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On the far infrared spectra of bacterial suspensions

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Fisica. — *On the far infrared spectra of bacterial suspensions* (*). Nota di MASSIMO CERDONIO e MARCO SAMPOLI, presentata (**) dal Corrisp. G. CARERI.

RIASSUNTO. — Vengono discusse le possibilità delle attuali tecniche sperimentali nel campo della spettroscopia ultra–infrarossa (FIR) adoperata sulle soluzioni. Gli AA. danno dei risultati sperimentali sui batteri nella loro soluzione madre e discutono sui limiti superiori che possono essere assegnati alla loro assorbenza « in vivo ».

§ 1. – FIR SPECTRA OF SOLUTION

The standard procedure to get the FIR absorption spectrum of some material dispersed in a host material is to record a spectrum of a dispersion, e.g. of bacteria, and a spectrum of the host material alone, e.g. the mother solution; then the ratio $R(\tilde{\nu}) = \frac{I_B(\tilde{\nu})}{I_S(\tilde{\nu})}$ is formed between the intensities transmitted by the sample with the bacteria $I_B(\tilde{\nu})$ and by the background $I_S(\tilde{\nu})$, that is the mother solution alone, in the same experimental conditions. This will be done with some resolution Δ, typically $\Delta \simeq 1 \div s\ \mathrm{cm}^{-1}$, and with some uncertainty δR on $R(\tilde{\nu})$, typically within a few parts in $10^{-3} \div 10^{-2}$.

We shall not discuss here the techniques needed to achieve the best performance for Δ and δR; a detailed discussion can be found in reference [1].

Let we consider here a solution and assume the thickness X of the sample and of the background to be the same; then we easily obtain for $R(\tilde{\nu})$ the relation

$$R(\tilde{\nu}) = \exp\left[-c_B(\alpha_B - \alpha_S)X\right] \tag{1}$$

where c_B is the concentration of the solute and α_B, α_S are the absorbivities at the wavenumber $\tilde{\nu}$ respectively of the solute and of the solvent.

In the limit of small concentration of the solute $c_B \ll 1$ and $\alpha_S X \approx 1$ (this last figure is needed to get a reasonable transmittance of the background) relations (1) can be written

$$R(\tilde{\nu}) - 1 = c_B(\alpha_S - \alpha_B)X\,. \tag{2}$$

In practice it is difficult to set the experimental conditions to be the same in recording the two spectra with an accuracy comparable with the error on $R(\tilde{\nu})$ due to instrumental noise; so it is convenient to arbitrarily set $R(\tilde{\nu}) = 1$ at some $\tilde{\nu} = \tilde{\nu}_0$ where $\alpha_B(\tilde{\nu}_0) = \alpha_S(\tilde{\nu}_0)$; it follows that the

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(**) Nella seduta del 18 giugno 1971.

measured quantity is

$$\Delta R(\tilde{\nu}) = R'(\tilde{\nu}) - 1$$

where $R'(\tilde{\nu})$ is the arbitrarily renormalised value of $R(\tilde{\nu})$.

From these simple considerations we learn the following things:

i) in the given limits for c_B and $\alpha_S X$, if $\alpha_B(\tilde{\nu})$ is constant over the whole range of measurements in $\tilde{\nu}$, which would be the case of a broad absorption band of the solute, no definite result can be given for $\alpha_B(\tilde{\nu})$.

ii) if $\alpha_B(\tilde{\nu}) \neq \alpha_S(\tilde{\nu})$ on a bandwidth smaller than that allowed, but larger than the instrumental resolution Δ, an absorption band in the solute can be resolved if at least

$$c_B |\alpha_B - \alpha_S| X \geq \delta R \tag{3}$$

around some wavenumber $\tilde{\nu}$.

iii) if $\alpha_B(\tilde{\nu}) \neq \alpha_S(\tilde{\nu})$ over a bandwidth d smaller than the resolution Δ we easily obtain the corresponding condition

$$\frac{d}{\Delta} c_B |\alpha_B - \alpha_S| X \geq \delta R \,. \tag{4}$$

We see that in the practical case of $\alpha_S X \approx 1$ and in the favourable case $\frac{d}{\Delta} \simeq 1$, if δR and c_B are of the same order of magnitude, as will be the case for dilute solutions, the upper limit which can be given for $\alpha_B(\tilde{\nu})$ is of the same order of the value of $\alpha_S(\tilde{\nu})$.

