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Effect of Low Power Microwave Radiation on Microorganisms and other Life Forms

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Abstract - Microwaves are a part of electromagnetic spectrum with multiple applications. Thermal effects resulting from high-power microwave radiation are well established, but considerable controversy surrounds the possible microwave specific athermal effects. Reports suggesting its presence or absence keep accumulating in literature. Exact mechanism through which low-power microwave radiation exerts its effect on different life forms including microorganisms yet remains to be elucidated. For this effective experimental strategies needs to be devised, where thermal and athermal effects can be studied separately. Identifying suitable biological model(s) for such studies is also required. This review focuses on the effects of low-power microwave radiation on different life forms, particularly microorganisms. Effects of low-power microwave on growth and enzyme activity of microbes are described. Factors affecting the interaction of microwaves with living systems, such as microwave power, frequency, exposure duration, continuous vs. pulsed treatment, etc. has also been covered. The possibility of using microwaves for mutagenesis (strain improvement) has also been indicated.

Keywords - Athermal effect, Microwave specific effect, Non-thermal effect, Microwave mutagenesis, Low-power microwave

1. Introduction

Microwaves (MW) are a part of electromagnetic spectrum with frequencies ranging from 300 MHz to 300 GHz corresponding to wavelength range of 1 mm to 1 m. They are non-ionizing radiation, and are placed next to infrared component of the electromagnetic spectrum (Fig.1). Out of the two perpendicular oscillating fields- the electric field and the magnetic field, former is responsible for heating [1]. The rapid heating achieved by MW has been exploited in several ways, and the mechanism underlying the thermal effect generated by MW is understood quite well. The challenge of understanding the non-thermal effects (particularly biological effects), and their mode of action still stands.

However, an appreciable degree of knowledge on MW has been generated, resulting in many biological and non-biological applications. Microwave irradiation can alter the rate of enzyme catalysed reactions, although the role of any non-thermal factors in such processes is controversial. The microwave assisted applications described in literature include use of lipases for esterification and transesterification reactions, protease for peptide synthesis. The so-called non-thermal effects are shown to increase the reaction rates by 2-5 fold at all water-activity levels. Microwave irradiation can be used in conjunction with other strategies for enhancing reaction rates. Miscellaneous applications of MW in bio-analysis, food processing and extraction of biological materials have also been reported. Enhancement of the rates of enzymatic reactions by pretreatment of insoluble substrates with controlled microwave irradiation is another interesting application [2].

MW was first applied to biological cells by Antonin Gosset in 1924, who along with his co-workers used these waves to destroy tumors in plants [3]. In modern world, MW radiations of specific frequency range are used for specific applications; those in 30-300 MHz range (VHF: very high frequencies) are used in FM radio and television, those with 300 MHz-3 GHz (UHF: ultra high frequencies) are used in MW ovens, and RADAR (Radio Detection and Ranging). MW frequency of 30 GHz-300 GHz (EHF: extra high frequencies) has been assigned for satellite-to-earth communications [4].

Apart from its use in domestic ovens, many other applications of MW in different areas have been identified. Applications of MW that are based primarily on its thermal effects include those in food processing industry [5], disinfection and sterilization [6,7], moisture removal [8], waste treatment [9], etc.

