The Effect of Low-Power Microwaves on the Growth of Bacterial Populations in a Plug Flow Reactor

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A plug flow reactor (PFR) which permits the growth of bacteria in a microwave environment is described. The apparatus is used to analyse the effect of microwave radiation at the frequency of 2.45 GHz and at 37°C on the growth rate of three bacterial species: Bacillus clausii*,* Pseudomonas aeruginosa*, and* Staphylococcus aureus*. The growth constant is determined for reacting mixtures irradiated with microwaves of different powers ranging from 0 to 400 mW. Analyses show that (a) the apparatus is able to perform the experimental runs maintaining an aseptic environment; (b) under the microwave power levels examined in the present study, no effects are detected for the* Bacillus clausii *and* Pseudomonas aeruginosa *species, while for the* Staphylococcus aureus *species, a few small effects are found (a minimum value of the growth constant at 200 mW and an increase of the constant between 200 and 400 mW).*
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Introduction

In the food industry, the reduction of bacterial counts or the reduction in the growth rate of bacterial populations is a key step for obtaining either an extension of shelf life or a safe product for consumption. For example, the shelf life of many dairy products (e.g., milk, ricotta, cheese, yoghurt, etc.) is reduced by bacterial spoilage; consequently, the shelf life of these products can be increased by a reduction in the bacterial growth rate. As a result, it is possible to say that the safety and quality of many products of the food industry could be increased by a reduction in the bacterial growth rate. Furthermore, if these operations were possible at temperatures lower or equal to 37°C, many of the nutritional and organoleptic properties of the raw materials would be saved.

Electromagnetic radiation having wavelength $\lambda = 0.1 \div 100$ cm [frequency $\nu = 300 \div 0.3$ GHz, known as microwave (MW)] does not have the high photon energy ($E = h\nu$) required to modify the atomic structure of the components in the radiated environment, but it can influence the behavior of the supermolecular structures. In fact, it can change the cell metabolism[1] as well as also being absorbed by RNA and DNA,[2] thereby interacting with bacterial populations. Also, the magnetic field promoted by the radiation can show some effects interacting with paramagnetic intermediates (such as radical or ion radical pairs) resulting in spin-mediated mechanisms.[3]

Certainly, microwaves are most widespread in the field of mobile phone telecommunications; it is probably from their large uptake in this field that interest has arisen regarding possible interactions with the human body,[4,5] particularly with the brain and with the hearing functions. Currently, no proof of this interaction is available, and the idea that this kind of interaction may exist is not accepted by many scientific circles,[6] mainly because the human brain is protected from the environment by the skull.[7] However, some researchers believe that MW radiation can interact with the human body; this consequently leads to the idea that if

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microwaves can produce interference with fully developed organisms with well-developed natural protection, then they could produce even more effects on microbial populations, which have less natural protection. Furthermore, the idea that microwaves can cause a reduction of the bacterial count or, at least, a reduction of the bacterial growth rate is acceptable since it is congruent with both experience and scientific evidence.[8,9]

It is known that microwave radiation can increase the temperature of aqueous solutions, even in a short time, causing a reduction of the bacterial count due to thermal effects.[8] However, the use of microwaves to reduce the bacterial count, by increasing the temperature, presents some disadvantages. Indeed, even if MWs give a volumetric transmission of the thermal energy, thus avoiding any need for a temperature gradient between the surface and the internal part of the body and any overheating effect, the fact that the radiation at the frequency of 2.45 GHz is obtained from electric energy with a yield of about 50% reduces the advantage of this property. The possibility of using MW radiation at a frequency of 2.45 GHz for reducing the growth rate of bacteria in food products only becomes very interesting, if it were possible to operate at low temperatures, thereby avoiding any degradation.

The first step in studying the effect of 2.45 GHz on food products is to study the effect that microwaves have on simple bacterial suspensions. In effect, many scientific investigations have been done either on bacterial populations[9–11] or on medical structures,[12,13] but most of the present information is about systems without temperature control; this, however, is an important aspect because, even though the temperature increase may not be very high, it could be sufficient enough to induce thermal effects on bacteria and also on proteins (for example, Bohr and Bohr[14] reported that MW radiation enhances thermal folding and unfolding of the globular protein β-lactoglobulin in the 5–44°C range).

In this work, suspensions containing growing microbial populations at the controlled temperature of 37°C (this temperature was selected because almost all key nutritional and organoleptic properties are retained) are irradiated with MW radiation at 2.45 GHz. Our objective is to see whether nonthermal effects on the growth rate of a bacterial population in an aqueous solution of a nutrient medium can exist. From a scientific point of view, the possible changing of the bacterial growth rate by the use of non-ionizing radiation at a frequency of 2.45 GHz at low temperatures could be an important fact because it would show the possible presence of a nonthermal effect of MW radiation on biological systems. Furthermore from a technological point of view, it could address the possibility of using this kind of radiation in raw food material processes and/or in the distribution phase, thereby extending the shelf life of the final products as well as the preservation of their nutritional and organoleptic properties.

