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Pseudomonas aeruginosa Growth in Microwave Irradiated Environment

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In this work we present the variation of the growth rate constant, of the microorganism *Pseudomonas aeruginosa* (PA) (a not spore forming and gram negative species), in microwave irradiated fields with respect to that obtained in the same experimental conditions but in absence of radiation. The microwaves, produced by a solid state oscillator, were used to irradiate a reacting suspension in a plug flow reactor positioned into a WR430 waveguide. The radiation powers used were 0, 100, 200, 300 and 400 mW. Also the frequency was varied (2.20, 2.30, 2.40 and 2.50 GHz). The reacting mixtures were obtained by adding 1 cc of a suspension containing 10^6 cells/cc of PA to a sterilized nutrient solution; the nutrient solutions were made by dissolving, in 200 cc of distilled water, 4.4 g of lyophilized Müller Hinton® broth. The growth kinetic constants were derived from optical density (OD) experimental determinations of the reacting mixtures. The measured OD values in the exponential growth region were processed by linear regression. To confirm the results, the growth rate values were also derived by the traditional method of the bacterial count (CFU/cc vs. t data). No effects of the microwave radiation were observed in the field of power 0÷300 mW. Low, but greater then experimental errors, reductions in the growth rate constant were observed with a radiation of 400 mW of power and in the range 2.3÷2.4 GHz of frequency.

1. Introduction

The aim of this work was to see if it is possible to reduce the growth rate of a bacterial population in an aqueous solution of a nutrient medium by radiating the reactor with low power microwaves at frequencies from 2.20 GHz to 2.50 GHz and at the controlled temperature of 37°C. The reduction of the bacterial growth rate by using low power microwave radiation could be an important fact because it would show the possible presence of non-thermal effects of this kind of radiation on biological systems; furthermore, it could address to the possibility of using that radiation in the food materials processing, so increasing the shelf life of the final products besides the preservation of their nutritional and organoleptic properties.
So our objective is to study the possibility of obtaining the reduction of the growth rate of bacteria using low power microwave (MW) radiation and working at temperatures lower than 37°C; in fact at these temperatures we are confident that all nutritional and organoleptic properties of interest for human being are saved.
The electromagnetic radiation of frequencies ν in the range between 2.20 and 2.50 GHz, notwithstanding it has not a photon energy ($E=h\cdot\nu$) high enough to modify the atomic

structure of the components in the radiated environment, can however interact with the super-molecular structures (like the cells), changing their characteristics; in the same way it can interact with the bacterial populations (Webb and Dodds, 1968; Webb and Dodds, 1969). Moreover, the accepted idea that interactions between the MW radiation and the human body (Hardell et al., 2006; Uloziene et al., 2005) can exist brings to the conclusion that if microwaves produce interferences with fully-developed and rich of natural protections organisms they can produce effects on the microorganisms populations that have less natural protections.

2. Experimental

2.1 Apparatus

The experimental procedure started with the inoculation of about 1 cc of seed to 200 cc of a sterile nutrient solution containing 4.4 g of lyophilized Müller Hinton (MH) nutrient. So the population of *Pseudomonas aeruginosa* could develop, and the growth process happened either in irradiated or in non-irradiated fields; to avoid any contamination all the process must run in a system that is completely isolated from the environment. Also the analysis of the bacterial concentration in the reacting suspension (after the growth starting the original nutrient solution becomes an heterogeneous mixture) leaving the reactor was made continuously without any drawing that could be a vehicle of contaminations. In Figure 1 the experimental structure is shown. The pirex bottle (5), filled with the MH nutrient solution, was hermetically sealed (4) with a cap and connected to the circuit, that could be sterilized as whole. The reacting fluid was