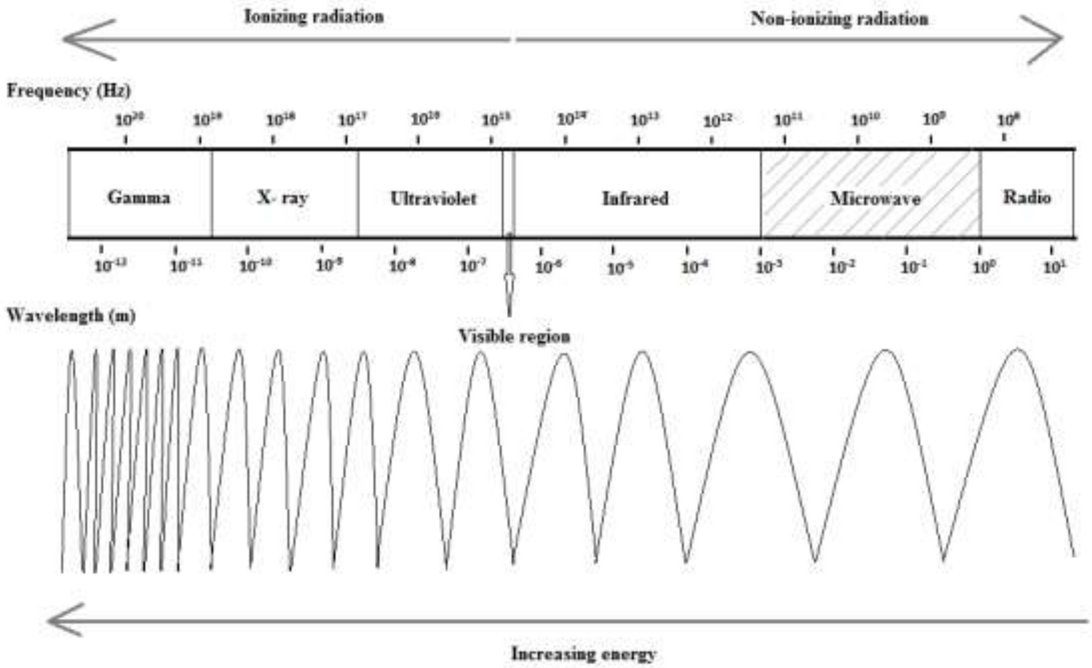

Fig. 1. Position of microwave radiation in electromagnetic spectrum (http://www.chem.ucalgary.ca/courses/350/Carey5th/Ch13/ch13-1.html)

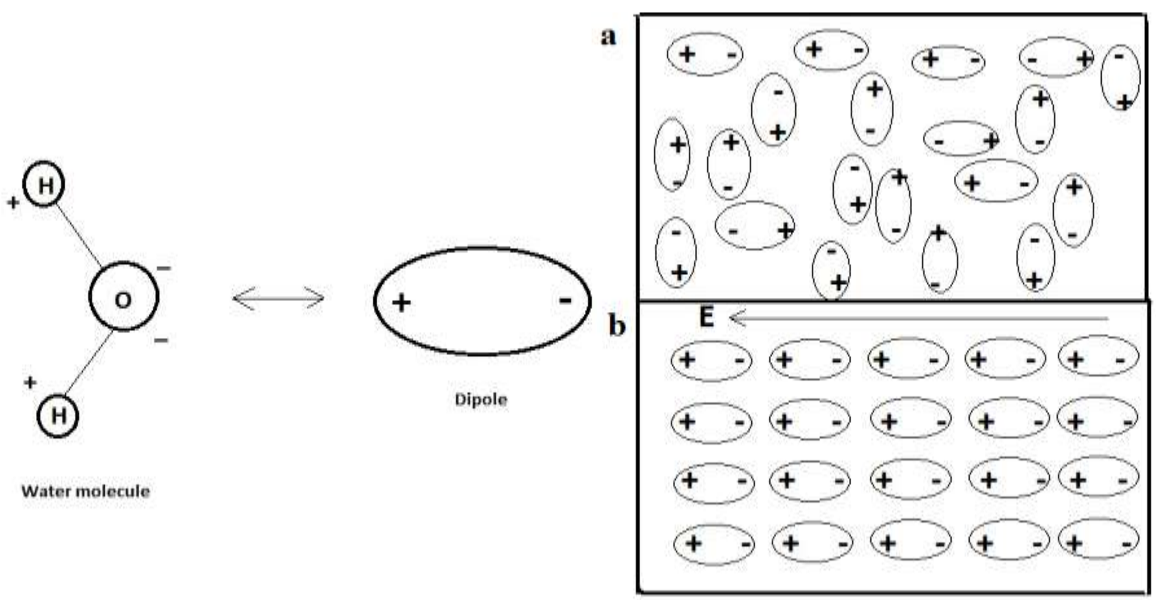

Fig. 2. The orientation of dipoles in absence (a) and in presence (b) of electric field (Williams J.M., 2009)

MW irradiation is also used to disinfect household products such as sponges, kitchen utensils, syringes and medicinal devices where bacterial viability is reduced by MW treatment. Microwave assisted extraction (MAE) has emerged as a promising method for preparation of bioactive plant extracts. It is considered suitable for fast extraction of phenolic compounds, and also for extraction of heat-labile phytoconstituents [10,11,12]. Research on non-thermal effects can open the door for new applications which may be based either solely on non-thermal effect, or both thermal and non-thermal effects. Either low power or high power (for very short duration, and with a provision of cooling water circulation to avoid heating) MW can be used for mutagenesis in plants [13] and microorganisms [14,15], protein unfolding [16], tumor detection based on electrical properties of tissues [17], rate enhancement of biochemical reactions [18,19] enzyme immobilization [20], and reduction of pathogenic population [21].