The experimental protocol aims to obtain optical density (OD) vs. t data for the reacting mixture, particularly in the exponential growth zone; the growth rate constant can then be derived from a linear regression. By comparing the growth rate constants derived both in irradiated and in non-irradiated systems at 37°C, the influence of the microwave radiation can be highlighted. The experimentation is carried out with the three bacterial species shown in Table 1. The three species were selected to collect information from different kinds of sources with the objective of obtaining information able to address the future developments of this research.

Table 1. Species Used and Their Characteristics

Species	Characteristics
Bacillus clausii (Commercial pruduct Enterogermina®, Sanofi-synthelabo®).	Spore forming
Pseudomonas aeruginosa (Local strain)	Non-spore forming, gram-negative, thin-walled
Staphylococcus aureus (ATCC 25923)	Non-spore forming, gram-positive, thick-walled

Materials Used

Electronic materials

The low-power microwave generating apparatus is composed of different electronic components. The main one of these is a Micro Lambda Wireless MLOM-0204 miniature permanent magnet YIG-Tuned (Yttrium Iron Garnet) oscillator. This device produces an output signal characterized by a power of 14 dBm ($\mathrm{dBm} = 10 \cdot \log\left(\frac{P}{1\mathrm{mW}}\right)$) and by a frequency that can be tuned in a range from 2 to 4 GHz. The operating frequency tuning is made by changing an input voltage signal in the range from 0 to 5 Vdc. It was found that the frequency is given by the linear function (with a coefficient of determination of 0.9999)

$$v = 2.0 + 0.4 \cdot \mathrm{V} \tag{1}$$

A DC Block (Suhner® 1100.01.A) was connected after the oscillator. The signal coming out from the oscillator passes through a 6-dB fixed attenuator (JFW Industries 4AH-06), and then through a rotary attenuator (JFW Industries 50R-248), allowing a further attenuation of the output power in a quantity ranging from 0 to 10 dB, with a step size of 1 dB. The consequent process generated signal is then transferred to a power amplifier (Herotek P/N AP271135), supplied with a voltage of 12 Vdc; the amplifier gain is 23 dB. After this, the signal encounters an insulator (RF & NC CI-200-172), which allows the incident signal to pass, but at the same time prevents the reflected signal from returning. All the connections were made using RG316 cables with SMA type connectors.

The microwave radiation coming from the amplifier goes through a coaxial cable to a waveguide adapter (MEC P/N LA 40-30-CH WR430). This apparatus converts the incoming cable signal and sends it from an antenna, through the waveguide type WR430 (MEC P/N LA 160-30N), to the biological reactor. At the end, the waveguide was closed by a stainless steel plate where the biological PFR was positioned.

The incident power measurements were made using a power meter (Hewlett Packard® 436A) with a power sensor (Hewlett Packard® 8481A); the power sensor can measure

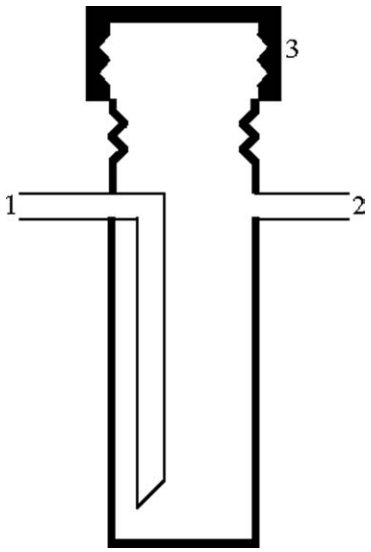

Figure 1. Flux cuvette that can be hermetically sealed (1 fluid entrance; 2 analyzed fluid exit; 3 sealing cap).

power levels from −30 dBm to +20 dBm (1 μW to 100 mW), at frequencies from 10 MHz to 18 GHz. The fraction of the microwave power absorbed by the reacting system, used to evaluate the specific absorption rate (SAR) of the system, was calculated as described in Appendix A.

Biological materials

A Müller Hinton (MH) broth and agar (Microbiol®) nutrient solution was used, made of heart infusion plus acid hydrolyzed casein and starch. It was prepared at a concentration of 22 g l^{-1} with deionized water; this value was selected to work in an excess of substrate, so that its consumption can be neglected. The microorganisms used are shown in Table 1; before inoculation, each of these was allowed to adapt in a MH solution (24–48 h at 37°C). For each culture used as a seed, the concentration of bacteria, before seeding the reaction system, was measured by a colony count on agar solidified MH medium.

Methods

The experimental procedure starts with the inoculation of 2–3 ml of seed into 200 ml of a sterile nutrient solution. The growth process happens either in irradiated or in non-irradiated fields. To avoid any contamination, the whole process develops under aseptic conditions. The analysis of the suspension (after the start of growth, the original nutrient solution becomes a heterogeneous mixture) leaving the reactor was carried out continuously without any sample removal that could allow contamination. The analysis was performed using a spectrophotometer (Varian Cary 50®). A sealed Pyrex glass flux cuvette was used for the analysis of the suspension; this material was selected so that the whole system could be sterilized. This cuvette was designed in such a way as to avoid any sedimentation of the heterogeneous reacting mixtures; the designed structure is shown in Figure 1.

