Detection of the effect of the sulphido-reducing pathogenic bacteria by an ultrasonic method

Jaouad Abrehouch[1,2], Bouazza Faiz[1], Ali Moudden[1], Rachid Latif[1],
Dominique Décultot[3] and Gérard Maze[3]
[1]*Laboratoire d'Instrumentation et Mesures, Université Ibnou Zohr d'Agadir (Maroc).*
[2] *Laboratoire d'assistance pour le développement de la qualité des produits de la mer, Délégation des Pêches Maritimes, Agadir (Maroc).*
[3]*Laboratoire d'Acoustique Ultrasonore et d'Électronique, LAUE UMR CNRS 6068, Université Le Havre (France, Email : gerard.maze@univ-lehavre.fr).*

Introduction

The sterilization applied to the tinned fishes is realized to the public health and the bacteriologic stability of foods. The clostridium botulinum, which is an anaerobic sulfito-reducing (ASR), is choice as reference bacterium because its pathogen spores are more heatproof. Its extermination induces the extermination of the others [1-2]. The microbiologic analyses of ASR require a sterilised material in addition to absolute work conditions and have the disadvantage to be slow and expensive, in the same way a microbiologic control of the ended produce does not permit to detect, in real time, a failure and to correct a manufacturing defect. Negative results on some samples subjected to a microbiologic analyse does not induce that the whole sample is completely healthy. Now, ultrasonic techniques are used, they are speedier, less expensive and particularly less constraining (asepsis conditions) [3,5,6].

Analyse method of the sulphido-reducing by anaerobic pathogenic bacteria

The clostridium botulinium is bacterium commonly found in the ground, the manhole water and the intestine of humans and animals. They can infect and shade off the tinned foods in anaerobic conditions. It is a pathogenic bacterium and has a great power to transform the sugars and proteins, releasing butyrique acid or sulphurous hydrogen H_2S [4]. The ASR pathogenic bacteria reduce the sulphites into sulphides. The ASR are strictly anaerobic, only their spores resist during 10 minutes at a temperature of 80°C. Their incubation temperature is 37°C.

The culture medium is constituted by a regenerated meat-liver with sodium sulphite 5%, ammoniac iron III alum 5% and sea water with clostridium botulinium. The necessary volume of water take place in a sterilised glass tank which is heated to a temperature of 80°C during 10 minutes to destroy all the germs. A part of the culture medium is added to the water in the analyse tank taking care of no air bubble enter in the mixture. The tank is closed with a paraffin stopper to isolate the mixture of air. This mixture is incubated at the temperature of 37°C during 24 or 48 hours. Black colonies of pathogenic bacteria are developed in the anaerobic medium producing sulphides from sulphites which form a precipitate with the iron ions.

Ultrasonic method

Figure 1 describe the experimental set-up constituted by an impulse generator which excites a broadband transducer with a middle frequency of 10 MHz.

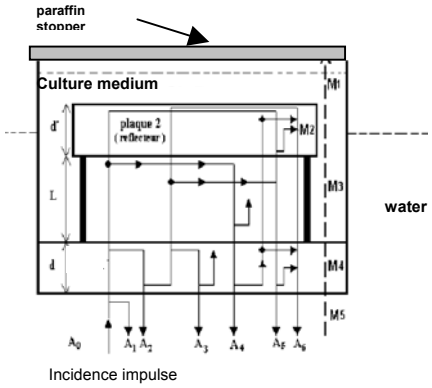

Figure 1 : propagation paths in sample tank.

The ultrasonic impulse is propagated in water and crosses the culture medium before to reflect on a glass surface (see Figure 1). The same transducer detects the signal which is amplified and digitalised by an oscilloscope Lecroy. This time signal is transmitted to a micro-computer. With this micro-computer, the phase velocity is calculated versus incubation time. The sample constituted by the culture medium takes place in a glass tank in part immersed in water at the temperature of 37°C.

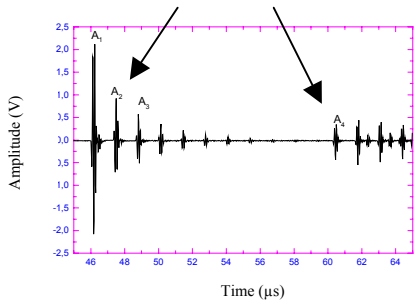

Figure 2: recorded ultrasonic signal.

