; Intracellular; Microalgae; lipid accumulation;

1. Introduction

Electric characterization of biological species is classically used as a non invasive way to extract physiological parameters (cell diameter, cell membrane properties...) but the main available techniques are based on low frequency ($f < 10$ MHz) measurements using direct impedance measurement [1] or electrorotation experiments [2]. By

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using dielectric spectroscopy based on Frequency Domain Reflectometry (FDR), cell intracellular characterization is possible by the penetration of the RF waves within the cell. Results depend strongly on cell composition [3]. Dielectric measurements at Radio Frequency allow intracellular and noninvasive characterization without the need of any sample treatment. In 2014, M. Al Ahmad *et al.* have presented a RF characterization of microalgal lipid content [4]. However, the method is based on a resonant technique which does not retrieve the dielectric spectrum over a wide frequency band. Here, the application of the RF spectroscopy to monitor *in situ* and in real time the lipid content of the microalgae “*Chlamydomonas reinhardtii*” in a bioreactor for algal biofuel production [5] is presented [6]. The set up description is given in section 2. RF measurements and an inversion model applied to estimate the complex permittivity spectrum of the microalgae are presented in section 3.

2. Set-up description

To address the increasing need in characterization of biological cells, innovative measurement techniques must be developed. Dielectric measurements at Radio Frequency can meet this challenge, and provide solutions to the limitations of some monitoring methods. Indeed, RF waves allow intracellular and noninvasive characterization without any sample treatment.

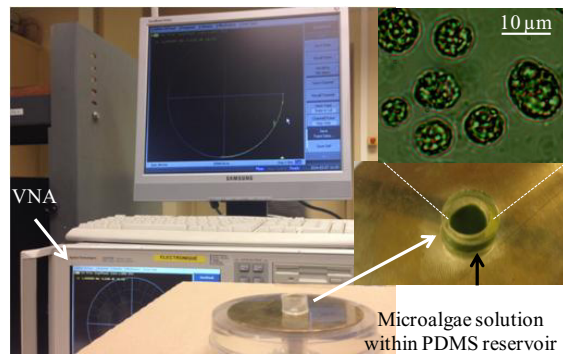


Fig. 1. Photography of the test bench. The inset is optical microscope photography of suspended microalgae solution. (1 ml – number of cells $\sim 7 \times 10^7$).

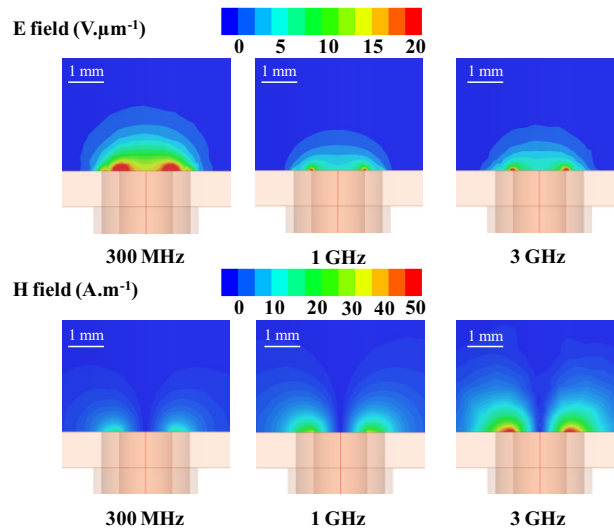


Figure 2: Magnitude of the electrical and magnetic field distributions at the test frequencies 0.3, 1 and 3 GHz in cross-section (on conductive medium; 0.1 S.m^{-1}).

The method presented in this paper is based on an adapted open-ended coaxial probe that is classically used to characterize porous material [4] and enables here a broadband measurement of RF scattering parameters \underline{S}_{11} of

microalgae suspensions. The Fig.1 shows the photography of the test bench using a VNA (E5061B) with the “homemade” probe. The microalgae suspension is inserted inside PDMS reservoir with 10 mm diameter above the probe.

The probe is a coaxial line and a modified SMA connector. The diameter of the inner conductor is equal to 1.5 mm, while the inner diameter of the outer conductor is equal to 4.2 mm. The distribution of the electrical and magnetic fields in the vicinity of the probe is investigated by means of electromagnetic simulations (@Ansys/HFSS) in the frequency range of 0.03 - 3 GHz. Fig. 2 presents the magnitude of the electrical and magnetic field distributions (on conductive medium; 0.1 S.m^{-1}) at the test frequencies of 0.3, 1 and 3 GHz. We can note that the fields are well confined at the end of the probe and the depth resolution depends directly on the probe geometry and size.

