Study of two sporulated *bacillus* species by THz time domain spectroscopy

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***Abstract*—Far Infrared spectra of *Bacillus subtilis* and *Bacillus thuringiensis* were recorded using Terahertz time-domain spectroscopy (THz-TDS). Multiple dilutions of each species were prepared to enable the analysis of spectral data up to 2,5THz. The optical constants of pure spores were then extracted using the well know Maxwell-Garnett effective medium model. We show that the refractive index can be used to discriminate the two sporulated species, contrarily to absorption coefficient.**

I. Introduction And Background

DURING the last decade, Terahertz Time Domain Spectroscopy (THz-TDS) became a highly active research field, owing to the availability of THz wave source. THz -TDS is a particularly interesting tool as it is a coherent method, enabling experimental determination of both absorbance and refractive index. This technology exhibits good prospects in security applications, since many wrapping materials are transparent, or nearly so, to THz[1] whereas drugs[2] and energetic substances[3] shows specific spectral features. We wish to report here on the THz –TDS transmission study for discrimination of two bacillus species in sporulated form: *Bacillus subtillis* and *Bacillus thuringiensis*.

II. RESULTS

The two Bacillus species shows the same global features, absorbance increases with frequency whereas refractive index slightly decreases without specific spectral signature. Frequency cut-off decreases with dilution of the absorbant biological material, allowing one to analyse the spectrum of each species up to about 2THz for 10% spores in HDPE (figure 1).

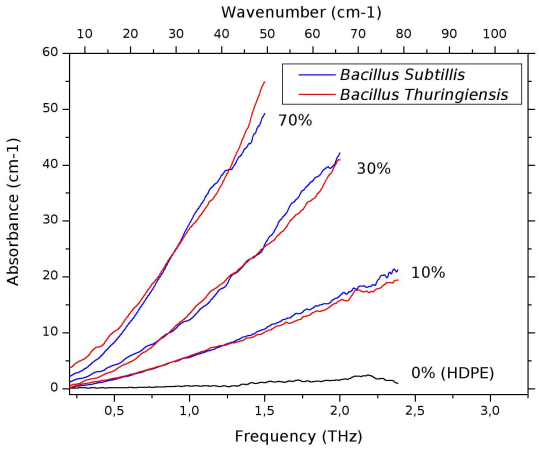

Figure 1: absorbance of the two spore species at various concentrations.

The lack of specific spectral features may be explained by the nature of the samples. Spores are complex assembly of many molecules, including proteins, sugars, lipids, nucleic acids, peptidoglycans, dipicolinic acid, etc. Since much of these compounds are macromolecules, they individually show number of vibrational modes in within the terahertz range. Thus, a tremendous number of vibrational modes occur very close so they can't be resolved by THz-TDS. Scattering of the THz wave can't explain these absorbances, particles sizes of both biological material and HDPE being much lower than THz radiation wavelength.

All extracted refractive index values are in good agreement with group refractive index.

Considering standard deviations calculated on 6 measurements of each spore's concentration, one can show that refractive index values began to be discriminative up to 30% of spore. However, absorbance is not a relevant parameter to differentiate the two spores, whatever the considered fraction of spore. Since the preparation of pure *Bacillus* spores pellets was problematic for high fraction of spore, the optical constants (refractive index and absorption coefficient) of pure spores were obtained using a Maxwell-Garnett effective medium model (figure 2).

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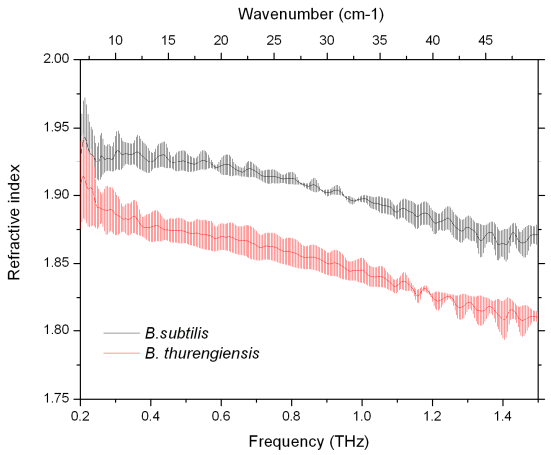

Figure 2: refractive index of the two spore species extracted by the Maxwell-Garnett effective medium model.

At 1THz, pure *Bacillus subtillis* shows a refractive index of 1,89 whereas pure *Bacillus thuringiensis* is 1,84. These values are close, but difference observed between them is relevant, as standard deviations calculated using the three concentrations are clearly separated within the 0,5-1,5THz range. As pointed out in dilutions study, the extracted absorption coefficient is not discriminative.

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