2. Thermal versus athermal effects

Biological effects of MW radiation can be divided into two categories: thermal effects and non-thermal effects. Thermal effect is the one in which the MW energy is converted into heat energy in the living systems. These effects can be macroscopic where whole organisms or major portions of them participate in the heat transfer process, or microscopic where a cellular component like bound water is vaporized by the selective application of the MW heating [22]. The dielectric effect of MW on polar molecules has been known for more than a century [23]. Polar molecules are present in the cells in the form of water, DNA, and proteins and they respond to an electromagnetic field by rotating. This rotation creates an angular momentum which results in friction with neighboring molecules, thereby developing a linear momentum (vibrational energy) [24]. In this way, radiation energy is converted into thermal energy. Effect generated from vibrational energy is thermal effect which occurs in a biosystem due to penetration of electromagnetic waves (MW) into biological materials and heating up the intra- and extra- cellular fluids by transfer of vibrational energy [25]. However, MW thermal effect is different from conventional heating effect. Dipolar polarization and rotation of molecules in an attempt to align the dipoles with applied MW field (Fig. 2) produces effects which cannot be achieved by conventional heating [26].

The non-thermal effect of MW is highly controversial and has been a matter of debate in scientific community. Non-thermal effects are postulated to result from a direct stabilizing interaction of electric field with specific (polar) molecules in reaction medium with no rise in temperature [27]. Thermal effects solely cannot explain the manner in which the MW affects biological systems. Several studies have revealed that MW radiation can kill microbial cells; however it is still not clear if non-thermal effects of MW have any contribution to this. One of the mechanisms involved in killing of microorganisms by MW is by altering the permeability of the cell membrane. The alteration in the permeability of the membrane of the cells is reflected by cell shape changes observed under electron microscopy or by detecting leakage of intracellular protein or DNA using spectroscopy [28]. Due to the paucity of information regarding the exact mechanism involved in *'nonthermal* microwave effects' (also referred to as *athermal* effects or MW specific effects), their existence is highly controversial. [29] attempted at detection of nonthermal effects of MW energy on microbes at low temperature in various test fluids. To separate thermal and nonthermal effects in a system, they developed a continuous experimental microwave process combining rapid energy input to the food system using microwave, with rapid removal of thermal energy utilising an efficient heat exchanger design. A continuous MW treatment (7 kW, 2450 MHz) was given to the test organisms in various test fluids like water, liquid egg, beer, apple juice, and tomato juice. They concluded that MW energy in the absence of other stresses did not kill microorganisms at low temperatures and there was no convincing evidence that MW energy could kill microorganisms without thermal energy. [30] studied the effect of MW at sub-lethal temperature on Staphylococcus *aureus* and suggested the existence of a phenomenon different from thermal heating resulting in altered activity of various metabolic enzymes. There has been a plenty of reports favoring the existence of non-thermal effects [30, 35, 37], but the studies refuting the possibility of athermal effects [29, 58, 62] can also not be neglected.

3. Factors affecting MW effects

Interaction of MW with biological entities is influenced by multiple factors viz., MW power and frequency, far-field versus near-field location, duration of exposure, polarization, etc. Reports suggest that continuous and pulsed MW treatments have different effects on cells. Human diploid fibroblasts and rat granulosa cells were exposed to intermittent and continuous waves. With the help of alkaline and neutral comet assay, DNA strand breaks were determined. Single and double strand breaks occurred in both cell types, but the intermittent exposure showed a stronger effect in the comet assay than continuous exposure [40]. Normal human lymphocytes isolated from the peripheral blood were exposed for 5 days to 2450 MHz microwave radiation in both continuous and pulsed form. Spontaneous lymphoblastoid transformation was determined with an image analysis system and it was found that pulsed wave exposure enhanced transformation to a greater extent than continuous wave exposure [38].

Duration of MW exposure seems to be a major determinant of MW effect on living cell. The time of exposure and power density are correlated in a way that decrease in power density (PD) could be compensated by increase in duration of exposure. Cells of *Escherichia coli* K12 AB1157 were irradiated with millimetre waves within the PD range of 10−20 to 104 W/cm^2 and it was found that decrease in PD could be compensated by increase in exposure time to achieve the same changes in chromatin conformation [33]. There have been reports suggesting that the length of post-treatment time following MW exposure is also important in determining response of living cells to MW radiation. Sub-lethal MW radiation studies on *E. coli* revealed that cell-surface undergo modifications which are electrokinetic in nature, and cells revert back to original stat after 10 min of exposure [57]. Low level MW radiation of 10 GHz frequency, 0.58 mW/cm^2 intensity, applied for 30-120 min caused loss of virulence in Agrobacterium tumefaciens strain B6, where 30-60% decrease in their ability to produce tumor and turnip disk in plant was observed. However, this loss in virulence was reversible within 12 h [52].

