Effects of Microwave Radiation on Living Microorganisms: Effects and Mechanisms

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The article analyzes the scientific literature on the effect of microwave exposure on the vital activity of microorganisms. The influence of the frequency of microwaves, the power of the applied impact and the total amount of absorbed energy on the viability of microorganisms and the features of their growth is considered. Possible mechanisms of interaction of microbial cells with the electromagnetic field in the ultrahigh frequency range are considered. It is noted that microorganisms die when exposed to high-energy and high-frequency microwaves, while low-energy and high-frequency microwaves contribute to the intensification of their growth. It is concluded that although many authors observe significant biological effects when exposed to microwaves on living systems, this issue has not been sufficiently studied in the scientific literature. It is of interest to conduct a systematic study of the effect of microwaves of a certain frequency on the biological, biochemical and growth parameters of the cells of microorganisms, in particular lactic acid organisms, in order to use the results of these studies in the food industry in the production of new food products.

Key words: microwave radiation; microorganisms; microbial culture; biological effect of microwaves; thermal and non-thermal effects of physical factors.

Introduction. Microwaves

Since the advent of technical devices that operate using electromagnetic waves, interactions between various types of electromagnetic radiation and living organisms have attracted the attention of scientists.

Microwaves are electro-magnetic energy sources. Microwave energy is relatively low and a non-ionizing radiation (In one quantum there is approximately 10-5 electron volts (eV) which is substantially lower than the quantum energy needed to eject an electron from a molecule or to break an intra-molecular bond (> 10 eV) (Michaelson 1974)) that induces molecular motion by ion migration and dipole rotation but does not alter the structure of the molecules. Microwave energy occupies a portion of the electromagnetic spectrum and is distinguished by being arranged between 1 mm and 1 m in the wavelength range and between 300 MHz and 300 GHz in the frequency range (Figure 1). They are usually used between 915 and 2450 MHz for industrial food processing and 2450 MHz for domestic use (Kalla et al., 2017).

In this review we will discuss how the microwave radiation will affects on bacterial cells and on the growth of microorganisms, the difference between thermal and nonthermal effects, the mechanisms of destruction by microwave radiation and the factors affecting microwave effects and another points.

Methods

We analyzed the articles published in the period from 1966 to 2020 in the international databases Scopus and Web of Science on the use of the electromagnetic field of the microwave range for the regulation of the various microorganisms' vital activity and their ability to produce enzymes. The following keywords were used: microwave radiation, microorganisms, biological effects of microwave treatment. The comparative analysis of the results of experiments by different authors on the effect of microwave processing on the biological, biochemical and growth parameters of the microbial mass is carried out.

The effect of microwave radiation on the bacterial cells

An ongoing area of both interest and controversy is the study of the interaction of MW radiation with biological

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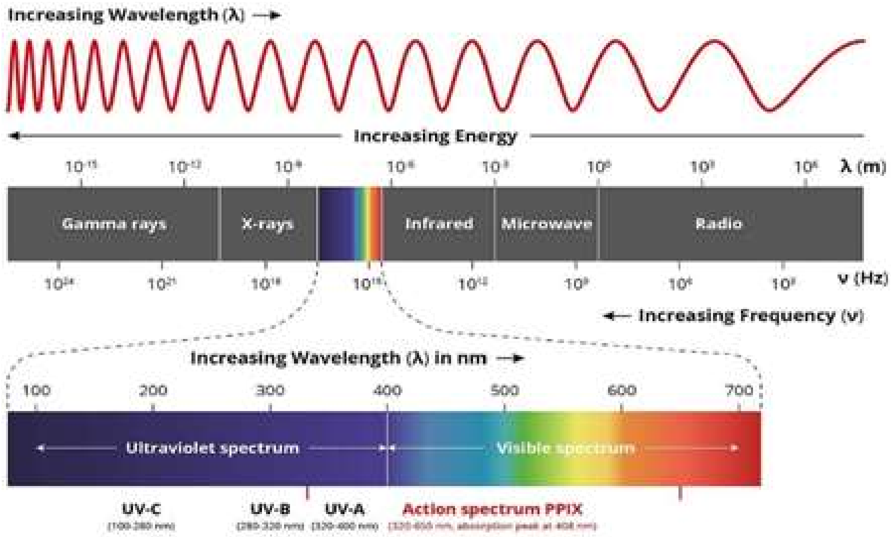
Figure 1
Electromagnetic spectrum



