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Biological Applications of Microwaves

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ABSTRACT:

Microwaves are increasingly being used for a variety of biological purposes. Due to their rapid heating ability, they have proven of significant utility in extraction, disinfection, tissue processing, biomedical imaging, etc. Major advantages provided by microwaves are reduced time for particular treatment, rate enhancement, and minimum degradation of the sample. Temperature monitoring and control remains one of the challenges to be solved during microwave operations. If ambiguity pertaining to non-thermal effect of microwaves can be solved, they can find more numerous and reproducible applications in biology.

Key words: Microwave assisted extraction (MAE), Non-thermal effect, Superheating, Sterilization.

INTRODUCTION

Microwaves (MW) are that part of the electromagnetic spectrum which occupy a frequency of 0.3 to 30 GHz, corresponding wavelength range is 0.01 m to 1 m [1, 2] (Box-1). For commercial use, a frequency of 2.45 GHz has been allotted by International Commission for Domestic and Industrial Ovens [1, 2]. Since many years, microwaves are being used for disinfection in hospitals [9], disinfecting contact lenses [10], and accelerating chemical reactions. Other applications of microwaves wherein their use can provide additional benefits include- sterilization, extraction, biochemical reactions, staining, tissue processing, etc.

History of microwaves

The existence of electromagnetic radiation was predicted in 1864 by Maxwell. The existence of microwaves was first demonstrated by H. Hertz [1]. Indian scientist Jagdish Chandra Bose demonstrated the existence of microwaves in laboratory, and also invented a detector for radio waves. He had actually reduced the electromagnetic waves to millimetre length. He was the pioneer of wireless communication and electromagnetism. He studied the effect of microwaves on plant tissues and cell membranes [11, 12, 13].

The first indication of using microwaves as a source of heat came up when Percy Spencer of Raytheon, on passing through a microwave tube found the candy in his pocket melting [14]. Some of the major historical developments on microwaves are summarized in Table 1. There has been a controversy about the development of the microwaves that the Nazis had first developed the machine [15].

How microwaves act?

Ion migration and dipole rotation are molecular motions induced due to microwave effects [2]. They may also involve distortion of electron clouds from a molecule [16]. The response time of permanent dipoles in organic and inorganic molecules for a particular microwave frequency, namely relaxation time is the factor determining interaction between microwaves and the solvent system. For polar molecules, on application of static electric field like microwaves, the molecules orient themselves randomly and the time required for them to do so is called relaxation time. For example, the relaxation time of water increases on increasing the concentration of ionic salts. These phenomena are involved in accelerating the reactions in chemistry [5, 16].

Box 1. Glossary

Disinfection: Destruction or removal of vegetative pathogens but not bacterial endospores, usually applicable for inanimate objects [3].

Dissipation factor (tan δ): A measure of the ability of the solvent to absorb the microwave energy [4].

Electromagnetic spectrum: The entire distribution of electromagnetic radiation according to frequency or wavelength. The various portions bear different names based on differences in behaviour in the emission, transmission, and absorption of the corresponding waves and also based on their different practical applications [5].

Microwave: Electromagnetic radiation having a frequency within the range of 300-300000 MHz and wavelength beginning at about 1000 μm [1, 2, 6].

Microwave dielectric heating: The phenomena which results in generation of heat directly in the exposed material by conversion of the electric energy into heat energy. Dielectric losses affect such heating [7].

Non-thermal effect: Accelerations of chemical transformations in a microwave field that cannot be solely accounted for by thermal/kinetic effect [8].

Microwaves heat the liquid through a phenomenon called dielectric heating and is related to the polar nature of the material undergoing microwave treatment. The energy transfer which is caused due to dipole moment, is a function of the relaxation time of the molecules. It is most efficient when the molecules are able to relax quickly [6]. Thermal Effect is the heating effect that produces alterations in the dielectric properties of the dipole molecules. The electric field of

the microwaves exerts a force on the charged molecules of the material [17].

Microwaves certainly exert thermal effects on materials. Although the existence of non-thermal effect (Box 3) has also been predicted, its influence is still under question. Oliver Kappe and his colleagues at Karl Franzens University, when used silicon carbide vials that separated the two effects of microwaves, i.e. allowed only the heating effect to transmit and blocked all the other effects, almost similar results were obtained in the vials as in pyrex containers when measured for 18 reactions [24].