The medium/matrix in which the cells are embedded during MW exposure can also have its impact. The efficiency with which different solvents absorb microwaves and pass it on as heat to the surrounding molecules is indicated by dissipation factor ($\tan \delta$), expressed as: $\tan \delta = \varepsilon'' / \varepsilon'$,

where, ε'' is the dielectric loss which indicates the efficiency of conversion of microwave energy into heat; ε' is the dielectric constant which is the measure of the ability of the material to absorb microwave energy. A reaction medium with a high tan δ at the particular operating frequency of a microwave synthesis reactor is required for good absorption [27]. Different cells, organs and tissues of biological entities have varying dielectric properties, and thus are affected differently by MW radiation.

4. Biological effects of MW radiation

Research is being done worldwide on thermal and athermal effects of MW on different biological systems. Phormidium spp. Kutzing ISC31 (a cyanobacterium) grown in BG-11 medium was treated at a frequency of 2450 MHz using a microwave oven by combining five different intensities (180, 360, 540, 720 and 900 W/cm^2) and three pretreatments (10, 20 and 30 s). The content of chlorophyll a decreased with increase in intensity and exposure time. Synthesis of phycobiliproteins, phycocyanin, phycoerythrin, and allophycocyanin increased in all exposures except in 720 and 900 W/cm^2 (30 s). Photosynthetic rate compared to nitrogenase activity increased by all microwave exposures except at 180 W (10 s) and 720 W (10 s) as compared to control [32]. Studies on E. coli and *S. aureus* suggested that physical damage caused by MW led to alterations in membrane permeability and consequently influx of extracellular Ca^{2+}. An increase in cell permeability of upto 89.8% and 19.7% was obtained after MW treatment in *S. aureus* and E. coli respectively [28]. Effect of MW on transformation efficiency was studied by [42], where calcium chloride competent cells of E. coli were given MW treatment at 180 W for 1 min, and the transformation efficiency was increased three-fold as compared to the classical method. [53] studied the effect of microwave (2450 MHz, 55 W) on the cellular differentiation of *Bacillus subtilis* YB 886 and its Rec derivatives YB 886 A4. It was found that organism's growth and amount of DNA decreased after MW exposure by 4% and 27% respectively, whereas the amount of RNA and plasmid was enhanced by 6.5% and 21% respectively. They noted an increase in the amount of specific protein synthesized during DNA damage by SOS repair system, and its binding to din C promoter region, following MW exposure in rec+ bacteria.

4.1 Effect of MW on growth and enzyme activity

MW effects on growth and other cellular activities are being studied for atleast more than 50 years. Growth rate of yeast *Saccharomycetes cerevisiae* either increased upto 15% or decreased up to 29%, when exposed to MW radiation having narrow frequency range of 41.8-42.0 GHz [44]. When *S. aureus* culture was exposed in a controlled temperature experiment to microwave radiation (24 GHz) for 10, 20, 30, and 40 s, the activity of various enzymes like malate dehydrogenase,cytoplasmic adenosine triphosphatase, glucose-6-phosphate dehydrogenase, and cytochrome oxidase increased in microwave treated cells than microwave non treated cells; whereas membrane adenosine triphosphate, alkaline phosphatase and lactate dehydrogenase activity remained unaffected [30]. The effect of MW radiation on 94 strains of Enterobacteriaceae was studied and it was found that microwave irradiation increased the enzyme activity of bacteria in suspension [60]. Low power MW treatment (2450 MHz; 90 W; 2 min exposure) on *Aeromonas hydrophila* decreased its total protease activity by 33%. Urease activity and aflatoxin production in *S. aureus* and *Aspergillus parasiticus* respectively was completely inhibited by MW exposure [39]. Low power (100 W, 60 s) microwave radiation reduced not only the cell number but also the acid resistance and verocytotoxin productivity in enterohemorrhagic E. coli [61]. [47] investigated influence of MW on soil bacteria. They observed both the suppression and the stimulation of the growth for different bacterial species under the impact of microwaves. Spore suspensions responded to microwave radiation upon a shorter time of exposure than suspensions of vegetative bacterial cells. The influence of microwave radiation on the biomass accumulation and the intensity of other physiological processes in streptomycetes species led to changes in the number and activity of these microorganisms in the soil microbial complex.