systems. Because of their rapid growth rate and the availability of a variety of experimental designs and testing protocols available for the elucidation of MW-induced effects, microorganisms are considered one of the best test systems for testing MW exposure. To date, a wealth of studies has shown evidence of some bio-effects arising from exposure to varying degrees of radiation in which variables such as MW frequency, power, temperature, and time of exposure all play important roles (Banik et al., 2003; Shamis, et al., 2008). Specific effects of MW are those that are reportedly independent of traditional mechanisms for thermal heating. Many studies have reported comparable findings about the presence of unique effects of MW radiation on biomaterials (Dreyfuss et al., 1980; Copty et al., 2006), although other researchers have reported contradictory results (Shazman et al., 2007; Shamis et al. 2012).

It is generally accepted that MW radiation causes dielectric heating in biological systems. This heat is generated as a result of the absorption of the MW energy by dielectric material (primarily water), with the MW energy transformed into heat due to the internal resistance of rotation (Heddleson & Doores 1994; Shamis et al., 2012). In other words, as a result of friction, dipolar molecule movement dissipates energy that has been obtained from microwaves. The thermal theory of MW effects claims that MW radiation bio-effects can be explained solely by differences in the temperatures or temperature profiles between MW and conventionally heated systems, and that no basis for direct electromagnetic interaction with living systems exists independent of such temperature-mediated effects (Shamis et al., 2012).

Thermal and nonthermal effects

Microwave radiation's biological effects can be classified into thermal and non-thermal effects. The thermal effect is one in which the MW energy in living systems is transformed into heat energy. Such effects may be macroscopic where whole or significant portions of organisms are involved in the heat transfer process, or microscopic where the selective application of MW heating vaporizes a cellular component such as bound water (Cleary, 1970). In the cells, polar molecules are present in the form of water, DNA, and proteins and through rotation they react to an electromagnetic field. This rotation produces an angular momentum that results in friction with adjacent molecules, producing a linear momentum (vibrational energy) (Saifuddin et al., 2009). Radiation energy is converted into thermal energy in this way. The effect caused by vibrational energy is a thermal effect that occurs in a biosystem due to the penetration of electromagnetic waves (microwaves) into biological materials and the transfer of vibrational energy to heat up intra- and extra-cellular fluids. However, the thermal effect of the MW varies from the traditional heating effect. In an attempt to match the dipoles with the applied MW field, dipolar polarization and rotation of molecules create results that cannot be accomplished by traditional heating (Zelentsova et al., 2006).

MW's non-thermal effect is extremely controversial and has been a subject of scientific controversy. It is postulated that non-thermal effects arise from a direct stabilizing interaction between the electric field and particular (polar) molecules in the reaction medium with no temperature increase (Herrero et al., 2008).

The manner in which the microwave influences biological systems cannot be explained by thermal effects alone. Several studies have shown that microwave radiation can destroy microbial cells, but it is still not clear if microwave non-thermal effects can lead to this. Altering the permeability of the cell membrane is one of the processes involved in the microwave killing of microorganisms. The alteration in cell membrane permeability is reflected by modifications in cell shape detected under electron microscopy or by spectroscopy to detect leakage of intracellular protein or DNA (Chen et al., 2007). Their nature is highly controversial due to the lack of knowledge about the exact mechanism involved in 'nonthermal microwave effects'. (Kozempel et al., 2000) attempted to detect the non-thermal effects of MW energy on low temperature microbes in different test fluids.

