Praise Worthy Prize

International Review of Chemical Engineering (I.RE.CH.E.), Vol. 4, N. 4
ISSN 2035-1755 *July 2012*

Possible Non-Thermal Microwave Effects on the Growth Rate of *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*

Renzo Carta[1], Francesco Desogus[2]

Abstract – *The present work reports the effects of low power microwaves in the frequency range of 2.20-2.50 GHz with an incident power range of 0-400 mW on the growth rate of the bacterial species Pseudomonas aeruginosa and Staphylococcus aureus. Since culture temperatures were maintained constant (37°C) during irradiation, these effects can be considered as non-thermal. A continuous recirculating experimental structure was used for the purpose. It is very difficult to find a mathematical correlation between the observed effects and radiation power or frequency.*

Keywords: *Bacterial Growth, Food Treatments, Growth Rate Constant, Optical Density Measurements, Radiated Biological Reactor*

I. Introduction

One of the main risk factors connected with the assumption of foods is the microbiological one, due to pathogenic organisms (or to different kinds of toxins), which can come into contact with foods because they are normally present in the environment, or as a consequence of inadequate hygienic production and conservation systems; in many cases, the microbiological risk can be due to the voluntary addition of grafts (natural or graded), as different species and biotypes are an integral part of many fermented foods, improving edibility and conservability characteristics. Thus, both the need to have an acceptable level of food safety and the continuous launch of new food products on the market need to make technological innovation more and more rapid and important in terms of investment consistency. Furthermore, in recent years an increasingly greater part of food production has focused on slightly treated foods, similar as far as possible to fresh ones, subject to just mild treatment and with (possibly) no additives or preservative substances: thus, microbial growth has to be controlled and limited below harmful thresholds while aiming at preserving, at the same time, the nutritional and organoleptic properties of foods. This is the context in which the present work is set, part of a wider study on the non-thermal biological effects of food irradiation with low power microwaves; here the influence of microwaves, ranging from a frequency of 2.20 to 2.50 GHz with power in the range between 0 and 400 mW, on the *Pseudomonas aeruginosa* and *Staphylococcus aureus* species is considered. The use of electric fields in food treatments has been reported ever since 1935 when Getchell [1] made alternating current pass through some milk with heat generation, thereby deactivating the microorganisms present.

Nowadays, food preserving technologies based on strong pulsed electric fields seem to be more and more promising with the increase in demand for commercial food products similar to fresh ones [2], [3]. As regards food irradiation with ionizing radiations, both gamma (with a higher penetration depth) and X (with a lower penetration depth) rays are used, however these cause the loss of liposoluble components and essential fat acids, while some foods, like dairy products, are not suitable for treatment with ionizing radiations due to the development of a rancid taste [4]. Instead, UV rays (limited to particular applications due to their small penetration depth) and microwaves are used as non-ionizing radiations.

Microwaves are widely diffused as dielectric heating technology, by which materials are heated due to the very quick vibrational movement of dipolar molecules (in particular those of water, but it can also interest lipids, proteins and sugars) induced by alternating magnetic fields; if the material dimensions are small, heating may be quite uniform [5]. Consequently, in industrial applications, microwaves can be used in order to obtain volumetric heating: this is more rapid and selective, especially for materials which are bad heat conductors; more attention needs to be paid if the food structure is heterogeneous, since heating velocity can vary in different parts.

In food processing, microwaves are used for operations like cooking, drying, pasteurization and sterilization. The use of microwaves implies greater electric energy consumption with respect to conventional heating techniques, but the more efficient heating process allows a saving of primary energy, thus an integration between conventional and microwave heating should also be promoted [4]. Having said this, the possible existence of non-thermal effects, besides being