The experimental apparatus was prepared to work in the previously described conditions. It should be highlighted that the complete experimental procedure runs without any contact with the external environment; in fact if contamination occurs, it cannot usually be detected by our analytical procedure, and only the incorrect final results show that something has gone wrong.

In Figure 2, the experimental structure is shown. The Pyrex bottle (5 in Figure 2) was filled with the MH nutrient solution; it was then hermetically sealed with a PVC high temperature-resistant cap (4 in Figure 2) so that it could be sterilized. The cap is provided with four holes that were used to perform the following functions:

- the influx of external air (1 in Figure 2) through a ceramic filter with a mesh of 0.22 μm (2 in Figure 2);
- the withdrawal of the reacting suspension from the bottle (10 in Figure 2) to be sent to the irradiated plug flow reactor (6 in Figure 2);
- the input of the reacting suspension (11 in Figure 2) coming from the reactor and from the spectrophotometric analysis system (12 in Figure 2);

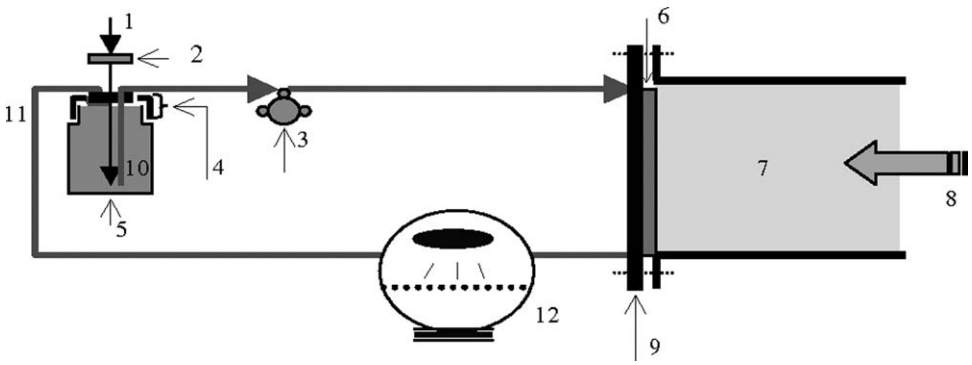

Figure 2. Sketch of the proposed experimental apparatus (1 external air, 2 ceramic filter of the external air 20 μm; 3 peristaltic pump; 4 sterilizable cap; 5 bottle containing the suspension to be irradiated; 6 PFR where the bacterial suspension is irradiated; 7 waveguide; 8 incident microwave radiation; 9 plate supporting 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).

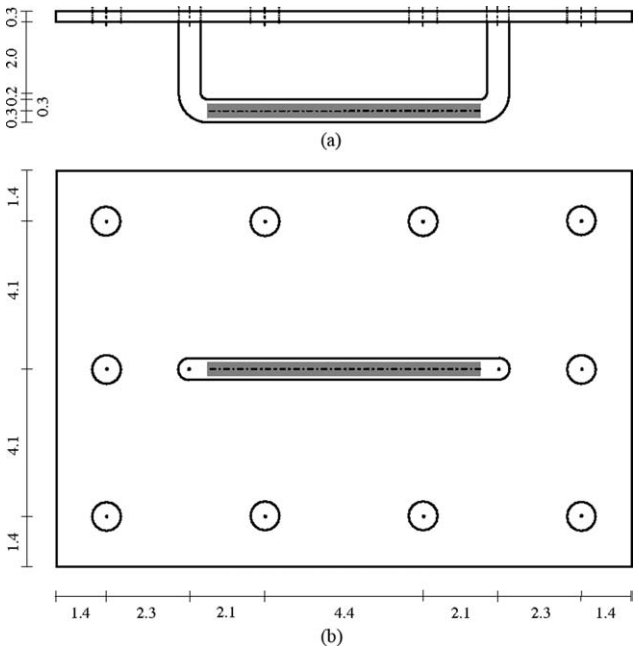

Figure 3. Top view (a) and front view (b) of the internal part of the plate.

PFR (ID 0.4 cm, $L = 7.6$ cm) (all the reported quotes are in centimeters).

• the escape of the excess air used for the oxygenation of the reacting suspension. This escape is provided with a U-shaped tube closed with a cotton cap in its terminal part, replacing the ceramic filter of 22 μm of mesh which can get blocked up after a few minutes of operation, due to moisture condensation on its filtration surface. The U-tube prevents both the cotton filaments and the condensed water from falling into the suspension and the over or upper pressures in the bottle from causing contamination.

The reacting fluid is pumped by a peristaltic pump (3 in Figure 2) with a flow rate of 40 ml min^{-1}. The circuit is made of silicon tubes (ID 2 mm; OD 4 mm) and, where necessary (for connections and soldered joints), tubes of stainless steel (ID 2 mm). The reactor was a PFR made with a silicon tube (ID 4 mm; OD 6 mm); it was placed on the irradiated part of the stainless steel plate (9 in Figure 2 and Figure 3), the residence time of the suspension in the reactor was 24 s. The stainless steel plate was also used to end the waveguide (7 in Figure 2). Silicon was selected because of its flexibility and its transparency for microwave radiation.[7] Junctions were made using Swagelok® products that allowed complete insulation from the environment and safe connections.