§ 2. – EXPERIMENTAL DETAILS AND RESULTS

We report FIR spectra of yeast and *escherichia coli* living in their mother solution in the spectral region between 10 cm^{-1} and 70 cm^{-1} where water is relatively more transparent. A typical result is shown in fig. 1; at glance no noticeable difference could be seen in respect to the spectrum of the mother solution alone. All the spectra were recorded and analized to give the ratio spectrum with a commercial Michelson interferometer equipped with Fourier transform facilities [1]. To eliminate water vapor bands the interferometer was evacuated except the sample cell, which was maintained in dried air. The samples were obtained reconcentrating a culture by centrifugation and partial redilution; a drop of the final product was pressed between two quartz windows 0.5 mm thin, held apart by 20 μm with a mylar spacer; it was obtained a coverage C of the living matter in respect to the mother solution of $C \approx \frac{1}{3} - \frac{1}{5}$ of the area exposed to the FIR beam. By visual observation of the mobility of the bacteria just before and just after the recording of the spectra it was checked that no appreciable fraction of them had died during all the procedure.

The results are shown in fig. 2 and fig. 3; as can be seen, no significant structure or band over the instrumental noise, which is marked by the dashed

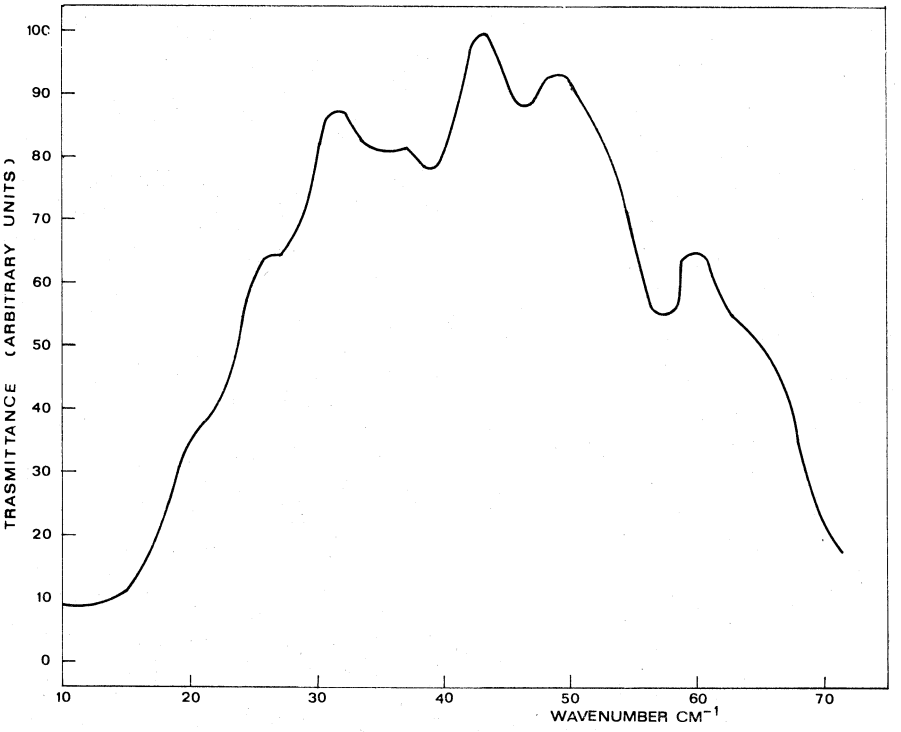

Fig. 1. – Trasmittance of yeast living in its mother solution; not corrected for instrumental background.

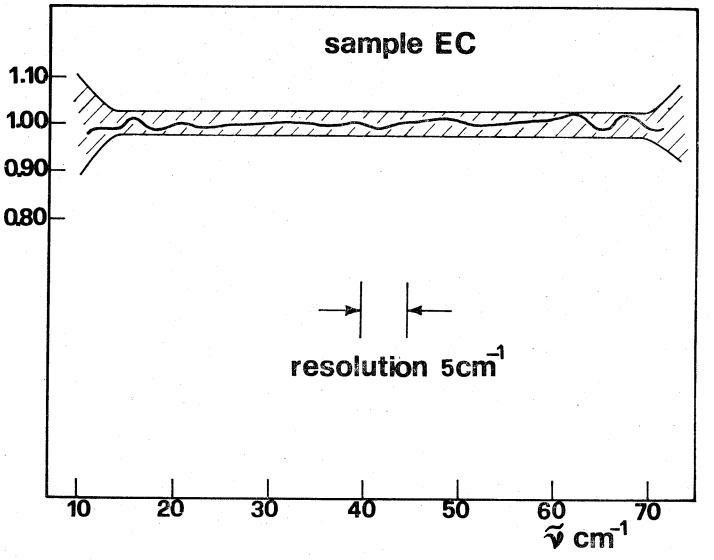

Fig. 2. – Ratio spectrum of *escherichia coli* to its mother solution; dashed region marks the instrumental error.

region, can be resolved in these spectra. In the spectral regions outside that reported, because of the high absorbivity of the mother solution (see fig. 1), the noise grows rapidly and so no results can be given. From the above results it can be concluded that no exceeding absorption above 3 % of the total can be ascribed to the living matter present in the culture in the spectral region 10 cm^{-1} to 70 cm^{-1} at a resolution of 5 cm^{-1}.