Manuscript received and revised June 2012, accepted July 2012

of great importance from the scientific point of view, could lead to innovative food treatments not based (or not based alone) on thermal heating, with certain qualitative advantages, since the nutritional and organoleptic properties of treated foods could be better preserved, thereby leading to the possibility of obtaining products more similar to the corresponding fresh ones: if the same effects as conventional thermal pasteurization can be obtained by using small doses of ionizing radiations [6], but with fewer effects on organoleptic properties, these properties could be reasonably even less damaged by using non-ionizing radiations; furthermore, potential economic advantages could arise. The reference context of this hypothesis has been made in many previous findings. The first point of view to be considered is a strictly chemical one. In fact, among the methods which promote and control chemical reactions, the use of magnetic fields has always been the least considered [7]: this could be due to the small quantity of energy that can be supplied to the reactants even by very high intensity magnetic fields, which can be several orders of magnitude smaller than the amount necessary for the chemical reaction [8]. Nevertheless, not just the absolute value of energy should be considered, but also the spin angular momentum of reactants electrons and nucleus. In fact, all chemical reactions are spin-selective, meaning that they are possible only under some spin conditions and not possible with others: identical chemical reactants can have completely different reactivities depending on spin conditions, and reactions with unfavourable spin conditions cannot develop in spite of the favourable energy conditions [9]-[11]. The only interactions able to modify the reactants spin conditions, changing a non-reactive into a reactive status (and vice versa) are magnetic ones, in spite of the fact that they do not supply energy to the reaction [12], [13]. The principle can be summarized in the conservation of the total spin in elementary chemical reactions, both in intensity and in orientation; for this reason, chemical reactions are spin-selective, and are not possible if the total spin were not maintained [14]. Of course, many chemical reactions with high biological relevance are also spin-selective, in other words, they are magnetic sensitive; an example of these are photosynthesis reactions, which show effects induced by the magnetic component of microwaves [5]. Furthermore, biological effects of electromagnetic fields can be due to the formation of free radicals as a consequence of magnetic field exposure [15], such as hydroxyl radical (·OH) [16]. It is worth saying that many bioeffects of magnetic field exposure on microorganisms are currently or potentially valuable in biotechnology and bioenergy applications [17], [18]. For example, it is now generally accepted that weak electromagnetic fields can activate DNA to synthesize proteins [19]-[21] or directly interact with electrons in DNA, sometimes leading to breaks in DNA strands [19]. In principle, the magnetic field can produce positive or negative effects on the growth and metabolism of living organisms [22].

The interest of scientists on the non-thermal effects of microwaves on biological tissues is relatively quite recent [23]-[26], but even so many researchers have already verified these kinds of effects. Webb and Dodds [27] and Webb and Booth [28] reported that *Escherichia coli* cells, grown in a culture medium and exposed to microwaves, showed a slower cell division and anticipated the inhibition of metabolic processes with respect to cell lifetime; at cell biology and enzyme chemistry levels, many observations of microwave associated magnetic field effects have been carried out, like those of [29]-[35]. It was also found that the microstructural heterogeneity proper to biological systems (whether at cell, organ or organism level) is of great relevance, as it has at least two important consequences.

The first one is that heterogeneity produces a non-homogeneous electromagnetic field distribution in the different structural elements, so its local amplitude can be much higher than its medium value, even at different orders of magnitude; the second one is that structural heterogeneity of cells and organs with respect to dielectric and conductivity leads to strongly non-homogeneous field absorption, so both temperature and local ionic currents can exceed, even by far, their medium values [35]. As a result of this, even if they are local phenomena and interest a microscopic scale not perceivable by standard measurement methodologies, they can nevertheless strongly condition cell functions; consequently, these effects can show at cell activity and membrane levels, and then at the whole organism level; the latter concept, in the opinion of Buchachenko and Frankevich [5], seems to agree with many experimental determinations; for example, Akoev [36] found biological effects induced by electromagnetic fields only on systems characterized by microscopic level heterogeneity while no effects were found on systems of the same chemical nature but with a homogeneous structure [37]. Also water molecules, which play a critical role in biochemical and biological reactions, can be affected by magnetic and electromagnetic fields [38]-[42].

One of the main difficulties in distinguishing between thermal and non-thermal effects is to maintain a constant temperature during microwave irradiation; this problem was faced by Kozempel et al. [43], who debugged a discontinuous process through which they reduced the bacterial count of *Pediococcus sp.* populations in sugary solutions maintaining a (nominal) temperature of 35°C for times up to nine minutes through an efficient heat exchange system. Another method with the same aim was set up by Sato et al. [44], who observed, at 45, 47.5 and 50°C (but not at 35) an increase in the death rate of *Escherichia coli* bacteria exposed to microwaves compared to the death rate at the same temperatures but in absence of irradiation, thereby suggesting

modifications in microorganism protein secondary and tertiary structures, induced by quick electric field fluctuations, as the controlling mechanism. Geveke et al. [45] developed an experimental continuous microwave process in order to isolate non-thermal effects from thermal ones during a hypothetical "non-thermal pasteurization" operation of liquids; the process is based on a rapid thermal energy supply to the system by microwaves combined with a likely rapid system of heat removal by external exchange; the authors found that, at 35°C and with exposure times between 3 and 8 minutes, no significant reduction of yeasts or bacterial count happened if microwaves were not coupled with a heat supply or with pH conditions unfavourable to microorganisms. Culkin and Fung [46] found that *Escherichia coli* and *Salmonella typhimurium* bacteria were destroyed if exposed to microwaves at the frequency of 915 MHz, and that cell death happened at lower temperatures and in shorter times with respect to conventional heating methods; besides, they noted different effects depending on the field intensity; the obtained results led them to suppose mechanisms that were justified not only by normal microwave thermal effect.