Then 200 ml of MH nutrient solution were put into the container which was sealed and the entire part, constituted by elements 4-5-6-9-10-11 (Figure 2) of the circuit, was sterilized. Sterilization was achieved by placing the complete block in an autoclave for 20 min at 121°C.

Without the ceramic filter, the bacterial inoculum suspension was put into the bottle using a syringe through the hole where the input of the external air occurs; then the peristaltic pump and, if necessary, the microwave generating apparatus were enabled. The OD of the reacting suspension was read every 5 min at a wavelength of 600 nm; the reading was made through the Pyrex cuvette which, as already said, avoids any accumulation of solid particles from the non-homogeneous reacting mixture.

Generation and transport of the microwave radiation

The microwave radiation was generated by the YIG (1 in Figure 4) oscillator at the frequency of 2.45 GHz; this frequency was fixed imposing an external constant voltage of 1.12 V, derived from Eq. 1, to the YIG driver circuit. The fixed attenuator (2 in Figure 4) of 6 dB joined to the rotating attenuator (3 in Figure 4) allows the produced signal to be tuned from -2 dBm to $+7$ dBm. After this, this signal goes to the amplifier (4 in Figure 4) producing a signal, with a power varying from 21 to 30 dBm. After the insulator (5 in Figure 4), to prevent any reflection, the signal coming from the amplifier goes to the cable waveguide converter (6 in Figure 4); then from the antenna of this apparatus the signal was given to the radiated reactor (6 in Figure 1) through the waveguide (7 in Figure 2; 7 in Figure 4). Theoretically, the maximum signal power delivered to the reactor could be 30 dBm (1000 mW) but, due to the global apparatus efficiency, to the compression of the signal by the amplifier, and to the yield of the single components, the maximum measured signal power was about 26 dBm (400 mW).

The radiation arrives at the plate to which the reactor is fixed through the waveguide (internal dimensions 109 $\times$ 55 mm); the plate is localized in the terminal part of the waveguide (8 and 9 in Figure 2) and joined to it by a flange; the incident signal power is measured by the power meter. The power meter can read the signal power coming from a directional coupler (coupler coefficient 10^{-3}) joined to the

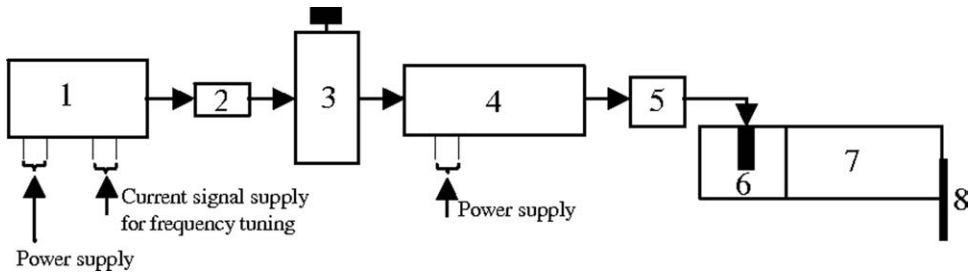

Figure 4. Sketch of the microwave generating apparatus (1 oscillator; 2 fixed attenuator; 3 rotating attenuator; 4 amplifier; 5 insulator; 6 cable—waveguide converter; 7 WR430 waveguide; 8 final plate with the reactor).

Connections of elements were made with RG316 cables.

waveguide by a sensor. From the power meter the signal power is read and saved through a NI board for further processes. The part of the whole incident energy, derived from the power meter readings, really absorbed by the reacting suspension, was measured as described in Appendix A; it represented about 55% of the energy derived from the power meter data. In Figure 4, a sketch of the apparatus is shown; the power supply given to the YIG oscillator was a 12 V voltage direct current, while the power supply given to the amplifier had a voltage of 15 V.

Analytical derivations

The growth rate of a microbial population can be derived from the equation of Monod as a function of the concentration of microorganisms and of the substrate, and it can be written in the form[15]

$$R_C = \frac{kC_C C_S}{K_M + C_S} \tag{2}$$

if it is applied to a system with a great concentration of substrate so that $C_S \gg K_M$, it can be simplified into the phenomenological form

$$R_C = kC_C \tag{3}$$

This means that the growth rate of the bacterial population can be seen as a first order chemical reaction rate. Under this condition by replacing R_C with its definition form, it is possible to derive the relation (starting time $t_0 = 0$).

$$\ln\left(\frac{C_C}{C_{0C}}\right) = kt \tag{4}$$

If the zero point of the spectrophotometer is made using the mixture of the MH nutrient and the inoculum at $t = t_0$, a relation between the OD and the biomass concentration can be written as

$$C_c = \alpha \mathrm{OD} \tag{5}$$

Equation 5 is valid only for low OD values.[16] The applicability of Eq. 5 was checked as described in Appendix B. Thus, Eq. 4 can be rewritten in the form

$$\ln\left(\frac{\mathrm{OD_t}}{\mathrm{OD_0}}\right) = kt \tag{6}$$

Equation 6 expresses the measurement of the OD of the reacting mixture as a function of time; the growth rate constant (kinetic constant) can be derived by a linear regression. In practice, the experimental derivation of k is not simple because many external causes can cause wrong determinations (principally due to contamination), which are sometimes difficult to detect. To reduce the incidence of external errors, the experimental runs were repeated nine times for each different set of experimental conditions; only data giving a coefficient of determination greater than 0.999 and k values comprised in the accepted field $\pm 5\%$, with respect to the medium values specified later, were accepted.