Table 1. Timeline for the development of microwave apparatus [1,12-14]

Year	Scientist	Event
1864	Maxwell	Prediction of Electromagnetic Waves
1888	H. Hertz	First to demonstrate the existence of electromagnetic waves
1894	J. C. Bose	Publicly demonstrated the radio control of a bell
1939	Alfred Loomis	Coined the term 'microwave'
1940s	Dr. Percy Spencer	First physical demonstration of microwave heating theory
1940s	Marvin Bock	Made the initial designs for Microwave Oven
1946	Dr. Percy Spencer *et al.*	Invented the first microwave oven, called 'Radarange', process was patented.
1947	Dr. Percy Spencer	First Commercial Radarange was manufactured
1955	Tappan	Microwave ovens built and marketed for home use
1967	-	First Amana 'Radarange' was introduced by Rayethon
1978	-	CEM introduced the first commercial laboratory microwave

Box 2. Various applications of microwaves

- Staining
- Biological tissue processing
- Biomedical imaging
- Extraction
- Disinfection & Sterilization
- Rate enhancement of biochemical reactions
- Medical applications
- Waste treatment
- Cancer detection [breast cancer]
- Protein folding and unfolding
- Protein hydrolysis and proteomics
- Methane production
- Moisture removal
- Enzyme immobilization
- Mutagenesis in plants

VARIOUS APPLICATIONS OF MICROWAVES

Among many applications of microwaves (Box 2), some have already found widespread acceptance, whereas others are waiting for being employed on large scale. A description of major applications follows.

Staining

Certain effective treatments promote penetration of stains till the periphery of the cell, thus better visualization becomes possible. One such effective treatments is microwave irradiation, which provides certain additional benefits, too. Chemical processes usually require longer time, which can be overcome by replacement with or by supplementation of microwave treatment [25-27]. Staining procedures using microwaves can be perfected by maintaining the accurate time and proper positioning of the staining vessel. The staining time can be reduced by 2-10% than the conventional methods [26].

Microwaves promoted faster staining of proteins spotted on a nitrocellulose membrane by dye based blue-black ink in 3 minutes [28]. Faster staining was also seen when microwave irradiation was used with nuclear probes like Syto 13 as these were initially not useful for plant and insect systems owing to the plasma membrane barrier. The usage of MW irradiation made this possible with intermittent exposures for ~2-4 min at 900 W. It also resulted in reduced time requirement for the process as compared to the conventional one [25]. Microwave enhanced staining used for plant virus characterization had two main advantages, better staining intensity and reduced time requirement. Mucicarmine staining using microwave oven reduced the staining time to ~3 minutes, in which the microwave treatment was provided for 30 seconds. Extra mammary skin tissues when used as the test sample, MW treatment lessened the time requirement and provided rapid diagnosis on frozen sections [27]. Similar advantage of lesser staining time was obtained by the Saffranin O staining method used for formalin fixed rabbit tissues. Tissues were stained in microwave oven for ~30 seconds at 360 W in fast green and 0.1% saffranin solutions, however microwave didn't affect the properties of cartilage as compared to conventional method [29]. Similarly organ of corti of mouse cochlea were stained using glycol methacrylate along with counter stain eosin. It gets completed using microwaves in 1-2 min instead of 45 min in cases where microwaves and colour extenders were not used. Microwaves also proved handy in hair cell and spiral ganglion cell counts [30]. Thus, the generalized advantages obtained for staining using microwaves are a reduced time requirement, and better maintenance of the tissue quality.

Biological sample processing

One of the most influential applications of microwaves has been in the field of tissue and sample preparation, which primarily provides reduced time requirement. Microwave use for tissue processing was first described by Mayer in 1970 [25, 31]. Initially there were considerable problems regarding the usage of microwaves in tissue processing due to lack of compatible apparatus design and protocols, but nowadays microwaves are being used for the processing of specimens for biopsy, surgical pathology, and electron microscopy.