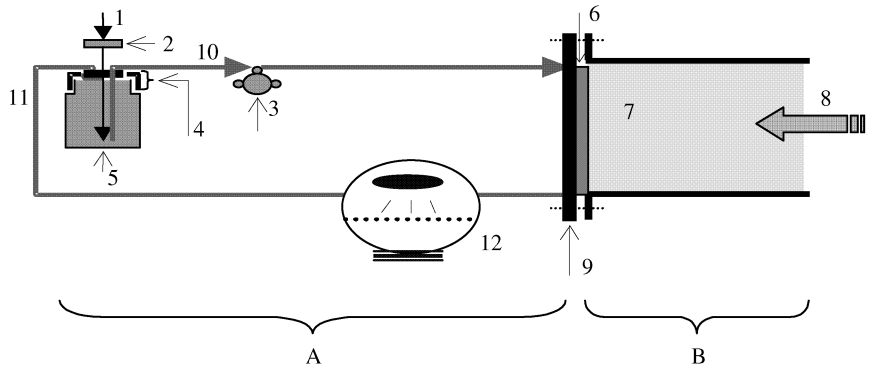

Figure 1. Sketch of the proposed experimental apparatus (1: external air; 2: ceramic filter of the external air 20 μm; 3: peristaltic pump; 4: cap that can be sterilized; 5: bottle containing the suspension to be irradiated; 6: PFR where the bacterial suspension is irradiated; 7: waveguide; 8: incident microwave radiation; 9: plate containing the PFR and closing the waveguide; 10: withdrawal of the bacterial suspension from the bottle; 11: recycle of the bacterial suspension to the bottle; 12: spectrophotometric analysis system).

pumped by a peristaltic pump (3) with a flow rate of 40 cc/min. The circuit was made of silicon tubes (ID 2 mm; OD 4 mm) and, if necessary, tubes of stainless steel (ID 2 mm). The reactor, from a fluidodynamic point of view, can be thought as a plug flow reactor (PFR); it was made with a silicon tube (ID 4 mm; OD 6 mm) and it was placed on the irradiated part of the stainless steel plate (9); the residence time of the suspension in the reactor was 24 s. The stainless steel plate was also used to close the waveguide (7). The silicon tubes were chosen because of their flexibility and their transparency to microwave radiation (Von Hippel, 1954, p. 361). Junctions were made using Swagelok® products, that made a full insulation from the external environment and safe connections possible. Then the 200 cc of MH nutrient solution were put into the container that was sealed and the entire part A of the circuit was sterilized. The sterilization was made by putting the complete block in an autoclave for 20 minutes at 121°C.

2.2 Generation and transport of the microwave radiation

The microwave radiation was generated by a YIG oscillator (1 in Figure 2) at the frequencies of 2.20, 2.30, 2,40 and 2.50 GHz; so the power of radiation was increased in an amplifier (4 in Figure 2) and then sent through a waveguide-cable converter to a WR430 waveguide where the PFR was lodged in the final part and the reacting mixture was irradiated.

3. Discussion

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

$$\mathrm{Ln}\,(C_C/C_{0C})=kt \qquad (1)$$

In Eq. (1) C_{0C} is the concentration of microorganisms at the time t=0. The analysis of the reacting mixture was performed by reading the optical density (OD) by a spectrophotometer (Varian Cary 50®); if the zero point of the spectrophotometer is made using the mixture of the MH nutrient and the inoculum (at t=0), a relation

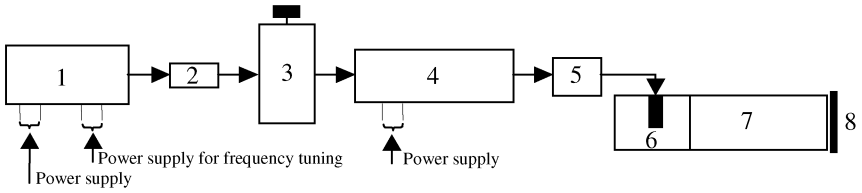

Figure 2. Sketch of the microwave generating apparatus (1: oscillator; 2: fixed attenuator; 3: rotating attenuator; 4: amplifier; 5: insulator; 6: cable–waveguide converter; 7: WR430 waveguide; 8: final plate with the reactor). Connection made with RG316 cables.

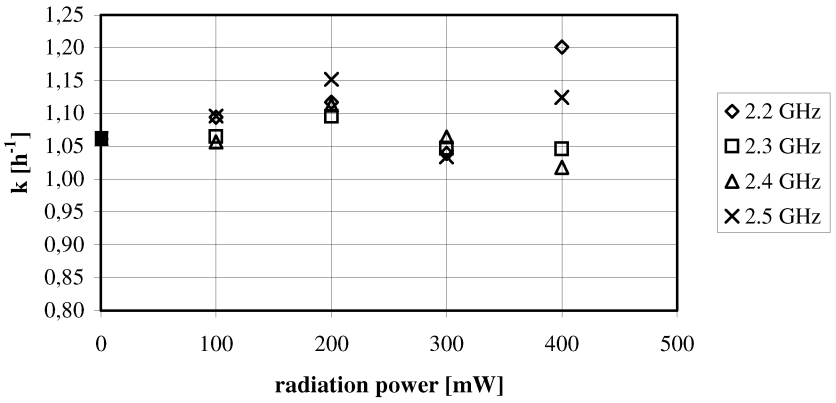

Figure 3. Experimental growth rate constant as a function of the radiation power and frequency.