A continuous experimental microwave method was designed to distinguish thermal and non-thermal effects in a system, combining rapid energy input into the food system using microwave and rapid thermal energy removal using an effective heat exchanger design. In various test fluids, such as water, liquid egg, beer, apple juice, and tomato juice, continuous MW treatment (7 kW, 2450 MHz) was provided to the test species. They concluded that MW energy did not destroy microorganisms at low temperatures in the absence of other stresses and there was no compelling proof that, without thermal energy, MW energy could kill microorganisms. The effect of MW on *Staphylococcus aureus* at sub-lethal temperature was studied by (Dreyfuss et al., 1980) and the presence of a phenomenon different from thermal heating, resulting in altered activity of various metabolic enzymes, was suggested. There have been a number of findings favoring the presence of non-thermal effects (Dreyfuss et al., 1980; Copty et al., 2006; Carta & Desogus, 2010), but it is also not possible to disregard research refuting the likelihood of nonthermal effects (Kozempel et al., 2000; Shazman et al., 2007).

Influence of microwave processing parameters on the vital activity of microbial cells

MW contact with biological entities is affected by many variables, such as MW power and frequency, far-field versus near-field position, exposure duration, polarization, etc. Continuous and pulsed MW therapies have different effects on cells, studies say. In intermittent and continuous waves, human diploid fibroblasts and rat granulosa cells were exposed. DNA strand breaks were measured with the assistance of alkaline and neutral comet assays. In both cell types, single and double strand breaks occurred, but in the comet assay, intermittent exposure had a greater effect than continuous exposure (Diem et al., 2005). Normal human peripheral blood isolated lymphocytes were exposed to 2450 MHz MW radiation for 5 days in both continuous and pulsed forms. With an image analysis method, spontaneous lymphoblastoid transformation was calculated and it was found that pulsed wave exposure improved transformation to a greater extent than continuous wave exposure (Czerska et al., 1992).

It seems that the length of exposure to MW is a significant determinant of the impact of microwaves on living cells. Exposure time and power density are associated in such a way that the decrease in power density (PD) could be offset by an increase in exposure duration. *Escherichia coli* K12 AB1157 cells were irradiated with millimeter waves within the PD range of 10-20 to 104 W/cm^2 and it was found that to achieve the same improvements in chromatin conformation, a decrease in PD could be compensated by increasing exposure time (Belyaev et al., 1992). There have been reports suggesting that in determining the response of living cells to MW radiation, the duration of post-treatment time following MW exposure is also significant. Studies of sub-lethal MW radiation on *E. Coli* has shown that cell-surface changes are electro-kinetic in nature, and after 10 minutes of exposure, cells return to their original status (Shamis et al., 2011). Low-level 10 GHz MW radiation, 0.58 mW/cm^2 intensity, applied for 30-120 min, caused virulence loss in Agrobacterium tumefaciens strain B6, where a 30-60 percent decrease in tumor and turnip disk production capacity was observed in plants. This loss in virulence was reversible within 12 h (Moore et al., 1979).

Whether microwaves affect the growth of microorganisms largely depends on the radiation level and the overall energy absorbed by the microorganisms. Thus, when microwaves are applied at specific frequencies, with high energy and for long enough time, their thermal effect is most likely dominant and destroys bacterial cells or yeasts. Numerous studies with microwave irradiation of different cultures of bacteria and yeasts in a wet environment such as water suspension did not display additional micro-wave killing compared to traditional microwave heating at the same temperature (Gorny et al., 2007; Duhain et al., 2012). However, in a dry environment, the killing effect of microwave radiation decreased dramatically and occurred only after a prolonged period of irradiation, most likely due to lower microwave energy transformation to heat. Some of the experiments also showed that the extent of killing of microorganisms was associated with the moisture content of the experimental specimens. In contrast, when microorganisms were irradiated at temperatures lower than the point of thermal destruction with microwaves; various effects were observed, from killing to enhanced growth, and also killing effect on *Escherichia coli*, distinct from the effect of hyperthermia was observed in many studies (Ramesh et al., 2002).