Our yet unpublished investigation on the effect of low power microwave (2450 MHz, 90 W) on growth and enzyme activity of different bacteria positively suggested existence of MW specific athermal effects (Table 1). Pectinase activity in *P. carotovora* and *B. subtilis* was greatly affected by MW treatment. Amylase activity suffered significant loss in *B. subtilis* and *S. mutans* due to MW exposure.

Table 1. Effect of low power MW on bacterial growth and enzyme activity

Duration of MW treatment (min)	*Bacillus subtilis*		*Streptococcus mutans*		*Pectobacterium carotovora*		*Bacillus subtilis*	
	% change compared to control (not exposed to MW)							
	Growth	Amylase activity	Growth	Amylase activity	Growth	Pectinase activity	Growth	Pectinase activity
2	-7.52	-47.28**	2.59*	7.36	8.28	215.10**	0.24	-100*
6	7.52	-67.43**	-3.89*	-13.43*	1.03	-51.19*	0.00	6404.61*

*p<0.05; **p<0.01

4.2 Effect of MW on higher organisms

A study on effects of low power MW radiation on germination and growth rate in seeds of wheat (*Triticum aestivum*), bengal gram (*Cicer arietinum*), green gram (*Vigna radiata*), and moth bean (*Vigna aconitifolia*) showed that different treatments stimulated the germination and seedling vigour. Increase in power density resulted in reduced rate of germination [55]. Effect of different MW power was studied on potato tuber biomass and it was found that for seed potatoes irradiated with microwaves (100 W) at frequency 2.45 GHz for 10 s, tuber weight was 7.9% higher compared to check sample. No significant change was observed at 38 GHz, 46 GHz, and 54 GHz [46]. Treatment with low-level MW (35 GHz; surface power density 30 $\mu W/cm^2$) radiation for 10 s has been reported to induce chromatin condensation (increase in number of heterochromatin granules) in human cells and increased membrane permeability. However, the number of heterochromatin granules decreased to its initial level and membrane permeability was recovered after a few hours [59]. Low-intensity MW radiation effectively changed membrane functions in striated muscle and cardiac pacemaker cells in rats [36]. Exposure at 0.1-0.15 mW/cm^2 for 90 s or lesser time (frequencies between 54-78 GHz) either decelerated the natural loss of transmembrane potential in myocytes, or even increased it by 5-20 mV. Low intensity MW was also found to suppress and alter the T-peak on electrocardiography of in situ exposed myocardium, enhance respiration, alter membrane calcium binding, and reduce the contractibility of cardiomyocytes. Drop in blood pressure of rats caused by MW pulses persisted for several weeks indicating a stable effect. The properties of blood plasma like dielectric permittivity and absorption coefficient could be altered by microwave radiation [50]. Hybridization profile of brain and testis DNA of mice exposed to low power MW (1 mW/cm^2; 2450 MHz) showed an additional band suggesting amplification of tandem sequences in particular region [56]. [41] concluded that exposure of rabbit eye to 2450 MHz MW at 150 W/kg for more than 30 min can induce cataracts. Induction of cataracts via thermal effects of high-power MW radiation is well established. Whether low-power MW are cataractogenic remains unclear. [63] reviewed non-thermal cellular effects of low-power microwave radiation on the lens and lens epithelial cells.

5. Microwave mutagenesis

A number of studies reveal that effects of MW can reach up to genetic level and can even result in stable mutation. Genetically stable mutant strains with higher nitrogenase activity and phosphate solubilizing capabilities of *Klebsiella pneumoniae* RSN19 were obtained by microwave (250W, 36 s) mutagenesis [49]. Mutagenic potential of MW has also been demonstrated with respect to cellulase production in *Trichoderma viride*, wherein a compound mutagenesis by MW (700W, 15-195 s) and ultraviolet radiation was employed, and the mutants were found to be stable up to 9 generations [15]. [14]studied the effect of MW (400 W for 3 min) on *Lactobacillus rhamnosus* which induced >50% increase in L-lactic acid production than the parent strain, and the mutant generated was found to be stable up to 9 generations. In our lab, we obtained a mutant of *Xanthomonas campestris* by continuous MW treatment (2450 MHz, 90 W, 6 min) and investigated it for xanthan gum production. The mutant produced 40% more xanthan gum than the parent strain upto 3 generations, and then reverted back to the parent type. Thus, in this case the mutation caused by MW treatment was not sustained beyond 3 generations (data yet to be published). Such studies together indicate the potential of MW as a substantial tool for strain improvement through mutagenesis. MW till now has not been exploited as widely UV has been as a mutagenic radiation. Identifying the frequencies, power range and exposure duration in MW region, which are most suitable for mutagenesis among microbes will certainly be of interest to fermentation industries.