One of the major advantages that microwaves provide for tissue processing is that of reduced turnaround times. Thus, after the entire preparation, processing and experiment, reports are obtained on the same day. In case of gastrointestinal and bronchial specimens, turnaround times were reduced to ~4 h. Specimens were given discontinuous microwave treatment for longer and shorter time intervals for about an hour [31]. Microwaves have been used to prepare samples of lipid A from *Helicobacter pylori*, a 50 W microwave treatment was used for about 5 min during enzymatic digestion. This gave a faster analysis and better sensitivity as compared to the conventional SDS promoted hydrolysis method. Total time for microwave assisted sample preparation followed by mass spectrometric analysis was less than 2 h [32]. The MW assisted labelling of proteins, especially in amino acids like glutamic acid, aspartic acid with ^{18}O occurred in less than 15 min replacing ^{16}O. This was verified by LC-MS/MS analysis prior to subjecting them to HPLC run [33]. Microwave irradiation was successfully used in the preparation of [3, 4-b] carbazoles, potent topoisomerase II inhibitors via Diels Alder reaction. Providing microwave radiation for ~2 h at 150^{o}C was a key step in the synthesis operation [34]. Microwaves are used for preparation of tissues for scanning electron microscopy as well as transmission electron microscopy. This is done by giving intermittent treatment of microwave power, maximum power is used most of the times [25]. In rat hippocampal portions, on using microwave irradiation, the samples took lesser time (few sec to min instead of 1-2 h) than when the conventional aldehyde treatment was used, and the latter even made the tissue more hypoxic which didn't turn out to be feasible for usage as electron microscopy samples. Fixation with microwaves produced high quality fixation in a short time, especially towards the centre of the slices [25].
When bone tissues are preserved in 70% ethanol, quality of the tissues is degraded. To improve the above preservation, microwave irradiation was used for immersion and dehydration of tissues in 70% ethanol. This provided better histological preservation after embedding, and processing time was reduced to ~7 h instead of 1 week [35].
Microwave irradiation is also used for antigen retrieval from tissues. As the amount of antigen obtained by proteolytic digestion was very less, microwave oven was used for this purpose at 720 W for 5-10 min with intermittent cooling. This provided better antigen recovery, as well as the freedom to use even 2 years old specimens [36].

Tissues of ileums and brains of mice were subjected to staining after preparation using microwaves. The tissues were kept in 20% sucrose solution, treated to boiling at full power and then 5 min microwave exposure at 50% power post boiling was given. This provided better tissue preparation on slides. In turn, it also gave better antigen retrieval. Intensity of fluorescent staining was not preserved following MW treatment as signals got diminished as compared to microwave untreated samples, but still visual clarity was acceptable [37].

Extraction

This is another field where microwaves are applied widely and slowly replacing the conventional techniques like Soxhlet. MW assisted extraction (MAE) is advantageous being simple, environment friendly, and economical [38]. It can also rapidly heat the solvent till elevated temperatures and holding it onto maximum temperature till the given time. Also, lesser time duration for experiments as well as reproducibility are the add-on advantages for MAE [39, 40].

MAE can be performed in two different modes- *open vessel* and *closed vessel*. In latter, a solvent can be heated at temperature above its boiling point by application of pressure. Usually the elevation reaches on the order of 150^{o}C for a vessel of 175 psig [6].The sample is in direct contact with the solvent. This method provides higher extraction speed and efficacy. In case of open vessel operation, which occurs at atmospheric pressure, the temperature can only be increased till the boiling point of the solvent [6, 39].
Some solvents such as hexane and chloroform, cannot absorb microwaves and hence don't undergo heating, they are called microwave transparent solvents [6]. Usually polar solvents are believed to be better for MAE than non-polar ones. However, according to the 'broken cell wall theory', microwave transparent solvents are superior to the microwave absorbing (polar) ones [40]. Binary mixtures of solvents (one component capable of absorbing MW, another not so) are also employed for MAE [41]. MW absorbing solvent will get heated in proportion to its dissipation factor gives (Box 1). MAE has been applied for extraction of essential oils from peppermint, and cedar. It proved better than steam distillation as latter ruptures the surrounding tissues along with the grandular and vascular plant tissues, while MAE keeps them intact

[39]. Steam generated during microwave heating can make the plant material more porous and result in opening of matrix of the plant tissues. Certain secondary metabolites are recovered better by giving the plant material MW exposure prior to or post-extraction [42]. MW may be made to directly reach the plant material without being absorbed by solvent, if a MW transparent solvent is applied for extraction, then contents from the plant matrix will directly leach out into the solvent. This happens as the moisture inside plant material after MW heating will create pressure on the cell wall causing it to rupture [43].

