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# The Influence of Low Power Microwave Radiation on the Growth Rate of *Listeria Monocytogenes*

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***Abstract*—**Variations in the growth rate constant of the *Listeria monocytogenes* bacterial species were determined at 37°C in irradiated environments and compared to the situation of a non-irradiated environment. The bacteria cells, contained in a suspension made of a nutrient solution of Brain Heart Infusion, were made to grow at different frequency (2.30÷2.60 GHz) and power (0÷400 mW) values, in a plug flow reactor positioned in the irradiated environment. Then the reacting suspension was made to pass into a cylindrical cuvette where its optical density was read every 2.5 minutes at a wavelength of 600 nm. The obtained experimental data of optical density vs. time allowed the bacterial growth rate constant to be derived; this was found to be slightly influenced by microwave power, but not by microwave frequency; in particular, a minimum value was found for powers in the 50÷150 mW field.

***Keywords*—**Growth rate constant, irradiated environment, *Listeria monocytogenes*, microwaves, plug flow reactor.

## I. Introduction

ELECTROMAGNETIC radiation of frequency ($v$) in the field between 2.30 and 2.60 GHz, although lacking a photon energy $E$ ($E=h\cdot v$) high enough to be able to modify the atomic structure of components that are present in the irradiated environment, can still interact with supramolecular structures, such as cells, modifying their developmental characteristics. It can also interact with bacterial populations in the same way [1], [2]. Furthermore, since the existence of interactions between microwave (MW) radiation and the human body have been ascertained [3], [4], it may be thought that if microwaves can create an interference with extremely developed organisms and with the natural protection of the human body, they may interfere even more with microorganism populations which have far less natural protection than more evolved organisms.

Therefore, the object of the present work was the study of the possibility of reducing the growth rate of the *Listeria monocytogenes* bacterial species, a common cause of food-borne infections, in a nutrient aqueous solution of Brain Heart Infusion at a temperature of 37°C; the microorganisms were made to grow in a PFR reactor irradiated with very low-power ($P$<400 mW) microwaves and at frequencies ranging between 2.30 and 2.60 GHz; similar studies have already been conducted with other bacterial species in similar experimental conditions [5]–[8].

Any reduction in the bacterial growth rate due to the use of low power MWs would have considerable importance, not only from a scientific point of view, as it would indicate the presence of the non-thermal effects of this type of radiation, but also from a technological one, since it would show the possibility of using low power MWs in food industry processes, thereby increasing the shelf-life of finished products while contemporaneously preserving their nutritional and organoleptic characteristics; in fact, the use of low power allows operation at low temperatures, both during production and in the storage phase, which preserves the afore-mentioned characteristics in the treated substances. This latter consideration has stimulated the authors' interest towards the study of the variation of the bacterial growth rate constant in environments that are irradiated with low power MWs and at a temperature of 37°C: in fact, at this temperature it is reasonable to assume that all the nutritional and organoleptic properties of interest for humans are preserved.

## II. Experimental

The nutrient solution was prepared by dissolving 3.7 g of Brain Heart Infusion (BHI) in 100 ml of distilled water; this aqueous solution, previously sterilized in an autoclave, was inoculated with 1 ml of a suspension of *Listeria monocytogenes* containing about $2\cdot10^7$ cells. The obtained reagent suspension was made to pass through a PFR reactor where it could react both in an irradiated environment and in a non-irradiated one.

In order to avoid any external contamination of the reacting suspension at any time, the process was made to evolve in a specially designed system and completely isolated from the external environment. The bacterial concentration in the reacting suspension exiting the reactor was continuously determined spectrophotometrically, without taking samples; in fact withdrawal operations could be the cause of contamination. The experimental structure in use is schematically shown in Fig. 1.

The Pyrex bottle (5) was filled with a nutrient solution containing 37 g/l of BHI and then hermetically sealed by means of the special cap (4) and connected to the circuit (part A in Fig. 1); all parts, together with the plate with the reactor,

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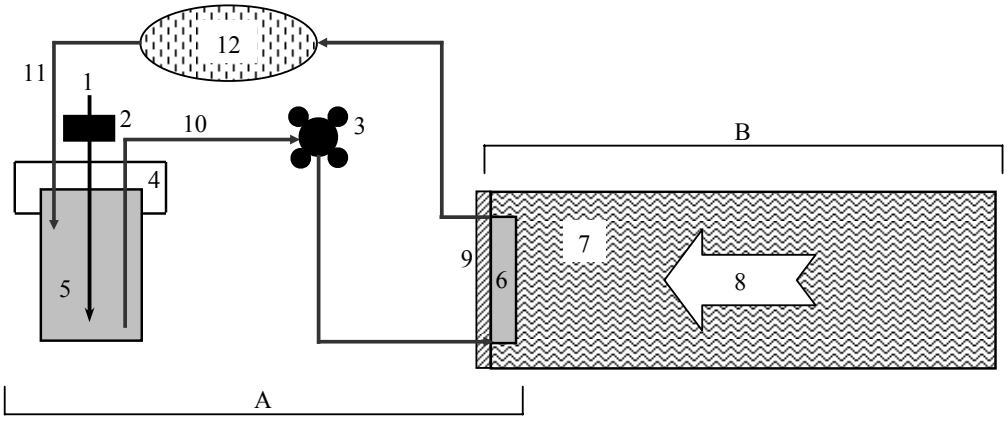