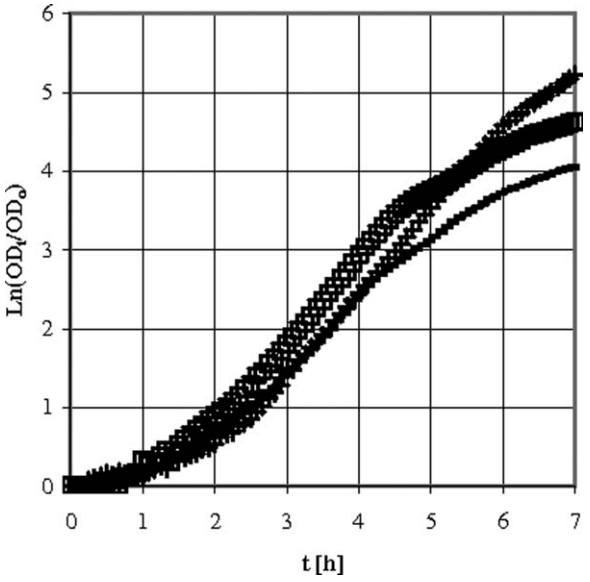

Figure 5. Typical $\ln(OD_t/OD_0)$ vs. t experimental curves for *Staphylococcus aureus* population (□ P = 100 mW; - P = 50 mW; + P = 0 mW).

Experimental Results

The experimental OD vs. t curves were obtained by using different power radiations.

In Figure 5, three typical growth curves are shown; the first one refers to a reaction under microwave irradiation with a power of 100 mW; the second one to an irradiated mixture with a power of 50 mW; the last one to a non-irradiated reacting mixture.

From these curves, three aspects must be highlighted:

- microbial growth follows the model of Monod (an induction period followed by an exponential growth zone and then by a stationary phase during which there is no longer any growth of the bacterial population);
- the exponential growth zone allows the kinetic constant to be derived for *Staphylococcus aureus*; the slopes of the three curves are in the order $\alpha_{100} > \alpha_{50} > \alpha_0$;
- the length of the lag time[15] depends on various factors (stress of the population, concentration of salts, etc.) and can differ from one test to another.

The kinetic constant k was derived using Eq. 6 by a linear regression of the experimental data points collected in the exponential growth zone and working in excess of substrate so that Eq. 3 can be applied. The results are presented, for the three considered species, in Table 2, where the kinetic constant is reported as a function of the radiation power for each studied microorganism. For each power level, the medium values of the results of the nine different experimental runs are reported; the deviation of the singular growth constant and the reported values is in the area of $\pm 5\%$. To confirm the validity of the obtained values for the kinetic constant, derived from the OD experimental measurements, in the exponential growth zone, a few samples of some runs were taken and analyzed at the point of exit from the PFR to obtain the concentration of the living microorganisms with the traditional methods. The kinetic constants derived from the OD measurements were compared to those

Table 2. Growth Constants at 37°C for Different Incident Powers

	P (mW)	k (h^{-1})
Bacillus clausii	0	0.43
	100	0.40
	200	0.39
	400	0.40
Pseudomonas aeruginosa	0	1.02
	100	1.08
	200	1.00
	400	1.01
Staphylococcus aureus	0	1.06
	50	1.04
	100	1.12
	130	1.14
	150	1.07
	180	1.07
	200	0.99
	220	1.12
	400	1.24

derived from the determination of the actual number of living bacteria. The comparison between the results obtained from the two measuring procedures highlighted the substantial correctness of the k values derived from OD measurement; in Figure 6 a typical experimental points series regression curve from OD vs. t measurement and the corresponding regression curve of N vs. t data are shown. The linear regression results in both cases confirm (as can be seen with very different R^2 values) the validity of the results obtained, consequently, the non-invasive method that did not require any withdrawal of reacting material was employed based upon the correctness of the obtained results. Furthermore, microscopic examination of samples of the reacting mixture (the same as that taken for obtaining the concentration of the living microorganisms) also confirmed the maintenance of aseptic conditions.

This comparison between the kinetic constants derived from OD vs. t and from N vs. t measurements was made for all the three bacterial strains used. There is a good agreement for *Pseudomonas aeruginosa* and *Staphylococcus aureus* species, but there is no concordance for the *Bacillus clausii* species; this is due, in our opinion, to the possibility that these bacteria can form spores. However, the k values derived from OD vs. t measurements were considered more reliable because the presence of spores does not significantly alter OD measurements, while on the contrary N values can be falsified if spores develop after samples have been taken.