MAE yielded good extraction efficiency when applied to *Annona squamosa* seeds in different solvents with total MW exposure time ranging from 50 sec to 5 min. Maximum extraction efficiency (17%) was achieved in chloroform-methanol mixture in just 50 s [40]. MAE proved effective for preparing extracts from certain plant seeds, which were further screened for antibacterial and antioxidant activity. Total MW exposure time required was less than 5 min [43, 44]. MAE has been applied for preparing plant extracts while evaluating them for antimicrobial activity [45]. as has been for extracting contents from *Phoenix sylvestris* Roxb and *Tricosanthes dioica* L seeds, with extraction efficiency falling in the range 2.8 - 13.6% in different solvents [46].

MAE for phenolic compound (gallic acid, vanillic acid, etc.) extraction from plants consumed lesser time and provided a better extraction in comparison to the conventional reflux method. The treatment was provided for 750 W for 4 min using methanol, acetone, and ethyl acetate as solvents. Resulting sample was amenable for RP-HPLC equipped with UV detection. Possibility of superheating leading to analyte degradation was indicated in this study [47].

Extraction efficiency for MAE of curcumin in acetone from *Curcuma longa* L. rhizomes was 98.05% in the first three hours while using microwave irradiation for 4 min at 800 W. Similar extraction efficiency was obtained without MW treatment, but employing constant stirring in acetone for 24 h. Analysis of different chromatographic parameters showed that curcumin was not degraded under microwave effect. [48].

Oils for use as biodiesel were easily extracted from dry algal biomass using microwaves. The algal samples before and after extraction were analysed on TEM. Catalyst concentration and other variables were analysed by response surface methodology (RSM). MW provided a higher degree of oil/lipid extraction. [49].

Extraction of genomic DNA from bacterial samples with laboratory microwave ovens is one of the upcoming methods in the field of molecular biology. In *Lactobacillus spp.* like *Lactobacillus plantarum* and *Lactobacillus fermentum*, a new method combining the chelex resins and microwave method has been devised, called Chelex-100 microwave method. This method gives a broader range in terms of species and also a reduced time requirement [50].

Disinfection and sterilization

If applied in proper dose and time, electromagnetic waves like microwaves, UV radiation, gamma rays, and electrons can effectively reduce microbial growth. The antimicrobial effects of microwaves are due, at least in part, to thermal effects [51]. The killing action of microwaves may solely be due to thermal effect [20-23, 52], or may result from a combination of thermal and non-thermal effects [53-55]. Lack of latter was indicated by Sasaki *et. al.* and Jeng *et. al.* [20, 22, 23]. The use of microwaves for the purpose of killing microbes has been suggested since years. Some investigators consider microwaves to be better at disinfection rather than at sterilization [56]. Standard sterilization cycles with moist heat (autoclave operations) are run at 121^{o}C and 15 lbs/square inch pressure. If microwave apparatus of larger volumes can be built which allow development of similar pressure, they can be very attractive option for routine sterilization of laboratory media and materials. This will take much lesser time than a typical autoclave operation, which may take 1 h or more. For ensuring terminal sterilization, any treatment must kill bacterial endospores. Heat resistance of bacterial spores vary from one species to another (Table 2). A useful killing of several indicator bacteria like *B. stearothermophilus*, *B. cereus* and *Staphylococcu aureus* under different conditions of sterilization duration and unequal intensity of microwave power had been reported [58]. Najdovski *et al.* reported microwaves to be more suitable for disinfection and less for sterilization. They found a treatment of 1400 W for 10-20 min to be adequate to kill the spores of *B. subtilis* and *B. stearothermophilus* in aqueous suspension [56]. The effect exerted on spore structures of *B. subtilis* by MW is different from that of the autoclave [59].