A patent by Hofmann [47] explains that an alternating magnetic field couples energy in magnetic active parts of biological macromolecules with oscillations; in this way, when a certain number of magnetic dipoles is located in only one molecule, the energy transmitted to it can be high enough for a covalent bond breaking; such molecules can be vital for microorganisms, such as DNA or proteins, thus the interested microorganisms can be destroyed or at least simply rendered unable to reproduce.

On the basis of this patent, Mertens and Knorr [48] tried to evaluate the effectiveness of microwave energy in killing bacteria in liquid foods at low temperatures, and to realize a process based on this mechanism, at the same time bringing thermal damage to food to a minimum; to pursue their aim, the two researchers studied the effect of microwaves on the *Pediococcus sp.* microorganism in a flux totally recirculating system. As regards *Staphylococcus aureus*, non-thermal effects were found by [49] and by [50]. It should be highlighted that *S. aureus* is a particularly strong microbial species since it has good heat resistance [51], it is not very sensitive to magnetic fields [15] and is able to grow even with very low water activity values and in environments with high (up to 7.5%) NaCl concentrations [52].

II. Materials and Methods

In the authors' opinion, in order to discriminate between thermal and non-thermal biological effects, the problem of the possible temperature rise in the medium and/or the cells becomes of primary importance. About the bacteria cells, a local temperature rise cannot be excluded, as it is not perceivable by standard measurement instruments, but this fact is limited if the applied electromagnetic field is weak and the medium temperature is lower than that of the microorganism cells, as the heat exchange from the cells to the culture medium is made easier.

Having said this, first of all, only the use of low enough power, combined with an efficient temperature control system, can really allow the best conditions for discrimination between the supposed non-thermal effects and thermal ones, which are, on the contrary, well known. Consequently, the following apparatus was developed.

II.1. Experimental Apparatus and Conditions

The designed experimental structure allows the continuous circulating of bacterial suspensions under different irradiation conditions.

The focus is a biological reactor positioned in the internal side of a steel plate closing a waveguide: this is the contact point between biological matter and the electromagnetic field in the desired experimental and safety conditions.

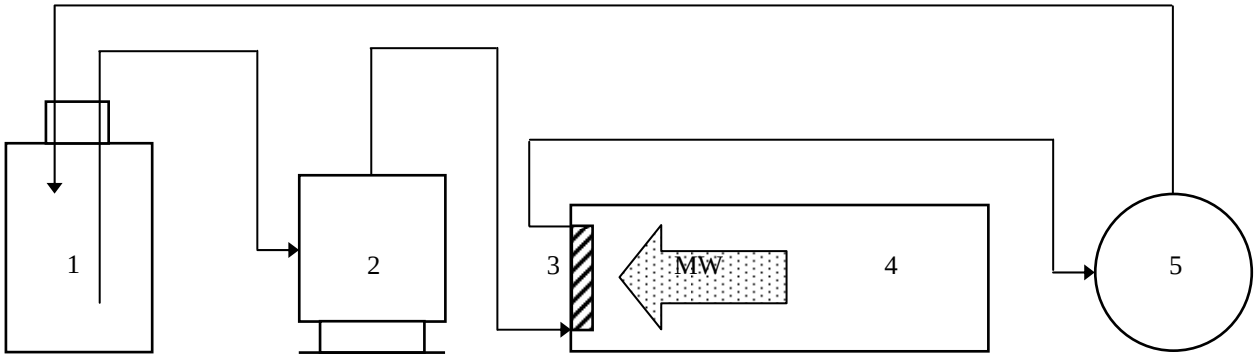

Fig. 1. Sketch of the experimental apparatus (1: container with the suspension to be irradiated; 2: peristaltic pump; 3: reactor; 4: waveguide; 5: spectrophotometric analysis system)

The bacterial suspension is contained in a Pyrex bottle that has previously been filled with the culture medium, hermetically sealed and sterilized, and then inoculated with microorganisms; the bottle cap has four passages with the following functions:

- insufflating of external air (previously filtered by a ceramic filter of 22 μm);
- input of the bacterial suspension from the irradiated reactor;
- output of the bacterial suspension to the reactor; output of the excess air.