Discussion and Future Work

The examination of the obtained data clearly demonstrates that the proposed apparatus (shown in Figure 2), and the cuvette design shown in Figure 1 can be used for studying the evolution of a biological process without any contamination from the external environment. Furthermore, the proposed apparatus allows the evolution of a complex experimental process to be carried out in a safe way for the operators, and to collect many experimental OD measurements in an automated way.

The results obtained and summarized in Table 2 lead to two main conclusions:

- as regards *Bacillus clausii* and *Pseudomonas aeruginosa*, no effect of the MW radiation in the field of power $0 \div 400$ mW was observed; in fact, the values of the kinetic constant for the two microorganisms show variations within the field of the error connected with the experimental determinations. This could be due to the excessively low values of MW power, so could address future research to the application of higher radiation power, according to other researchers' studies[17];
- as regards the *Staphylococcus aureus* microorganism, a minimum of the growth constant can be seen at the irradiation power of 200 mW. Nevertheless, this reduction is no greater than the experimental errors, so this fact is not enough to lead to the conclusion that we have a significant reduction in the growth rate for *Staphylococcus aureus*. On

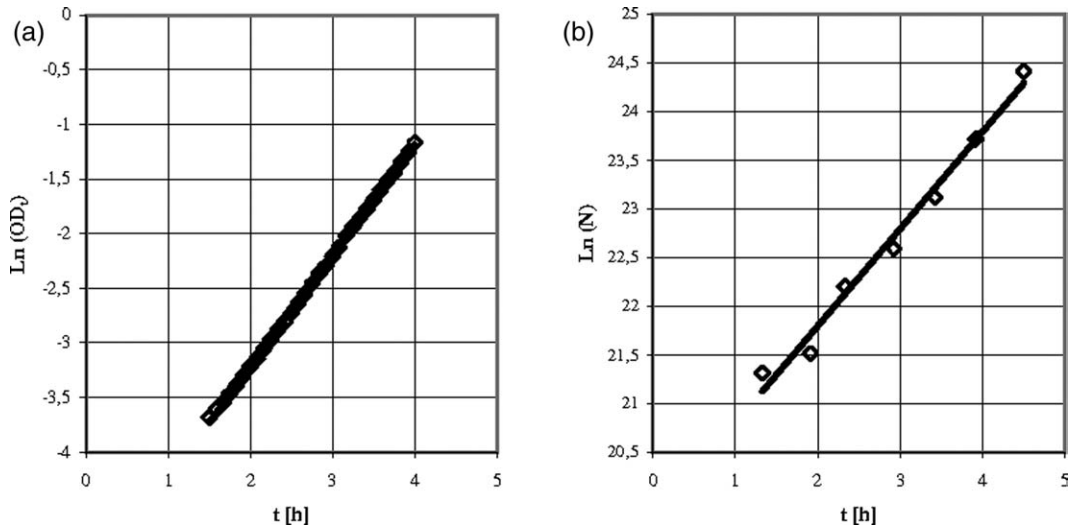

Figure 6. Regression curves of experimental ln(OD) vs. *t* (a) and ln(N) vs. *t* (b).

(a) slope = 1.0148, $R^2 = 0.9997$; (b) slope = 1.0017, $R^2 = 0.9852$ (species: *Pseudomonas aeruginosa*; power of the MW radiation: 400 mW).

the other hand, for powers greater than 200 up to 400 mW, an increase in the growth rate constant was found. An increase in cell proliferation has already been found by Cleary et al.[17] and Stagg et al.[18]; the latter, even though in different conditions of frequency and power, also pointed out the presence of a localized maximum with respect to power, thus confirming the possibility that localized non-thermal effects may exist.

The determinations contained in the last point should be confirmed by other experimental runs, but it is necessary to inform the scientific community about the possibility that the growth rate constant of a bacterial population could be modified by MW radiation, due to the huge consequences that this fact could have on the food industry.

Future research plans will concern the extension of the power field, the study of other bacterial species and the influence of the residence time in the irradiated reactor.

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Notation

C = concentration (g dm^{-3})
c_p = specific heat of water (J g^{-1} °C^{-1})
E = energy (J)
f = dilution factor
h = Plank constant
k = kinetic constant in the Monod equation (h^{-1})
K_M = Monod constant (g dm^{-3})
m = mass of the fluid in the container (g)
N = number of CFU (Colony Forming Units) (cm^{-3})
OD = optical density
OD_{app} = read optical density (sum of the transmitted and the fraction of the diffuse light)
OD_{or} = optical density of the nondiluted solutions
$P\%$ = percentage of incident power absorbed from the reacting mixture
$\overline{P}$ = medium value of $P\%$
q = power (W)
R = growth rate of bacteria/biomass (g dm^{-3} s^{-1})
S = exchange surface (m^2)
t = time (s)
Tr = transmittance
ΔT = increase in the temperature (°C)
V = electric voltage (V)