A solution undergoing microwave treatment may get subjected to superheating, a phenomena in which it reaches a temperature exceeding its normal boiling point at atmospheric pressure (Box 4). Superheating cannot be strictly controlled by the operator, which along with possible non-thermal effect, if involved, may lead to lack of reproducibility in microwave operations.

Table 2. Destruction times for different bacterial spores by moist heat [57]

Organism	Destruction time at 100°C (min)	Destruction time at 105°C (min)	Destruction at higher temperatures	
			Temperature (°C)	Destruction time (min)
B. anthracis	2-15	5-10	105	5-10
Clostridium tetani	5-90	5-25	105	5-25
C. welchii	5-45	5-27	120	1
C. botulinium	300-530	40-120	120	4-20
C. sporogenes	150	45	134	1
Soil bacteria	Many hours	420	134	1.5-10

MW are used for sterilizing drug solutions in ampoules. This has been accomplished using a microwave continuous sterilizer (MWS) capable of sterilizing 150 ampoules in a min. It also monitored temperature of the ampoules and was claimed to achieve terminal sterilization [64, 65]. Certain areas of an ampoule appeared to be as the 'cold spots' for microwave sterilization in the MWS [65]. This instrument is claimed not to deteriorate the quality of ascorbic acid and pyridoxamine phosphate solution (3%), which does occur during autoclaving [64]. Terminal sterilization using MWS was confirmed by ensuring killing of indicator organism *Bacillus stearothermophilus*, which is the most resistant one to MW killing and steam sterilization [23]. *B. subtilis* is also considered to be a suitable indicator organism for microwave sterilization [35]. In the dry state, microwaves do not kill by any other effects specific to themselves and only utilize their heating effects, as indicated in a study on spores of *B. subtilis* and *B. stearothermophilus*, while attempting ampoule sterilization in the MWS [23, 52].

MW have been applied for preparing antibiotic containing LB agar plates. These plates did not show contamination till 10 days, in comparison autoclaved plates were contaminated after 14 days. Microwave sterilization may be considered more suitable for short-term sterilization when media are required for immediate use [66]. Though MW treatment being economical and environment friendly [67], a common sterilization protocol using microwaves applicable to a wide variety of biological media has not yet been available. Experimental data from our own laboratory [67a] indicates that MW treated media can support better bacterial growth than autoclaved media. For example, *Escherichia coli* and *S. aureus* achieved higher cell densities in MW treated media. There was a 57.96% and 124.61% increase in cell density respectively for *E. coli* (in tryptone yeast extract broth) and *S. aureus* (in nutrient broth). These organisms also registered lesser generation time in MW treated media. Better microbial growth in MW sterilized media was also reported for *Aggregatibacter actinomycetemcomitans*, in solid as well as liquid culture [9].

Medical applications

Microwaves have been applied for sterilization of the dentures infected by *Candida albicans*, instead of other disinfectants. MW treatment reduces the microbial load but results in the development of increasingly brittle dentures [68]. Microwaves can help in effective detection of diseases, for example, toxoplasmosis infected DNA samples were subjected to PCR using both microwave and standard genomic DNA extraction methods, and out of 42, 7 samples were obtained positive through microwave method after PCR treatment, whereas through standard extraction, no positive results were registered. MW treatment resulted in an increase in sensitivity and specificity of detection method [69].

MW are used in the coagulation and ablation of tumor cells. The technique is called microwave coagulation therapy (MCT), in which a thin antenna providing microwave energy causes coagulation of the tumor as a result of heating. A MW generator of 2.45 GHz is used at 40 W for about one min. The reflective coefficients measured during MCT help in real time monitoring of the extent of coagulation, which is comparable to ultrasonography [70]. The killing of tumors using microwaves potentiates the ionizing radiation further, which is traditionally used to kill tumors. Application of heat before or after radiation affects the response of a tumor to radiation. In cases where MW radiation is followed by ionizing radiation, a combination microwave of 433.92 W is used for 20 min [71]. Ablation of tumor cells is caused by heating the tissues to cytotoxic temperatures, based on their water content and dielectric properties. The amount of water present followed by ionizing radiation, a combination microwave of 433.92 W is used for 20 min [71]. Ablation of tumor cells is caused by heating the tissues

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Box 3. Controversy over non-thermal effects

In the wet state, the thermal and non-thermal effects are the two main effects associated with microwave action. The thermal effect is mainly caused due to the movement of charged molecules in a solution, in which dielectric heating occurs. The dielectric heating is attributed to the dipole movement of the charged molecules in the solvent, the higher the compaction, lesser movement. More the losses like conductivity loss, dipolar loss, and dielectric loss factor, higher amount of heating is obtained [18].