In field of thermal effects, numerous studies were performed attempting to determine the minimum dose of microwave energy that could be used for disinfection or sterilization purposes because it was noticed that the heating of microorganisms to a certain temperature by microwaves could kill them. When cultures of *Escherichia coli* and *Bacillus cereus* spores in a home microwave oven were exposed to the maximum power of the microwave, they were completely destroyed after two and four minutes, respectively (Iuliana et al., 2015; Kalla et al., 2017).

The understanding of the non-thermal action mechanism of microwaves on microorganisms is very important for the potential future use of microwave technology or for the avoidance of its deleterious effects on humans living in symbiosis with microbes. In a study in which *Escherichia coli* cultures were exposed to microwaves (18 GHz, absorbed power 1500 kW/m^2, electric field 300 V/m) at temperatures below 40°C to avoid microwave thermal effects, transient morphological changes (dehydrated appearance) and openings of pores in the cell membrane about 20 nm in diameter allowing dextran molecules to pass were observed (Shamis et al., 2011). It seems that this effect is electro kinetic in nature caused by increased anions and cations movements, causing localized structural disarrangements of the cell membrane, resulting in the emergence of pores. Large membrane pores allow vital intracellular molecules to leak out of the bacterial cells which may lead to their death. The effect was reversible because more than 87% of exposed cells remained viable. This effect could be attributed to the non-thermal action of microwaves because these changes in the spore coat and inner membrane were not caused by the same temperature (as produced by the microwaves). When microwave radiation of the same characteristics was applied to forms of *Bacillus subtilis* vegetative, the cell walls were disrupted, and cytoplasmic (intracellular) protein aggregation was observed on electron microscopy transmission. Both the aggregation of cytoplasmic proteins and the swelling of the cell wall were observed after microwave irradiation in *Escherichia coli* and *Bacillus subtilis* (2450 MHz and 600 W at 40, 60 and 80 °C); these results could not be due to thermal damage because same temperatures from other sources of heat did not show similar changes. In addition to its effects on cell membranes, microwaves cause non-thermal acceleration of enzymatic reactions in microbial cells, such as non-aqueous esterification (forming of ester-type chemical compounds without water involvement), and this effect is concentration-dependent on the substrate. Moreover, microwaves can accelerate the synthesis of glycopeptides in vitro (out of the living cells) that may occur in microbial cells, altering their functions (Kim et al., 2008, Garcia-Martin et al., 2012).

Biological effects of microwaves

Research is being done worldwide on thermal and nonthermal effects of MW on different biological systems. *Phormidium spp. Kutzing* ISC31 (a cyanobacterium) grown in the medium BG-11 was treated using a MW oven at a frequency of 2450 MHz by combining five different intensities (180, 360, 540, 720 and 900 W/cm^2) and three pretreatments (10, 20 and 30 s). With increased strength and exposure time, the content of chlorophyll has decreased. Phycobiliprotein, phycocyanin, phycoerythrin and allophycocyanin synthesis increased in all exposures, with the exception of 720 and 900 W/cm^2 (30 s). The photosynthetic rate increased by all MW exposures compared to nitrogenase activity except at 180 W (10 s) and 720 W (10 s) compared to control (Asadi et al., 2011). Studies on *E. coli* and *S. aureus* indicated that microwave-induced physical damage contributed to improvements in membrane permeability and ultimately to extracellular Ca2+ inflow. After MW treatment with *S. aureus* and *E. coli* respectively (Chen et al., 2007), an improvement in cell permeability of up to 89.8% and 19.7% was obtained. The transformation efficiency effect of the MW was studied by (Fregel et al., 2008), where calcium chloride competent *E. coli* cells received MW treatment at 180 W for 1 min, and the transformation efficiency increased three-fold compared to the classical method. MW effects (2450 MHz, 55 W) on the cellular differentiation of *Bacillus subtilis* YB 886 and its Rec derivatives YB 886 A4 have been studied by (Otludil et al., 2004). It was found that the growth and amount of DNA of the organism decreased by 4% and 27% respectively after MW exposure, while the amount of RNA and plasmid increased by 6.5% and 21% respectively. They noted an increase in the amount of specific protein synthesized by the SOS repair system during DNA damage, and its binding to the promoter region of din C, following exposure to microwave in rec+ bacteria.