The suspension contained in the bottle can be maintained at the fixed constant temperature by a water thermostation, then it is sucked and circulated by a peristaltic pump with a flow rate of 40 ml min^{-1}; the circuit was made of silicone tubes, since it is flexible and transparent to microwave material [53]. The experimental temperature was fixed at 37°C as it is a value near to the optimal one for most pathogenic examined, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This choice has another important (from an experimental procedure point of view) consequence: possible differences between microbial growth rates in the presence and absence of microwaves are enhanced with temperature in these conditions, though this is not itself a growth limiting factor; at the same time, the influence of possible experimental errors due to temperature control by the thermostatation is limited, as the sensitivity of the growth rate with respect to temperature in the optimal growth temperature zone is minimum. Furthermore, another important element should be considered: 37°C is the medium value of human body temperature, so it is the maximum temperature to which food is exposed in the passage from the mouth to the digestive system; as a consequence, carrying out food treatment at 37°C produces nutritional losses at least equal to those produced by its consumption. The fixed temperature is the same as that of the previous work by Carta and Desogus [54]. Information about microbial concentration was obtained at fixed times by measurements of optical density.

II.2. Biological Materials

The following culture mediums were used:

- "Mueller Hinton Broth" (Microbiol®) at a concentration of 22 g/l for *Pseudomonas aeruginosa*;
- "Brain Hearth Infusion Broth" (Microbiol®) at a concentration of 37 g/l for *Staphylococcus aureus*.

Bacterial suspensions for inoculation were prepared in the following ways:

- for *Pseudomonas aeruginosa* from a local strain, with bacteria previously made by adapting in MH at 37°C for 24-48 h;
- for *Staphylococcus aureus* from an ATCC 25923 strain, with bacteria previously made by adapting in BH at 37°C for 24-48 h.

II.3. Microwave Apparatus

The low power microwave generating apparatus, already presented by the same authors [54], is composed of the following electronic components:

- Micro Lambda Wireless MLOM-0204 miniature permanent magnet YIG-Tuned (Yttrium Iron Garnet) oscillator;
- DC Block (SuhnerVR 1100.01.A);
- 6-dB fixed attenuator (JFW Industries 4AH-06);
- rotary attenuator (JFW Industries 50R-248);
- power amplifier (Herotek P/N AP271135);
- insulator (RF & NC CI-200-172);
- waveguide adapter (MEC P/N LA 40-30-CH WR430);
- waveguide type WR430 (MEC P/N LA 160-30N).
- Connections were made using RG316 cables with SMA type connectors; the incident power measurements were made using a power meter (Hewlett PackardVR 436A) with a power sensor (Hewlett PackardVR 8481A).

II.4. Experimental Data Analysis

To describe the kinetic behaviour of the examined microorganisms populations the equation of Monod [55] was chosen:

$$R_M = \frac{k \cdot C_C \cdot C_S}{K_M + C_S} \tag{1}$$

where R_M is the growth rate as a function of microorganism concentration (C_C) and of substrate concentration (C_S) for a determined temperature (which significantly affects the growth rate constant), pH, aeration and carbon and other nutrient source conditions; k and K_M are respectively the growth rate constant and the so called "Monod constant". If $C_S >> K_M$, the growth rate is maximum (unlimited growth), so the following linear relationship can be considered:

$$R_M = k \cdot C_C \tag{2}$$

It can be linearized as:

$$Ln\left(\frac{C_C}{C_{C,0}}\right) = k \cdot (t - t_0) \tag{3}$$

where t represents time and $C_{C,0}$ is the concentration at t_0, that is the time at which the exponential growth phase begins. The beginning of the exponential growth phase was empirically identified for each experimental run, as it depends on the length of the lag time which is difficultly determined beforehand; some researchers, like Baranyi [56]-[57], tried to foresee its length by stochastic models, but it is beyond the purposes of the present work. The growth rate constant, for each experimental run, can be obtained as the slope of the data regression line. To calculate the growth rate constants for the

examined bacterial species, experimental optical density data were obtained at a wavelength of 600 nm and with a 5 minute interval. Experimental runs were performed at frequencies of 2.20, 2.30, 2.40 and 2.50 GHz and at incident powers of 100, 200, 300 and 400 mW, as well as in the absence of irradiation.