3. RF measurements

The complex relative permittivity $\epsilon_r^* = \epsilon_r' - j\epsilon_r''$ can be expressed through the reflection coefficient \underline{S}_{11} in the following form:

$$\epsilon_r^* = \frac{c_1 \underline{S}_{11} - c_2}{c_3 - \underline{S}_{11}} \quad (1)$$

c_1 , c_2 and c_3 are three complex calibration constants which are determined using three measurements of perfectly known dielectric liquids [7]. An Open-Water-Liquid (OWL) calibration procedure is conducted and (1) can be rewritten as:

$$\begin{aligned} S_{11}^O c_1 - c_2 - \epsilon_{r,O} c_3 &= -\epsilon_{r,O} S_{11}^O \\ S_{11}^W c_1 - c_2 - \epsilon_{r,W} c_3 &= -\epsilon_{r,W} S_{11}^W \\ S_{11}^L c_1 - c_2 - \epsilon_{r,L} c_3 &= -\epsilon_{r,L} S_{11}^L \end{aligned} \quad (2)$$

Suspensions of *Chlamydomonas reinhardtii* of 7×10^7 cells/ml with cells presenting two levels of lipid content (30%, 70%) were tested. Cells with different lipid contents observed by Confocal Laser Scanning Microscopy (CLSM) are shown in Fig 3. Lipid droplets emitting a green fluorescence were stained with Bodipy 505/515 [7].

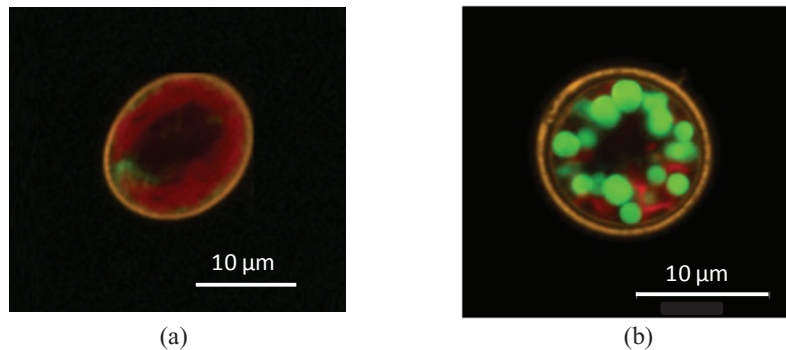


Fig. 3. Confocal Laser Scanning Microscopy of *Chlamydomonas reinhardtii* cells with different lipid content. (a) no lipid droplets, (b) high lipid content. (Green fluorescence: lipid droplets stained with Bodipy, red fluorescence: chlorophyll autofluorescence, yellow fluorescence: cell wall stained with concanavalin A).

All measurements were obtained at room temperature from 30 MHz to 3 GHz (Fig. 4). Each calibration standard is measured using the average of 50 runs and input source is set to 0 dBm. The temperature of the liquids was also checked. The two different evolutions of the measured reflection coefficient \underline{S}_{11} are associated to cell lipid content and thus the intrinsic physical properties of the microalgae.

Thanks to the model given in Eqs. (1)–(2), the complex permittivity of the microalgae suspensions with different lipid contents can be easily determined. Three calibration measurements were carried out: open, deionized water ($\epsilon_{r,\text{water}}=78$) and methanol.

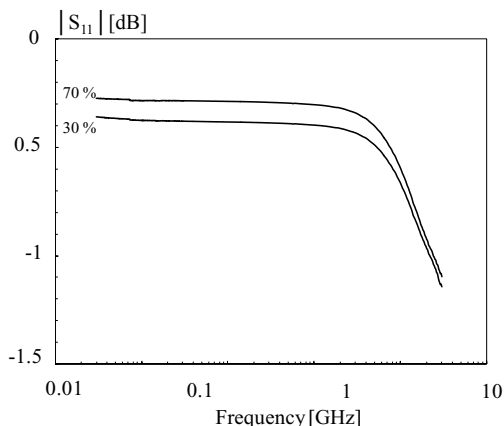


Fig. 4. Measured reflection coefficient S_{11} for suspensions of microalgae with different lipid contents.

After obtaining these three complex parameters, the unknown relative complex permittivity can be found from the measured reflection coefficient S_{11} at each frequency. Table 1 summarizes the resulting complex permittivity at 0.3, 1 and 3 GHz for the different suspensions of microalgae. We can note that the complex dielectric permittivity of microalgae suspension decreases when the lipid content concentration ($\epsilon_{r, \text{lipid}}=2.5$) increases. Results demonstrate that the lipid content of *Chlamydomonas reinhardtii* can be evaluated by the developed method.

Table 1. Complex dielectric permittivity ϵ_r^* determined.

Lipid content	ϵ'			ϵ''		
	0.3 GHz	1 GHz	3 GHz	0.3 GHz	1 GHz	3 GHz
30%	77.18	76.62	73.97	9.6	6.4	12.03
70%	75.23	74.59	71.86	9.32	6.35	11.99

4. Conclusion

In this work, an open-ended coaxial line probe is used to obtain dielectric spectra of microalgae suspensions with different lipid contents in a large frequency range (0.03 – 3 GHz). The frequency dependent complex permittivity was deduced from the measured complex reflection coefficient S_{11} after a calibration procedure using three standards: air and two well-known liquids. Results demonstrate that biological cells can be non-invasively characterized by the developed RF method in a broadband range of frequencies. New applications in a wide range of different fields such as medicine, biology, chemistry and food industry can therefore be envisaged.

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