The cell death caused by microwaves is not only due to heat but also due to electric field. Microbial destruction has also been shown to occur at lower temperatures and shorter time periods in comparison to conventional heating methods [19]. This effect is called the non-thermal effect, which may be due to the radiofrequency of the microwaves. It doesn't depend on the dipole molecules, it plays a very important role in the inactivation of microorganisms in suspension [20]. But however, the existence of non-thermal effect is still under question. It also called 'microwave specific effect'.

When *E.coli* and *B. subtilis* spores were subjected to 30 KW microwave power at 2540 Hz frequency, no existence of non-thermal effect was observed in spore killing [21]. It has been demonstrated that sterilization with the help of microwaves is attributed to 'microwave dielectric heating'. The substances are heated very quickly in the presence of microwaves as compared to the conventional methods [2, 22]. Also, the absorbed electromagnetic energy is transformed into heat energy [20]. According to Sasaki *et al.*, the microbes get killed only due to the heating effect and non-thermal effect did not exist [23]. They found that the spores of *B. stearothermophilus* are the most resistant to microwave killing as well as to autoclave (which employs solely heating for killing) and hence denied any existence of non-thermal effects prevailing through microwaves [22]. With reports both in favour and against existence of non-thermal effect available, it is difficult to draw a definitive conclusion.

and dielectric properties. The amount of water present in them will determine the amount of heating caused. Microwave ablation usually occurs in close proximity of the antenna of the applicator, hence providing better ablation of tumors, especially those developing in liver and kidney [72].

Other miscellaneous applications

- *Protein folding studies*: When protein unfolding was induced by microwaves, it was better and faster as compared to conventional heating. The unfolding was verified with the help of chaperone binding [73].
- *Protein hydrolysis and proteomics*: The hydrolysis of proteins to obtain peptides, their further analysis and mass spectrometry can be done in a better way under the influence of heat. This heat is mainly provided with the help of MW for 4-12 min. MW assistance is also used for in-gel digestion. MW exposure is provided for about 20 min during staining, destaining and enzyme incubation. The hydrolysis effect was enhanced due to the effect of MW [1].
- *Waste treatment*: MW irradiation is one of the new means being tried for pretreatment and conditioning of wastewater sludge. MW are nowadays being used for solubilisation, biodegradation, and anaerobic digestion of wastewater sludge from food industries. MW *Mutagenesis in plants*: MW are found to be inducing mutagenesis in plants. When *Vigna aconitifolia* was treated with microwaves at 800 W for maximum 7 sec, up regulation of one polypeptide was observed. Delayed callusing was observed after 7 sec MW exposure whereas enhanced callusing was observed after 5 sec exposure. Overall frequency of mutation was 1.6% for all the explants [76].

  Rate enhancement of biochemical reactions: MW irradiation was used for the synthesis of ether cross linked chitosan. MW caused crown ether and chitosan get converted to ether cross linked chitosan with a higher frequency as well as a shorter reaction time [77]. When microwaves were used for performing the Akabori's reaction, it happened in minutes instead of hours and also helped in the intermittent analysis of the amino acids. This is a time reduction modification to the lengthy conventional process [78]. MW were used to study antioxidant activities of the maillard reaction products between proline and glucose [79].