6. MW and cell phones

Within last decade, the world population has adopted the use of cell phones in a horrendous way. Increasing use of radiofrequency devices has become a trend as well as a need in a large section of society. Cellular phones transmit radiofrequency waves of very low intensity and there has been a lot of discussion on possible adverse effects of these radiations on health. Effect of electromagnetic radiation from communication towers on human and other forms of biodiversity has been a matter of concern. [54] exposed fruit flies to cell phone radiation (Global System for Mobile communication-GSM-900 MHz) at very low levels (3-7 mW/cm^2) for 6 min/day before hatching. The exposed groups in their adult life showed a loss in reproductive activity varying between 15-60%. [43] reported that exposure of heads of volunteers to low level of radar from a radar horn resulted in headaches, and that low level of radar can interact with nerve tissue to cause dysaesthesiae. [45] reported a neurological abnormality in a patient after accidental exposure of left side of the face to cellular phone radiation, which led to long-lasting loss of tactile sensitivity of the facial skin, unilateral left blurred vision, and pupil constriction. The worker took virtually 6 months to recover.

7. Final comments

The controversy underlying the non-thermal effects of MW has invoked the interest of scientists in different parts of the world. Notable interest among research community about MW is evident from the rising number of papers involving MW radiation (Fig. 3). At present a large part of general population is exposed chronically to non-thermal MWs from different types of mobile communication including GSM and UMTS/3G phones and base stations, WLAN (Wireless Local Area Networks), WPAN (Wireless Personal Area Networks e.g., Bluetooth), DECT [Digital Enhanced (formerEuropean) Cordless Telecommunications] wireless phones. With such frequent exposure to MW radiation, the current safety standards set by ICNIRP (International Commission for Non-Ionizing Radiation Protection) which are based largely on thermal effects of MW does not seem to be totally safe [34]. In light of recent reports about MW effects (particularly athermal effects) on different life forms, the safety standard of exposure needs to be re-evaluated. Further research is needed on the exact mechanism(s) behind non-thermal effects of MW. Before shifting to large scale epidemiological studies for possible health hazards of MW radiation at low-level, a valid model needs to be established for studying the non-thermal effects alone, as there are few inherent methodological deficiencies in such studies focusing on athermal effects [48]. Also, it is almost impossible to select control unexposed groups for such experiments because people are continuously exposed to various signals/frequencies including non-effective ones. Children and pregnant women may represent the most sensitive group to non-thermal effects of MW. Significance of acquiring reliable information regarding the effects of low power MW needs to be well acknowledged by the society. Due to the ease of handing them in laboratory, microorganisms can be conveniently used to study the effect of MW on living systems. Besides, employing mutagenic frequencies of MW radiation for microbial strain improvement can be of considerable industrial significance.

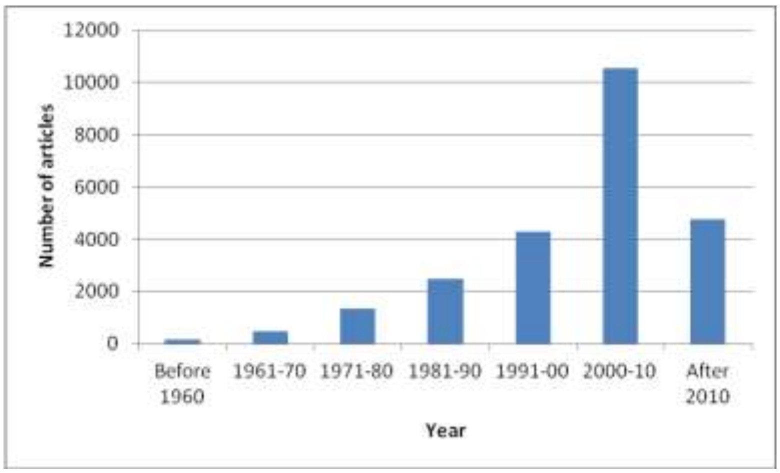

Fig. 3. Number of publications related to MW over last 60 years (till March 20, 2013) (Based on search in PUBMED using *microwave* as keyword

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