The effect of microwave radiation on the growth and enzymatic activity of the microbial mass

When exposed to MW radiation with a small frequency range of 41.8-42.0 GHz *Saccharomycetes cerevisiae* yeast growth rate has either increased up to 15% or decreased up to 29% (Grundler et al., 1977). When *S. aureus* culture was exposed to MW radiation (24 GHz) for 10, 20, 30, and 40 s in a controlled temperature experiment, the activity of various enzymes such as malate dehydrogenase, cytoplasmic adenosine triphosphatase, glucose6-phosphate dehydrogenase, and cytochrome oxidase increased in MW-treated cells as compared to MW non-treated cells, while membrane adenosine triphosphate, alkaline phosphate, and cytochrome oxidase increased in MW-treated cells as compared to MW non-treated cells (Dreyfuss et al., 1980). The effect of MW radiation on 94 Enterobacteriaceae strains was studied and it was found that the enzyme activity of suspended bacteria was increased by MW irradiation (Spencer et al., 1985). On *Aeromonas hydrophila*, low-power MW treatment (2450 MHz; 90 W; 2 min exposure) decreased its total protease activity by 33%. MW exposure completely inhibited the activity of urease and aflatoxin production in *S. aureus* and *Aspergillus parasiticus*, respectively (Dholiya et al., 2012). In enterohemorrhagic *E. coli*, low power (100 W, 60 s) MW radiation decreased not only the number of cells, but also acid tolerance and verocytotoxin productivity (Tsuji & Yokoigawa 2011). (Komarova et al., 2008) researched the effect of MWs on soil bacteria. Both the suppression and the enhancement of growth under the influence of MWs were observed for different bacterial organisms. After a shorter exposure time than suspensions of vegetative bacterial cells, spore suspensions responded to MW radiation. In the soil microbial complex, the effect of MW radiation on the accumulation of biomass and the intensity of other physiological processes in species of streptomycetes has led to changes in the amount and activity of these microorganisms.

The mechanism of destructive action of microwave radiation

The mechanism by which microwaves destroy microorganisms is still unknown but most researchers will agree that most of the killing effect is exerted by heat produced by microwaves in the food systems. One group of researchers suggested that heat alone was responsible for the killing effect of microwaves on microorganisms, while another group stated that heat might not be the only agent, although the latter group did not provide clear evidence of alternative agents. However, a third party, which examined cellular and subcellular events in microorganisms subjected to microwave irradiation, obtained a variety of findings, some of which were due to microwave effects and others to heating effects resulting in the injury of sub lethally treated microorganisms. The difficulty in comparing those findings is that the frequencies of the microwaves used, the microorganisms tested, and the media suspended used by different researchers were usually different. Microwaves heat foods unevenly, thereby compounding the issue of those variables. It is also difficult to determine the temperature of the suspended menstruum under irradiation since most temperature measurement instruments are influenced by microwaves at irradiation time.

The idea of pasteurizing or sterilizing foods at lower temperatures and shorter periods than those needed by traditional heating methods has been one of the more intriguing aspects of microwave energy usage. That could only be accomplished if microwaves selectively destroy microorganisms independently of the heating of the suspended menstruum by some still undetermined means. Another possibility is that the microenvironment surrounding the microbial cells may reach a higher temperature (thereby destroying the cell) than the macro-environmental temperature that conventional temperature measurement devices can measure.