  Industrial aspects: Microwaves are widely used for moisture removal. In this case, the maximum temperature obtained is a function of dielectric properties of the material and the power applied. MW were used to remove moisture from mint leaves i.e., drying of mint leaves. The use of MW provides 120 fold increase over the conventional solar energy method. The output power was used in a series of 180-900 W and consequently, the drying time also decreased. For 900 W, the drying time was 76% shorter than that for 180 W [80]. MW are also used for moisture removal from peanuts, by subjecting them to MW curing chambers at 1.2 and 2 KW. The extent of moisture removal depends on power of MW applied, and also on the dielectric properties and moisture contents of the peanuts. MW are used to extend t he shelf life of bread after treatment for 10 sec. Here, no detectable mold growth was noted even after 60 days [81].
- *Enzyme immobilization*: In certain cases, the native enzyme when used as such, without immobilizationis either costly or it doesn't give the required proportion of activity when needed. Thus, in order to increase the experimental productivity in the native state or post-immobilization, MW irradiation is being used. MW irradiation was provided at ~50W for enzyme 2-Deoxy-D-ribose-5-phosphate aldolase (DERA) which provided about 157% increased activity as compared to the MW untreated free enzyme and 149.2% higher as compared to that of MW untreated immobilized enzyme. Thermal stability of DERA was more for

Box 4. Superheating and wall effects associated with microwaves

Microwaves may exert a superheating effect on the liquids causing them to reach an average temperature higher than their atmospheric boiling point. This phenomenon is called superboiling or superheating [60-61]. As a result, it becomes difficult to determine the actual temperature of the material exposed to microwaves. Some special gas thermometers (or IR based devices) are used to determine temperature in microwave operations [62]. As superheating cannot strictly be controlled by the operator, it may prove a factor contributing towards lack of reproducibility in microwave based experiments. Practically, superheating effect can be diminished by stirring the liquid during treatment [8]. Different solvents have different extents of superheating and have their particular nucleation boiling points while undergoing microwave treatment (Table 3). Superheating occurs as the excess thermal energy is lost only at the gas-liquid interfaces and not in the bulk liquids. This is believed to be the reason for the rate enhancement found during chemical reactions. During the initial phase, the increase in heating is confined to certain parts of the reaction vessel [60-62].

During superheating, the system stays at equilibrium and hence it is termed as 'nucleation limited boiling point' which is the temperature achieved after superheating. Microwave heating is a function of the free liquid surface area. A glass balloon thermometer or infrared sensor is used to monitor the temperature during microwave treatment. In our laboratory, superheating effects were experimentally measured using a thermometer and the maximum temperature obtained due to superheating for media, namely nutrient broth was found to be 104^{o}C. For terminal sterilization to occur, the temperature should reach at least 121^{o}C, which is not possible in case of water [63].

Wall Effects are associated with the lesser temperature of the inner surface of the wall of the reactor as compared to the bulk liquid. The reason for this is the dissipation of energy in the bulk of the liquid as a result of which, the thermal energy doesn't remained retained inside the reactor wall and keep it heated. Perhaps this lead to better chemical conversions than the conventional heating methods, but it has not been proved [8, 63].

Table 3: Nucleation limited boiling points of various solvents due to superheating [60-61]

Solvent	Standard Boiling Point (SBP) (^{o}C)	Nucleation Limited Boiling Point (NLBP) (^{o}C)	NLBP-SBP (^{o}C)
Water	100	104	4
Acetone	56	81	25
Chlorobutane	78	100	22
Butanol	118	132	14
Trichloroethylene	87	108	21

lower power of MW treated immobilized enzyme than the high power treated enzyme. Immobilization was done on mesocellular silaceous foams [82].

LIMITATIONS AND CHALLENGES

MW ovens, if not properly closed or tampered, may lead to radiation leakage, then it may lead to exposure of radiation to skin. Also, artificial heart pacemakers are affected due to exposure to MW [83]. But this aspect is still under controversy as microwaves are not ionising radiations [84], thus the radiation damage may be neglected. Even if they provide a good alternative, MW devices require a high power input. It has yet not been possible to develop a common sterilization or disinfection protocol by using MW, due to various reasons like lack of reproducibility, ability to monitor exact temperature, controversy on the existence of non-thermal effects, etc. (Box 3). Large volume MW devices capable of being operated under high pressure and with real-time temperature monitoring facility are still not widely available. Metal containers cannot be put inside a MW device. Glass and Teflon are the only compatible materials. If a liquid gets heated above its boiling point inside the MW apparatus, then it may undergo explosion [85]. If above limitations can be overcome, then MW are very much likely to find more numerous applications in biology.

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Contribution made by both the authors listed at first and second place was equal to that of each other.