III. Results

For each power level, a number of runs sufficient to provide 21 giving growth rate constants close enough to each other were performed; about 85-90% of runs gave an acceptable result, that is, a growth rate constant with a maximum deviation of 5% from the medium value and obtained with a regression coefficient (linear regression made by equation 3) not lower than 0.999; a maximum deviation of 5% was assumed taking into account the high inaccuracy (not improvable) levels of many variables (radiation power and frequency, specific adsorption rate, reacting mixture flow rate into the circuit, mixture volume, nutrient concentration, initial physiological state of cells, etc.) and the possibility of occurrence of external origin contamination.

Furthermore, the acceptability of results from optical density measurements was checked by performing some withdrawals and following the bacterial counts on plates: as well as revealing the real consistency of bacterial populations, it also made it possible to show (or exclude) the occurrence of external contamination.

Fig. 1 and 2 report the medium values of the growth rate constants for *Pseudomonas aeruginosa* and *Staphylococcus aureus* species in the different experimental conditions. As can be deduced from the data in Fig. 1 and 2, precise evidence of non-thermal microwave effects on the examined bacterial species can be found with difficulty, in particular for *Pseudomonas aeruginosa*, since variation of growth rate constants with respect to the correspondent value in the absence of irradiation are relatively small.

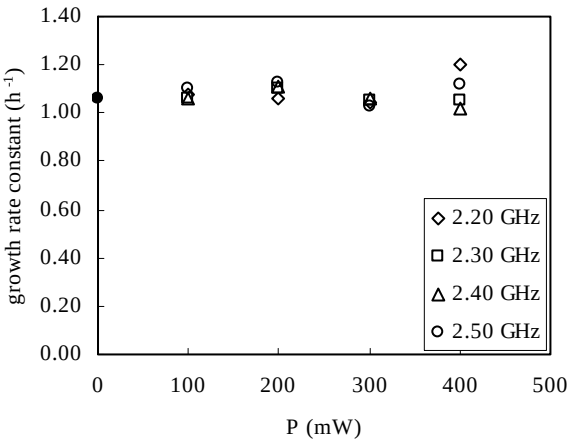

Fig. 2. Growth rate constants for *Pseudomonas aeruginosa* for different values of incident power (P) and frequency (F); error of ±5%

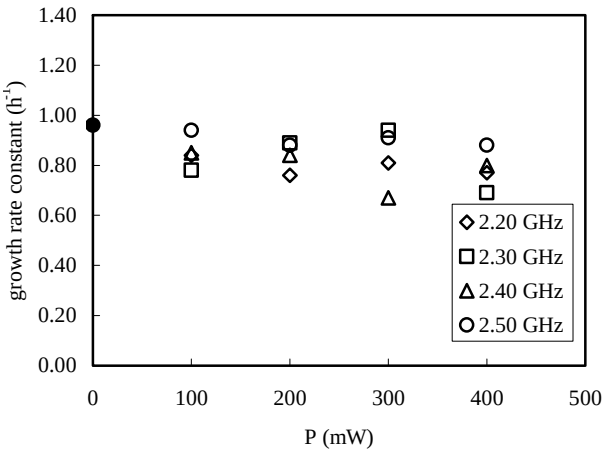

Fig. 3. Growth rate constants for *Staphylococcus aureus* for different values of incident power (P) and frequency (F); error of ±5%

Nevertheless, the presence of some non-thermal effects can likewise probably not be refused, in particular for *Staphylococcus aureus*, since variations are greater and all negative with respect to the reference value obtained in the absence of radiation. In both cases a relationship between microwave power and frequency and the size of their effects on the examined bacterial species cannot be identified.

However, the latter statement does not express a dissatisfied necessity, as different literature evidence reports similar results (absence of correlation between experimental conditions and measured effects) and "window effects": a very interesting fact is that a good part of the experimental results suggests that the degree of non-thermal effects is almost independent of the absorbed power, and this is also demonstrated by the presence of "window effects", like those identified by [58] concerning cell growth rate and by [59]-[63] regarding Ca^{2+} ion efflux; in other words, from a theoretical point of view, some effects may be demonstrated in particular experimental conditions but may not show in different, even if only slightly different, experimental conditions. With regard to the presented results, it could be considered a "window effect" the behaviour of *Pseudomonas aeruginosa* species at 400 mW of power (apparent greater sensibility to frequency with respect to other power values) and the response of *Staphylococcus aureus* species to 300 mW of power and 2.40 GHz of frequency (the growth rate constant is much lower than those obtained for other frequencies and than the reference value obtained in the absence of radiation).