Earlier research (1930-1950) in this field included calculating the viability of microorganisms in relation to the final temperature attained in the suspended media after microwave irradiation and the association of these two attributes in the understanding of the microorganism killing impact. Another approach was to keep the suspended media temperature below the microorganisms' thermal death point under investigation, while applying microwave irradiation to determine the killing effect of microwaves on microorganisms independent of the thermal effects.

Recent findings of microorganism destruction by microwaves at temperatures below the microorganism thermal destruction level were made by some researchers, where (Robe & Marketing. 1966) recorded sterilization of yeast-inoculated beer and wine using 27.12 MHz. The temperatures were 46.2-48.8 °C, though the author claimed that conventional heat required 60 °C for 30 min to kill the yeast. However, (Culkin et al., 1975) observed that destruction of *Escherichia coli* and *salmonella typhimurium* in microwave-cooked soups, did not directly correlate with the achieved temperature in soup after irradiation. A frequency of 915 MHz, they cooked tomato soup, vegetable soup, and beef broth. The temperature in the middle region of the soups was warmest for any given exposure period, that of the bottom was moderate, and that at the top was warmest, but the species in the top of the soups were killed more rapidly than those in the center and the bottom. The authors concluded that heat emitted during microwave exposure was insufficient to fully account for the microwaves' lethal effects. Authors (Cunningham, 1978) also observed reduction of microorganisms following brief microwave exposure with minimum temperature increases. Authors (Wayland et al., 1977) demonstrated variations in the rate of inactivation of *Bacillus subtilis* spores when treated with microwave and traditional heating and they noticed a nonthermal effect and concluded that heat and electromagnetic effects were strongly interdependent.

In a recent study on the impact of microwave radiation on metabolic activities, survival, enzyme productions and other factors of *Escherichia coli*, *Staphylococcus* aureus, Salmonella enteriditis, and Bacillus cereus, it reported that heat is a major factor in the effects of microwave radiation on test organisms and that there is little difference in survival and absorption between microwave treatment and conventional microorganism heat treatment when approaching thermal death point. For example, he found that cell numbers increased dramatically when cells were suspended in nutrient broth and irradiated for 20 s (46 °C) (Dreyfuss, 1978).

The thermal and nonthermal effects of microwaves on microorganisms are not clarified in those studies. Apparently, microwaves cause certain changes in biological systems that are difficult to completely explain by heat generated by dielectric molecules.

Conclusion

Thus, although many authors have observed significant biological effects when exposed to microwaves on living systems, this issue has not been sufficiently studied in the scientific literature. The data is fragmented and non-systematic. In this regard, it is of interest to conduct a systematic study of the effect of microwaves of a certain frequency on the biological, biochemical and growth parameters of cells of microorganisms, in particular lactic acid organisms, in order to use the results of these studies in the food industry in the production of new food products.

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Воздействие микроволнового излучения на живые микроорганизмы: эффекты и механизмы

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В статье проведен анализ научной литературы по вопросу о влиянии микроволнового воздействия на жизнедеятельность микроорганизмов. Рассмотрено влияние частоты микроволн, мощности приложенного воздействия и общего количества поглощенной энергии на жизнеспособность микроорганизмов и особенности их роста. Рассмотрены возможные механизмы взаимодействия клеток микроорганизмов с электромагнитным полем в сверхвысокочастотном диапазоне. Отмечено, что микроорганизмы погибают при воздействии высокоэнергетических и высокочастотных микроволн, в то время как низкоэнергетические высокочастотные микроволны способствуют интенсификации их роста. Сделан вывод о том, что хотя и наблюдаются многими авторами значительные биологические эффекты при воздействии микроволн на живые системы, этот вопрос в научной литературе изучен недостаточно. Представляет интерес провести систематическое исследование влияния микроволн определенной частоты на биологические, биохимические и ростовые показатели клеток микроорганизмов, в частности молочнокислых организмов, для использования результатов этих исследований в пищевой промышленности при производстве новых продуктов питания.

Ключевые слова: микроволновое излучение; микроорганизмы; микробная культура; биологическое действие микроволн; тепловые и нетепловые эффекты действия физических факторов

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