Fig. 1 Scheme of proposed experimental structure (A: sterilizable circuit; B: irradiated environment; 1: external air; 2: 20 μm ceramic filter for external air; 3: peristaltic pump; 4: cap; 5: Pyrex bottle containing the suspension to be irradiated; 6: PFR where the bacterial suspension is irradiated; 7: waveguide; 8: incident radiation; 9: PFR supporting plate positioned as the waveguide closure ; 10: extraction of the bacterial suspension from bottle; 11: recycling of the bacterial suspension to the bottle; 12: continuous system of spectrophotometric analysis)

were sterilized by placing the entire block in an autoclave at 121°C for 20 minutes. After the sterilization and reassembly of parts A and B, the solution was inoculated through the external air duct (1) devoid of the filter (2). The reacting suspension was made to circulate by means of a peristaltic pump (3) with a flow rate of 40 ml/min.

The reactor was made of silicone and placed in the irradiated part of the stainless steel plate (9) placed in the waveguide closure (7); residence time is equal to 24 s; silicone was used because of its transparency in the range of used frequencies [9]. The experimental circulating apparatus was made with silicone tubing (ID 2 mm; OD 4 mm) and, where necessary, with stainless steel tubing (ID 2 mm).

The apparatus for MW generation and transportation has already been used in a previous work [7] and is schematically shown in Fig. 2; MW radiation was generated by means of a YIG oscillator (1) at the frequencies of 2.30, 2.40, 2.50, and 2.60 GHz; thereafter the power of the radiation was increased by an amplifier (4) and sent, via a cable-guide converter (6), to a type WR430 waveguide (7), which had a closing plate (8) collocated in its final part, supporting the PFR in which the mixture could react in the irradiated or non-irradiated environment.

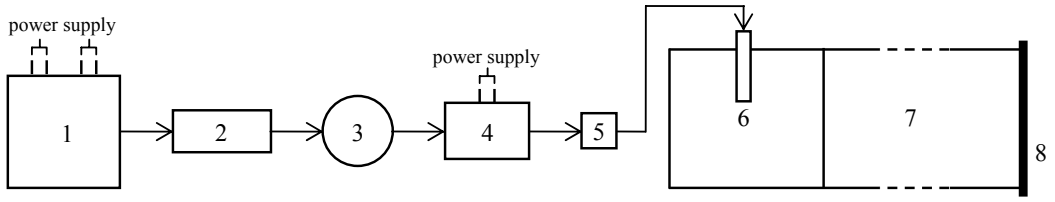


Fig. 2 Representation of the microwave generating apparatus (1: YIG oscillator, 2: fixed attenuator, 3: rotating attenuator; 4: amplifier; 5: insulator; 6: cable-waveguide coupler; 7: type WR430 waveguide; 8: end plate supporting the reactor); the connections were made by means of type RG316 cables

## III. Discussion

Starting from the Monod equation, if the growth of a bacterial species takes place in an environment with a large excess of substrate, it is possible to write:

$$\ln\left(C_C / C_{0C}\right) = k \cdot t \,. \tag{1}$$

In (1), $C_C$ is the concentration of microorganisms at time $t$, while $C_{0C}$ represents the concentration of microorganisms at time $t$=0. The analysis of the reacting mixture was carried out by reading the optical density (*OD*) by using a spectrophotometer (Varian Cary 50®); if the zero of the spectrophotometer is set using the nutrient solution of BHI before being inoculated, the relationship between optical density and the concentration of biomass can be written as:

$$C_C = \alpha \cdot OD \,. \tag{2}$$

Equation (2) is only valid for low values of *OD* [10]; if this condition is satisfied, Equation (1) can be rewritten in the

form:

$$\ln\left(OD_t / OD_0\right) = k \cdot t \,, \quad (3)$$

where $OD_t$ is the optical density of the reacting solution read at the time $t$, and $OD_0$ is the reference value.

The experimental curves of $OD$ vs. $t$ were obtained with power values equal to 0, 50, 100, 150, 200, 250, 300, 350, and 400 mW and frequencies equal to 2.30, 2.40, 2.50, and 2.60 GHz. The growth rate constant $k$ was derived from (3) by means of linear regression of the experimental points reported in the form of Ln ($OD_t$) as a function of $t$. In Fig. 3 the values of $k$ obtained under the different experimental conditions are shown; each point represents the medium value of nine different experimental results.