The echoes recorded in the hard disk of the micro-computer are presented on Figure 2: A_1 : the specular echo reflected on the bottom face of the plate 1, A_2 : the reflection on the top face of the plate 1 and the culture medium, A_4 the reflection

echo on the bottom face of plate 2. For the measure of the phase velocity, the echoes A_2 and A_4 (arrows) will be used because these two echoes have a same path in the plate 1, the A_4 echo has a double path in the culture medium. To test the experimental set-up, the sample tank has been filled with water and its velocity has been measured: C_{water} = 1475 m/s.

Experimental results and discussion

To determine the ultrasonic phase velocity in the culture medium in the sample tank, the echoes A_2 and A_4 are used [3,5,6]. The phase of these signals can be written:

Phase of A_2: $$\phi_{A_2} = \frac{2d\omega}{C_{glass}} + \phi_0 ,$$

Phase of A_4: $$\phi_{A_4} = \frac{2d\omega}{C_{glass}} + \frac{2L\omega}{C_{sample}} + \phi_0 .$$

The phase velocity can be written:

$$C_{sample} = \frac{2L\omega}{\phi_{A_4} - \phi_{A_2}} .$$

To obtain experimentally the phases, the FFT of signals A_2 and A_4 are calculated. These phases are written:

$$\phi_{A_2} = Arc\tan\left[\frac{I_2(\omega)}{R_2(\omega)}\right], \quad \phi_{A_4} = Arc\tan\left[\frac{I_4(\omega)}{R_4(\omega)}\right]$$

with R_2 and R_4 are the real parts and I_2 and I_4 are the imaginary parts respectively of echo A_2 and echo A_4. These phases vary between $-\pi/2$ and $+\pi/2$. To use these phase it is necessary to have continuous phases, an algorithm is developed to obtain this result. It is possible to calculate the phase velocity as function of the frequency ω for different incubation time in the culture medium.

The clostridium bacteria can reduce the sulphite, this chemical reaction displaies the iron sulphide in the making from the sodium sulphite and the iron salt. The bacterium growth is obtained in a reducing medium at the temperature between 30°C and 37°C during a period of 24 to 48 hours. It is described in 5 phases (Fig. 3):

- phase 1: it is a latence phase, the growth is null. The period delay depend on the quality of bacteria and the quality of culture medium;
- phase 2: an exponential growth;
- phase 3: the speed of growth is reduced;
- phase 4: the growth is stationary, the number of bacteria does not increase;
- phase 5: it is a declining phase, the growth is negative, the nutritive resources are consumed.

In Figure 4, the measures of the phase velocity obtained in previous conditions are presented (curves 2 and 3). These results are compared with those obtained for a sterilised culture medium (curve 1). The velocity on curve 1 is constant, the medium is not modified. The curve 3 is obtained with a microbial quantity larger than in the case of the curve 2. The curves 2 and 3 describe the next phases:

- the first part of the curves are related to the phase 1, 2, 3;
- the second part of these curves is related to the cellular destruction, the phase velocity shows a sharp transition (phase 4);
- the third part, for which the phase velocity is smaller, is related to the phase 5, the declining phase.

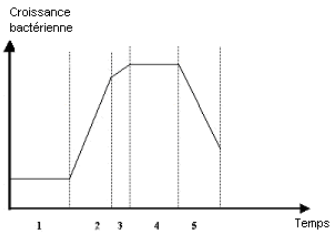

Figure 3: growth of bacteria.

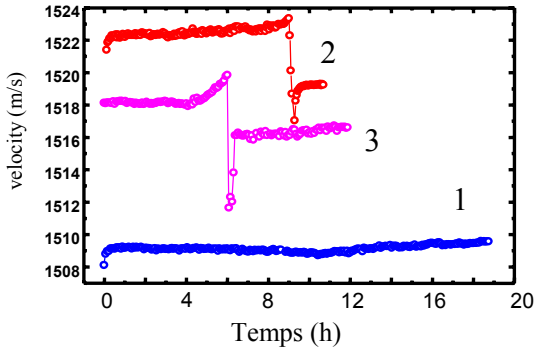

Figure 4: Evolution of the phase velocity versus time for different concentrations of bacteria.

Conclusion

The ultrasonic method based on the measure of the phase velocity allows to detect the action of sulphido-reducing pathogenic bacteria in a culture medium. It also permits to determine the quantity and the virulence of the bacteria.

Acknowledgments

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