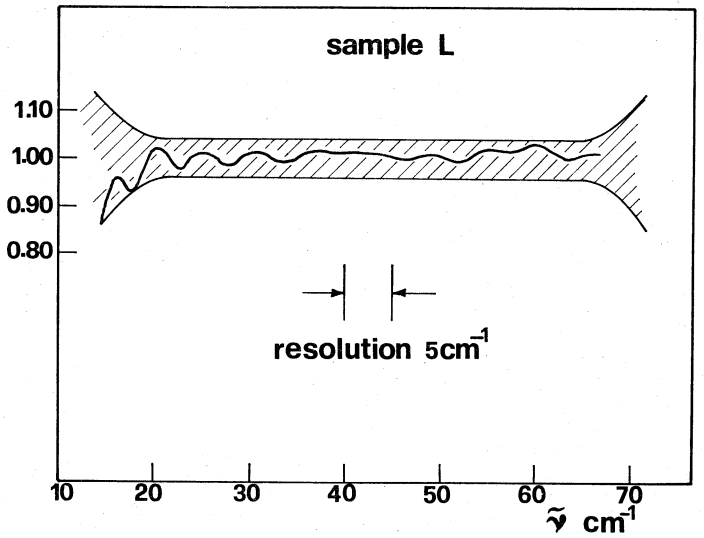

Fig. 3. – Same as fig. 2 for yeast.

§ 3. – DISCUSSION

To discuss the above negative results let us consider our suspension of bacteria as true solutions; this assumption is justified by the fact that we expect the refractive index of the bacteria to be not so much different from that of the mother solution; so FIR radiation will not be scattered away by the suspended particles but may indeed be absorbed by themselves.

The volume concentration c_B of living matter is given by the relation

$$c_B = C \frac{x}{X}$$

where C is the coverage, x a characteristic thickness of the living cells and X is the thickness of the mother solution film: as $C \lesssim 0.3$, $x \approx 2$ μm and $X = 20$ μm, we get $c_B \approx 3$ %.

If we assume that some characteristic band arise in the living cells as a whole as proposed by Frölich [2] from our experimental limitations and relation (3) for $\delta R \simeq 3$ %, we get

$$|\alpha_S - \alpha_B| X < 1 .$$

Now an average figure for α_S, the absorbivity of the mother solution, in the investigated frequency range is [3] $\alpha_S \simeq 200$ cm^{-1} so we get the following

limits for the absorbivity of our living cells as a whole

$$0 \leq \alpha_B \leq 700 \text{ cm}^{-1}$$

in the spectral range between 10 cm^{-1} and 70 cm^{-1} at a resolution of 5 cm^{-1}.

That is, due to experimental limitations no difference in the absorbivity of living cells in respect to that of the mother solution can be detected with an error smaller than 350 %.

§ 4. – Conclusions

From the above discussion we can conclude that the absorbivity of *escherichia coli* and yeast is not different, in the quoted limits, from the absorbivity of the mother solution; this excludes, with the quoted accuracy, the existence of any strong transversal absorption band due to living cells as a whole, in the region between 10 cm^{-1} and 70 cm^{-1}; obviously this does not exclude the existence in this spectral region of strong longitudinal modes as recently proposed by Frölich [2].

It should be noted that considerable improvements [4] can be made for this type of experiment. By a proper choice of a liquid helium cooled solid state detector, together with a careful optic design and using a computer to analize the data, one can hope to achieve a resolution $\Delta \simeq 1 \text{ cm}^{-1}$ and, at the same time, an experimental error on the renormalized ratio of the order $\delta R \simeq 10^{-3}$; it is clear from the given formulae that the absorbivity $\alpha_B(\tilde{\nu})$ could be bracketed between more strict limits.

We thank Prof. G. Careri in suggesting this experiment and for continuous discussions; we are most grateful to Prof. Tonolo for his kind help in preparing and testing the samples and to Dr. G. Signorelli and V. Mazzacurati for helpful discussions.

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