between the optical density and the biomass concentration can be written as:

$$C_C=\alpha OD \quad (2)$$

Eq. (2) is valid only for low OD values (Ageno, 1992, pp. 44-49); if this condition is respected, Eq. (1) can be rewritten in the form:

$$\text{Ln}\,(OD_t/OD_0)=kt \quad (3)$$

where OD_t is the optical density of the reacting solution read at the time "t", and OD_0 that read at the beginning of the experimental run (t=0).
The experimental OD vs. t curves were obtained by using radiation powers of 0, 100, 200, 300 and 400 mW and frequencies of 2.20, 2.30, 2.40 and 2.50 GHz. The kinetic constant k was derived from Eq. 3 by a linear regression of the experimental data points in the form Ln (OD_t) vs. t. In Figure 3 the values obtained for k in the different experimental conditions are reported; each data point is the medium value of nine different experimental data. To confirm the validity of the obtained values for the kinetic constant, derived from the OD experimental measurements collected in the exponential growth zone, at the exit of the PFR some samples were withdrawn and analysed to obtain the concentration of the living microorganisms with the traditional methods of the bacterial count, then the kinetic constants derived from the OD measurements were compared to those derived from the determination of the real number of living bacteria. The comparison between the two measuring procedures highlighted the substantial methodological correctness of the k values derived from the OD measurements; in Figure 4 a typical experimental points series regression curve

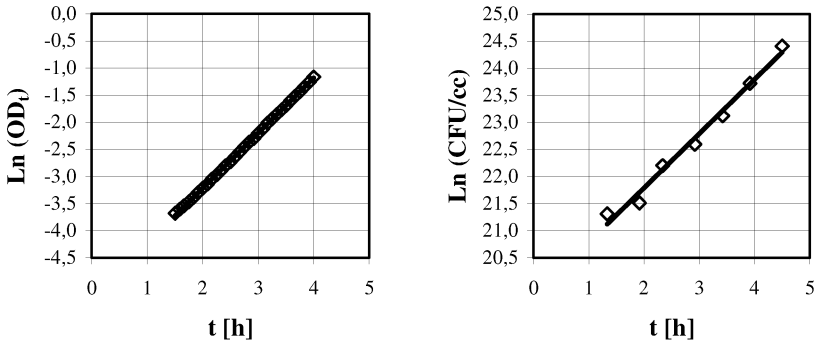

Figure 4. Regression curves of experimental Ln (OD_t) vs. t (part a) and Ln (CFU/cc) vs. t (part b). Part a: slope=1.0148, R^2=0.9997; part b: slope=1.0017, R^2=0.9852.

from OD vs. t measurements and the corresponding regression curve of Ln (CFU/cc) vs. t data are shown; the linear regression results in both cases confirms (as can be seen with very different R^2 values) the goodness of the obtained results, so the not invasive method that did not require any withdrawn from the reacting material was employed rest upon the correctness of the obtained results.

4. Conclusions

The obtained results bring to three main conclusions:

1) about the microorganism *Pseudomonas aeruginosa* we did not see any effect of the MW radiation power in the field 0÷300 mW; in fact the values of the kinetic constant show variations into the field of error connected with the experimental determinations. The value of the kinetic constant obtained at powers 100, 200 and 300 mW are about equal to that obtained for non irradiated mixtures;
2) the effect of frequency, as shown in Figure 3, can be seen only at 400 mW; at this power we noted, for frequencies in the field 2.30 – 2.40 GHz, a low, but greater than the experimental errors, reduction of the growth rate constant with respect to those obtained at powers lower than 400 mW,
3) at frequencies out of the range specified in the previous point the experimental values of k are very different from those obtained at 2.30 and 2.40 GHz. This can be a poor but important rally of a possible specific effect of the radiation at frequencies in the field 2.30 – 2.40 GHz on the growth rate of the bacillus *Pseudomonas aeruginosa*.

In future we plan to extend our experimental work in the field of frequencies 2.30 – 2.40 GHz and for power greater than 400 mW to highlight, if exist, a possible MW effect.

5. References

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