IV. Conclusion

From the obtained experimental results, possible microwave non-thermal effects on the growth rate of *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacterial species, in the frequency range of 2.20-2.50 Ghz and incident power of 100-400 mW should be highlighted; however, the size of these effects cannot be

directly related to microwave frequency or power; one possible explanation could be the showing of "window effects". As an obvious consequence of what has been discussed previously, the study needs to be continued and extended: radiation frequency and power ranges will be extended by modifying the microwave generating apparatus and other bacterial species will be studied; furthermore, the use of different culture mediums could be of interest, especially those more similar in their characteristics to foods subject to possible contamination, or even real liquid foods themselves. Obviously, no information regarding biological mechanisms induced or influenced by microwaves can be obtained from macroscopic kinetic information alone, and these mechanisms should also receive further investigation in order to better explain (if possible) kinetic behaviour.

References

[1] B.E. Getchell, Electric pasteurization of milk, *Agric. Eng. 16 (1935) 408-410*.

[2] Q. Zhang, A. Monsalve-Gonzalez, G.V. Barbosa-Canovas, B.G. Swanson, Inactivation of *E. coli* and *S. cerevisiae* by pulsed electric fields under controlled temperature conditions, *Trans. ASAE 37 (1994) 581-587*.

[3] Q. Zhang, G.V. Barbosa-Canovas, B.G. Swanson, Engineering aspects of pulsed electric field pasteurization, *J. Food. Eng. 25 (1995) 261-281*.

[4] P.J. Fellows, *Food Processing Technology – Principles and Practice (2nd ed.)* (CRC Press, 2000).

[5] A.L. Buchachenko, E.L. Frankevich, *Chemical Generation and Reception of Radio- and Microwaves* (VCH Publishers Inc., 1993).

[6] J.H. Steele, R.E. Engel, Radiation processing of food, *J. Am. Vet. Med. Assoc. 201 (1992) 1522-1529*.

[7] L. Sterna, D. Ronis, S. Wolfe, A. Pines, Viscosity and temperature dependence of the magnetic isotope effect, *J. Chem. Phys. 73 (1980) 5493-5499*.

[8] A.L. Buchachenko, *Chemically Induced Polarization of Electrons and Nuclei* (Nauka, 1974).

[9] A. Carrington, A.D. McLachlan, *Introduction to Magnetic Resonance* (Harper & Row, 1967).

[10] K.M. Salikhov, Y.N. Molin, R.Z. Sagdeev, A.L. Buchachenko, *Spin Polarization and Magnetic Effects in Radical Reactions* (Elsevier, 1984).

[11] P.W. Atkins, R.S. Friedman, *Molecular Quantum Mechanics (4th ed.)* (Oxford University Press, 2004).

[12] N.E. Geacintov, M. Pope, F. Vogel, Effect of magnetic field on the fluorescence of tetracene crystals: exciton fission, *Phys. Rev. Lett. 22 (1969) 593-596*.

[13] N.E. Geacintov, M. Pope, S. Fox, Magnetic field effects on photo-enhanced currents in organic crystals, *J. Phys. Chem. Solids. 31 (1970) 1375-1379*.

[14] U.E. Steiner, T. Ulrich, Magnetic field effects in chemical kinetics and related phenomena, *Chem. Rev. 89 (1989) 51-147*.

[15] L. Fojt, L. Strašák, V. Vetterl, J. Šmarda, Comparison of the low – frequency magnetic field effects on bacteria *Escherichia coli*, *Leclercia adecarboxylata* and *Staphylococcus aureus*, *Bioelectrochemistry 63 (2004) 337-341*.

[16] M. Kohno, M. Yamazaki, I. Kimura, M. Wada, Effect of static magnetic fields on bacteria: *Streptococcus mutans*, *Staphylococcus aureus*, and *Escherichia coli*, *Pathophysiology 7 (2000) 143-148*.

[17] R.W. Hunt, A. Zavalin, A. Bhatnagar, S. Chinnasamy, K.C. Das, Electromagnetic biostimulation of living cultures for biotechnology, biofuel and bioenergy applications, *Int. J. Mol. Sci. 10 (2009) 4515-4558*.

[18] V. Perez, A. Reyes, O. Justo, D. Alvarez, Bioreactor coupled with electromagnetic field generator: effects of extremely low frequency electromagnetic fields on ethanol production by *Saccharomyces cerevisiae*, *Biotechnol. Prog. 23 (2007) 1091-1094*.

[19] M. Blank, R. Goodman, Electromagnetic fields stress living cells, *Pathophysiology 16 (2009) 71-78*.

[20] M. Blank, Protein and DNA interactions with electromagnetic fields, *Electromagn. Biol. Med. 28 (2008) 3-23*.