In order to confirm the validity of the growth constant values, obtained from experimental measurements of $OD$ carried out in correspondence with the exponential growth region, $k$ values were compared with those derived from the determination of the concentration of live bacteria by counting (Colony Forming Unities, CFU) performed with the traditional methods [11]. The comparison between the two techniques revealed the coincidence of the growth constant values derived from experimental measurements performed with the two techniques. Fig. 4 shows two typical regression curves of

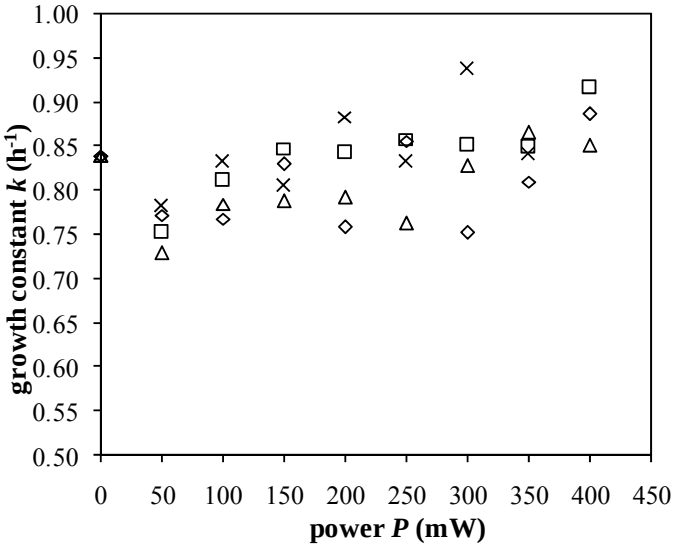


Fig. 3 Experimental values of the growth constant as a function of power and frequency of the radiation (◊ ν=2.30 GHz; □ ν=2.40 GHz; Δ ν=2.50 GHz; × ν=2.60 GHz).

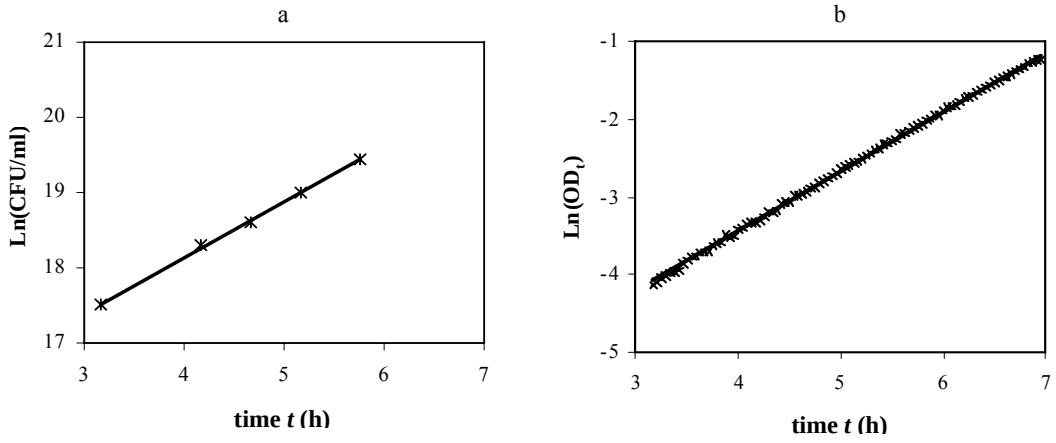


Fig. 4 Linear regression of Ln (CFU/ml) vs. $t$ (part a) and Ln ($OD_t$) vs. $t$ (part b) experimental data. Curve "a" has a slope of 0.74 ($R^2$=0.996), curve "b" has a slope of 0.77 ($R^2$=0.999). Both curves were obtained at $P$=100 mW and $\nu$=2.40 GHz

experimental data in the form of Ln(CFU/ml) vs. *t* (part a) and Ln($OD_t$) vs. *t* (part b); in both cases (even with $R^2$ values significantly different from each other) the linear regression results confirm the merit of the obtained results; this element has led to the choice of the non "invasive" method (using *OD* readings), since it does not require any withdrawal of reacting material, and does not prejudice result validity.

## IV. Conclusion

As shown in [11] it is possible to conduct the experimentation by opting for the non invasive option and thereby limiting the possibility of external contamination; the results presented in Fig. 4 show how the values of the growth constant, either derived by the traditional method of bacterial count or by the experimental procedure used here, are substantially equal.

In Fig. 3 the values of the growth constant calculated from the experimental data of *OD* vs. *t* are presented. For powers in the range of 50-150 mW one can see a minimum value of the growth constant; this minimum value is not influenced by the frequency of radiation.

However, it should be highlighted that the observed changes are of the same order of magnitude as the experimental errors, so further investigation is needed. This will be conducted by changing the experimental system in such a way [12] as to allow a more accurate control of the radiation power and the possibility of operating in a wider range of frequencies.

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