Greek letters

α = slope of the line C_C vs. OD
β = fraction of the non-transmitted light read by the sensor
δ = slope of the line ΔT vs. t
λ = wavelength (nm)
ν = frequency (GHz)

Subscripts

C = relative to microorganisms
S = relative to the substrate
0 = relative to the starting time
E = exchanged
f = friction
NR = non-radiated
MW = referred to the microwave radiation
R = radiated
S = relative to the nutrient (or substrate)
T = total
t = relative to time "t"
v = in the void
xx = quantity relative to xx mW of power

Literature Cited

1. Webb SJ, Dodds DE. Inhibition of bacterial cell grow by 136 gc microwaves. *Nature*. 1968;218:374–375.
2. Webb SJ, Booth AD. Absorption of microwaves by microorganisms. *Nature*. 1969;222:1199–1200.
3. Buchachenko AL, Frankevich EL. *Chemical Generation and Reception of Radio- and Microwaves*. New York: VCH Publishers Inc., 1994.
4. Hardell L, Hanson Mild K, Carlberg M, Soderqvist F. Tumor risk associated with use of cellular telephones or cordless desktop telephones. *World J Surg Oncol*. 2006;11:4–74.
5. Uloziene I, Uloza V, Gradauskiene E, Saferis V. Assessment of potential effects of the electromagnetic fields of mobile phones on hearing. *BMC Public Health*. 2005;19:5–39.
6. Kainz W, Alesch F, Dias Chan D. Electromagnetic interference of GSM mobile phones with the implantable deep brain stimulator. *ITREL-III Biomed Eng OnLine*. 2003;7:2–11.
7. von Hippel A. *Dielectric Materials and Applications*. Cambridge: Technology Press of MIT, 1954. p 361.
8. Wang J-C, Hu S-H, Lin C-Y. Lethal Effect of Microwaves on Spores of Bacillus spp. *J Food Prot*. 2003;66:604–609.
9. Sato T, Doi A, Urayama T, Nakatani T, Miyahara T. Sterilization of Escherichia coli by coaxal microwave plasma flow. *IEEE Ind Appl Soc Annual Meeting*. 2005;2:761–770.
10. Kuo SP, Tarasenko O, Poposich S, Levon K. Killing of bacterial spores contained in a paper envelope by a microwave plasma torch. *IEEE Trans Plasma Sci*. 2006;34:1275–1280.
11. Heun G, Tinkov S. Sterilization of parenterals with microwaves as an alternative to autoclavation. *Pharm Ind*. 2003;65:977–983.
12. Guven RG, Guven K, Dawe A, Worthington J, Harvell C, Popple A, Smith T, Smith B, de Pomerai DI. Effect of radio-frequency fields on bacterial cell membranes and temperature-sensitive mutants. *Enzyme Microb Tech*. 2006;39:788–795.
13. La Cara F, Scarfì MR, D'Auria S, Massa R, D'Ambrosio G, Franceschetti G, Rossi M, De Rosa M. Differents effects of microwave energy and conventional heat on the activity of a thermophilic β-galactosidase from bacillus acidocaldarius. *Bioelectromagnets*. 1999; 20:172–176.
14. Bohr H, Bohr J. Microwave-enhanced folding and denaturation of globular proteins. *Phys Rev E*. 2000;61:4310–4314.
15. Levenspiel O. *Chemical Reactor Omnibook*. Corvallis: Oregon OSU Book Stores, 1984.
16. Ageno M. *La macchina batterica*. Roma: Lombardo Editore, 1992:44–49.
17. Cleary SF, Liu L-M, Merchant RE. Glioma proliferation modulated *in vitro* by isothermal radiofrequency radiation exposure. *Radiat Res*. 1990;121:38–45.
18. Stagg RB, Thomas WJ, Jones RA, Adey WR. DNA synthesis and cell proliferation in C6 glioma and primary glial cells exposed to a 836.55 MHz modulated radiofrequency field. *Bioelectromagnetics*. 1997;18:230–236.

Appendix A

The power absorbed by the reacting mixture was measured as follows:

- in the experimental apparatus shown in Figure 2, the part for the optical density (OD) analysis of the reacting mixture (12 in Figure 2) was removed;
- all the circuit components were thermally isolated using a commercial isolating tube except for those parts that could not be isolated (the silicon tube in the peristaltic pump and the reactor in the radiated zone) which were left without any

insulation. A preliminary tuning phase without MW radiation accounts for the contribution of friction on to the internal energy increase;

- the container was filled with 200 ml of distilled water, and the water was circulated using the same structure as the reacting mixture; consequently, it was made to pass through the reactor (6 in Figure 2);
- the sensor of a digital thermometer (Hanna Instruments® HI 92710C) was placed into the hole destined as the exit point for the excess air from the container; the temperatures were collected and registered.

After this, the reactor placed in the stainless steel plate was radiated and the increase in the water temperature was collected at various radiation powers.

The recorded temperatures of the water in the container vs. time were found to vary linearly; Figure A1 shows the experimental values for the non-irradiated system and for an irradiated one with a power of 200 mW.