[21] M. Blank, R. Goodman, A mechanism for stimulation of biosynthesis by electromagnetic fields: charge transfer in DNA and base pair separation, *J. Cell Physiol. 214 (2008) 20-26*.

[22] J. Hristov, Magnetic field assisted fluidization – a unified approach. Part 8. Mass transfer: magnetically assisted bioprocesses, *Rev. Chem. Eng. 26 (2010) 55-128*.

[23] J.A. D'Andrea, O.P. Gandhi, J.L. Lords, C.H. Durney, C.C. Johnson, L. Astle, Physiological and behavioral effects of chronic exposure to 2450-MHz microwaves, *J. Microwave Power Electromagn. Energy 14 (1979) 351-362*.

[24] W.R. Adey, Tissue interactions with nonionizing electromagnetic fields, *Physiol. Rev. 61 (1981) 435-514*.

[25] J.A. D'Andrea, J.R. De Witt, R.Y. Emmerson, C. Bailey, S. Strensaas, O.P. Gandhi, Intermittent exposure of rats to 2450 MHz microwaves at 2.5 mW/cm^2: behavioral and physiological effects, *Bioelectromagnetics 7 (1986) 315-328*.

[26] J.A. D'Andrea, J.R. De Witt, O.P. Gandhi, S. Strensaas, J.L. Lords, H.C. Nielson HC, Behavioral and physiological effects of chronic 2,450-MHz microwave irradiation of the rat at 0.5 mW/cm^2, *Bioelectromagnetics 7 (1986) 45-56*.

[27] S.J. Webb, D.D. Dodds, Inhibition ob bacterial cell growth by 136 gc microwaves, *Nature 218 (1968) 374-375*.

[28] S.J. Webb, A.D. Booth, Absorption of microwaves by microorganisms, *Nature 222 (1969) 1199-1200*.

[29] P.W. Atkins, T.P. Lambert, The effect of a magnetic field on chemical reactions, *Annu. Rep. Prog. Chem. (Sect. A, Inorg. and Phys. Chem.) 72 (1975) 67-88*.

[30] J.M. Barnothy, Growth-rate of mice in stati magnetic fields, *Nature 200 (1963) 86-87*.

[31] J.M. Barnothy, M.F. Barnothy, I. Boszormenyi-Nagy, Influence of a magnetic field upon the leucocytes of the mouse, *Nature 177 (1956) 577-578*.

[32] M.F. Barnothy, *Biological Effects of Magnetic Fields* (Plenum Press, 1964).

[33] M.F. Barnothy, J.M. Barnothy, Biological effect of a magnetic field and the radiation syndrome, *Nature 181 (1958) 1785-1786*.

[34] M.F. Barnothy, I. Sumegi, Abnormalities in organs of mice induced by a magnetic field, *Nature 221 (1969) 270-271*.

[35] C.B. Grissom, Magnetic field effects on enzymatic reactions, *Symposium on Magnetic Field and Spin Effects in Chemistry. Konstanz, Germany, July 26-31 (1992)*.

[36] I.G. Akoev, *Biological Effects of Electromagnetic Fields* (USSR Academy of Sciences, 1986).

[37] F.S. Sarvarov, K.M. Salikhov, Theory of spin-dependent recombination of radicals in homogeneous solution, *React. Kinet. Catal. Lett. 4 (1976) 33-41*.

[38] V.N. Binhi, Theoretical concepts in magnetobiology, *Electromagn. Biol. Med. 20 (2001) 208-216.*

[39] V.I. Lobyshev, Water is a sensor to weak forces including electromagnetic fields of low intensity, *Electromagn. Biol. Med. 24 (2005) 449-461*.

[40] M.F. Chaplin, The memory of water: an overview, *Homeopathy 96 (2007) 143-150*.

[41] A.V. Tschulakow, Y. Yan, W. Klimek, A new approach to the memory of water, *Homeopathy 94 (2005) 241-247*.

[42] C. Cardella, L. De Magistris, E. Florio, C.W. Smith, Permanent changes in the physicochemical properties of water following exposure to resonant circuits, *J. Sci. Explor. 15 (2001) 501-518*.

[43] M. Kozempel, O.J. Scullen, R. Cook, R. Whiting, Preliminary investigation using a batch flow process to determine bacteria

destruction by microwave energy at low temperature, *Lebensm. Wiss. U. Technol. 30 (1997) 691-696*.
[44] S. Sato, C. Shibata, M. Yazu, Nonthermal killing effect of microwave irradiation, *Biotech. Tech. 10 (1996) 145-150*.
[45] D.J. Geveke, M. Kozempel, O.J. Scullen, C. Brunkhorst, Radio frequency energy effects on microorganisms in foods, *Innovat. Food Sci. Emerg. Technol. 3 (2002) 133-138*.
[46] K.A. Culkin, D.Y.C. Fung, Destruction of *Escherichia coli* and *Salmonella typhimurium* in microwave cooked soups, *J. Milk Food Technol. 38 (1975) 8-15*.
[47] G.A. Hofmann, *Deactivation of microorganisms by an oscillating magnetic field*, Patent WO 85/02094 (1985).
[48] B. Mertens, D. Knorr, Developments of non-thermal processes for food preservation, *Food Technol. 46 (1992) 124-133*.
[49] M.S. Dreyfuss, J.R. Chipley, Comparison of effects of sublethal microwave radiation and conventional heating on the metabolic activity of *Staphylococcus aureus*, *Appl. Environ. Microbiol. 39 (1980) 13-16*.
[50] U.R. Pothakamury, A. Monsalve-Gonzalez, G.V. Barbosa-Canovas, B.G. Swanson, Inactivation of *Escherichia coli* and *Staphylococcus aureus* in model foods by pulsed electric field technology, *Food Res. Int. 28 (1995) 167-171*.
[51] H.S. Ramaswamy, F.R. Voort, S. Ghazala, An analysis of TDT and Arrhenius methods for handling process and kinetic data, *J. Food Sci. 54 (1989) 1322-1326*.
[52] K.J. Ryan, C.G. Ray, *Sherris Medical Microbiology (4th ed.)* (Mc-Graw Hill, 2004).
[53] A.R. von Hippel, *Dielectric Materials and Applications* (Technology Press of MIT, 1954).
[54] R. Carta, F. Desogus, The effect of low-power microwaves on the growth of bacterial populations in a plug flow reactor, *A.I.Ch.E. J. 56 (2010) 1270-1278*.
[55] J. Monod, The growth of bacterial cultures, *Annu. Rev. Microbiol. 3 (1949) 371-394*.
[56] J.M. Baranyi, Comparison of stochastic and deterministic concepts of bacterial lag phase, *J. Theor. Biol. 192 (1998) 403-408*.
[57] J.M. Baranyi, Stochastic modelling of bacterial lag phase, *Int. J. Food Microbiol. 73 (2002) 203-206*.
[58] R.B. Stagg, W.J. Thomas, R.A. Jones, W.R. Adey, DNA synthesis and cell proliferation in C6 glioma and primary glial cells exposed to a 836.55 MHz modulated radiofrequency field, *Bioelectromagnetics 18 (1997) 230-236*.
[59] S.M. Bawin, L.K. Kaczmarek, W.R. Adey, Effects of modulated VHF fields on the central nervous system, *Ann. N. Y. Acad. Sci. 247 (1975) 74-81*.
[60] S.M. Bawin, A. Sheppard, W.R. Adey, Possible mechanism of weak electromagnetic field coupling in brain tissue, *Bioelectrochem. Bioenerg. 5 (1978) 67-76*.
[61] C.F. Blackman, J.A. Elder, C.M. Weil, S.G. Benane, D.C. Eichinger, D.E. House, Induction of calcium-ion efflux from brain tissue by radio-frequency radiation: effects of modulation frequency and field strenght, *Rad. Res. 14 (1979) 93-98*.
[62] C.F. Blackman, S.G. Benane, J.A. Elder, D.E. House, J.A. Lampe, J.M. Faulk, Induction of calcium-ion efflux from brain tissue by radiofrequency radiation: effect of sample number and modulation frequency on the power-density window, *Bioelectromagnetics 1 (1980) 35-43*.
[63] C.F. Blackman, S.G. Benane, W.T. Joines, M.A. Hollis, D.E. House, Calcium-ion efflux from brain tissue: power-density versus internal field-intensity dependencies at 50 MHz RF radiation, *Bioelectromagnetics 1 (1980) 277-283*.

Authors' information

[1]Università degli Studi di Cagliari, Dipartimento di Ingegneria Meccanica, Chimica e dei Materiali, Piazza D'Armi, 09123 Cagliari.
Tel.: 0706755068
E- mail: renzo.carta@dimcm.unica.it

[2]Università degli Studi di Cagliari, Dipartimento di Ingegneria Meccanica, Chimica e dei Materiali, Piazza D'Armi, 09123 Cagliari.
Tel.: 0706755070
E- mail: f.desogus@dimcm.unica.it