The derived slopes are shown in the second column of Table A1. The water temperature change is due to:

- the power transmitted by the microwave radiation;
- the friction the system opposes to the circulating water (q_f);
- the amount of heat exchanged with the external environment per unit of time (q_E).

Then for the irradiated systems, when the power given to the circulating water by the microwaves is q_{MW}, the power transmitted is

$$q_R = c_P m \frac{\Delta T_R}{t} = q_{MW} + q_f + q_E \tag{A1}$$

On the contrary for the non-irradiated situation when the power given to the circulating water by the microwaves is 0 W we have

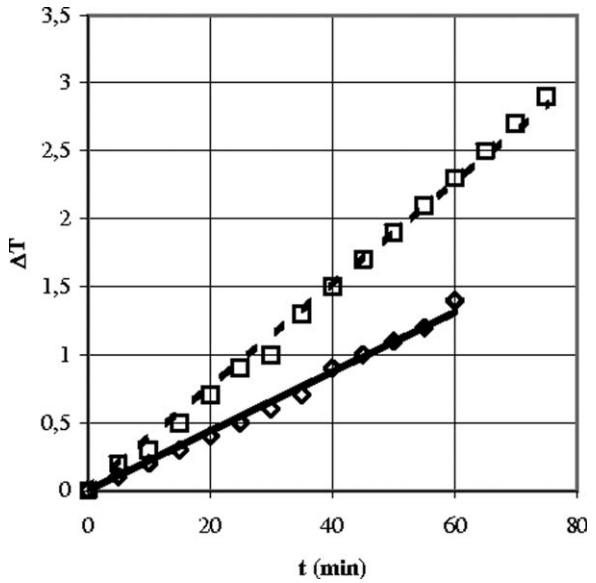

Figure A1. Temperature increases vs. time in the container in radiated (- - -; power 200 mW) and non-radiated (___) mixture.

Radiated mixture slope 0.039, $R^2 = 0.9966$; nonradiated mixture slope 0.0219, $R^2 = 0.9913$.

Table A1. Medium Slopes of the Straight Line ΔT vs. t at Various Microwave Powers

P (mW)	δ (°C min^{-1})	*P%*
0	0.022	
100	0.0264	61.3%
200	0.0309	62.0%
300	0.0338	54.8%
400	0.0363	49.9%

$$q_{NR} = c_P m \frac{\Delta T_{NR}}{t} = q_f + q_E \tag{A2}$$

where $\Delta T_{NR} = \delta_{NR} \cdot t$ is the temperature change for non-irradiated systems, and $\Delta T_R = \delta_R \cdot t$ for irradiated ones (the experimental slopes for all the studied radiation powers are reported in Table 3), the fraction of the microwave powers absorbed by the circulating water (which can be considered equal to that absorbed by the reacting suspension) with respect to the total incident power is

$$P\% = \frac{q_R}{q_T} = c_p m \cdot \frac{[\delta_R - \delta_{NR}]}{q_T} \cdot 100 \tag{A3}$$

By substituting the experimental medium values of δ_R and δ_{NR} (second column in Table 3), it is possible to calculate the P% values (third column in Table 3). By considering the low variation of the reported values and the difficulties of the experimental runs, a medium value

$$\bar{P} = 55\% \tag{A4}$$

was assumed. Thus the SAR (Specific Absorption Rate) level for the reacting systems varies in the range $5.5 \times 10^{-4} \div 4.4 \times 10^{-4}$ W g^{-1}.

Appendix B

As highlighted by Ageno,[16] the read OD for heterogeneous mixtures is affected by an error for large suspended particles but proportional to the ratio between the dimension of the particle that absorbs the incident radiation (to the order of some micrometers) and the wavelength of the radiation (0.6 μm).

The OD is defined as

$$OD = \log\left(\frac{1}{Tr}\right) \tag{B1}$$

the fraction of the non-transmitted (diffused) light is $(1 - Tr)$, of which a small part $\beta \cdot (1 - Tr)$ hits the light sensor of the spectrophotometer causing an increase in the read OD. Thus, the read OD is

$$OD_{app} = \log\left[\frac{1}{Tr + \beta \cdot (1 - Tr)}\right] = \log\left(\frac{1}{Tr}\right) + \log\left[\frac{Tr}{Tr + \beta \cdot (1 - Tr)}\right] \tag{B2}$$

So the real OD can be written as

$$OD - OD_{app} = \log\left(1 + \beta \cdot \frac{1 - Tr}{Tr}\right) \tag{B3}$$

The difference between OD and OD_{app} increases as the OD OD increases. Furthermore, since the coefficient β in Eq. B3 depends on many factors that cannot be detected, the correction was calculated by a tuning operation. For each bacterial species the OD of a sample of a concentrate suspension was measured. Then, the same sample was diluted by a factor "f," and its read OD value (OD_{or}) multiplied by the dilution factor should give the OD of the diluted sample ($OD = f \cdot OD_{or}$). If this equation is not verified the difference between OD and OD_{app} gives us the correction term (right part in Eq. B3). The operation was continued in this way, taking as an original sample the previous diluted mixture until the correction was about 0 (error accepted 1%).

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