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Microwave sterilized media supports better microbial growth than autoclaved media

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Microwave treated media were compared with media sterilized through conventional autoclaving in terms of their ability to support microbial growth, spore germination, and revival of lyophilized bacterial cultures. Microwave sterilized media were found to support better microbial growth. Both bacteria and yeast were able to achieve higher cell density at a faster growth rate in microwaved media. Microwave treatment was found to be suitable for media of varying compositions. Better retention of nutrient quality in microwave treated growth media due to shorter heat exposure seems to be the major reason for better microbial growth in it. Microwave sterilization can prove an attractive alternative of conventional autoclaving, especially when media are needed for immediate use, and also when high biomass yield is of particular interest.

Keywords: Microwave sterilization; microbial growth; generation time; autoclaving; growth media

Autoclaving as a means of media sterilization is a universally accepted simple method. However this method can be inconvenient when the plates are needed for immediate use (Iacoviello and Rubin, 2001). Microwave (MW) radiation has been used for effectively disinfecting gauze pieces and hospital white coats, aseptic packaging of food (Bhattacharjee *et al*. 2009). We performed a study to investigate whether the sterilization of media by autoclave can be replaced with MW treatment. Use of microwaves for preparation of growth medium for *Aggregatibacter actinomycetem-comitans* (Bhattacharjee *et al*. 2009), and LB agar plates (Iacoviello and Rubin, 2001) has been reported. Emergency sterilization of media using a microwave oven has been discussed by Hengen (1997) too. But to the best of our knowledge, there is no published report on whether MW treatment is suitable for media of varying compositions, and for growth of different microorganisms. The necessity to study the MW sterilization conditions through the experiments for different media and bacteria, as well as research on sterilization mechanism (especially non-thermal sterilization mechanism) has been emphasized by Xi *et al*. (2002). Microwaves have already been applied for sterilization of injection ampoules (Sasaki *et al*. 1995; Sasaki *et al*. 1998). Through present study we show that the MW method of sterilization is not just an alternative method of sterilization but also promotes faster microbial growth and higher cell density.

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Materials and Methods

Microwave treatment of media

200 ml media (HiMedia, Mumbai) filled in a 500 ml screw capped glass bottle was given continuous microwave (2450 MHz) exposure at 900 W for 10 min using a microwave oven (Electrolux EM30EC90SS). Cap of the bottle was kept slightly loose so that steam generated during heating can get released. Baseplate (30 cm diameter) of the oven rotated at a speed of 5 rpm. Volume of medium left in the bottle after MW treatment was ~140 ml. It took ~135 s for media to start boiling.

Test organisms

Following microbial cultures were procured from Microbial Type Culture Collection (MTCC) Chandigarh: *Aeromonas hydrophila* (MTCC 1739), *Bacillus subtilis* (MTCC 619), *Escherichia coli* (MTCC 119), *Escherichia coli* (MTCC 118), *Pseudomonas aeruginosa* (MTCC 1688), *Pseudomonas oleovorans* (MTCC 617), *Salmonella paratyphi A* (MTCC 735), *Shigella flexneri* (MTCC 1457), *Staphylococcus aureus* (MTCC 737), *Staphylococcus epidermidis* (MTCC 435), *Streptococcus pyogenes* (MTCC 442), *Candida albicans* (MTCC 3017), *Saccharomyces cerevisiae* (MTCC 170).

Growth promotion

One set of autoclaved and microwaved medium, each containing 3 test tubes (4 ml medium in each) was inoculated with test organism. Inoculum standardized to 0.5 McFarland turbidity standard was added at 1% v/v. After proper mixing tubes were incubated under static condition for 20 h at 35°C for all bacteria except *A. hydrophila*. Incubation temperature for latter and yeasts was set at 30°C. After incubation, optical density for all the tubes was measured at 625 nm (Spectronic 20D+, Thermoscientific). Before measuring OD a loopful from tubes containing microwaved medium inoculated with test organism was streaked on to a sterile nutrient agar plate to confirm purity of the growth (i.e. if microwaved medium was not completely sterile, few contaminant colonies should grow along with test organism). Such experiments were performed with 5 different media (Nutrient broth, Luria Bertani broth, Mueller-Hinton broth, Tryptone soya broth, and Tryptone yeast extract broth) for bacteria, and 3 different media (Yeast malt broth, Potato dextrose broth, and YPD broth) for yeasts. All media were procured from HiMedia, Mumbai.

Growth curve

Test organism was inoculated into 140 ml of autoclaved and microwaved medium. Both the media were filled in 500 ml screw capped glass bottle. After inoculation (inoculum added at 1% v/v) contents were distributed into sterile test tubes, and incubated under static condition at appropriate temperature as described above. Each tube contained 4 ml of medium. OD was measured at regular time intervals to prepare growth curve. Such experiments were performed with 5 different media for bacteria, and 3 different media for yeasts.

Spore germination

Both microwaved and autoclaved media (140 ml medium in 500 ml bottle) were inoculated with *Bacillus coagulans* spore (SPORLAC®) suspension. Before inoculation spore suspension was standardized to 0.5 McFarland turbidity standard. Contents from bottles were then distributed into sterile test tubes, and incubated under static condition at 35°C. Each tube contained 4 ml of medium. OD_{625} was measured at regular time intervals to follow spore germination. Similar experiment was performed with spores of *Bacillus stearothermophilus*. Here one intact spore strip (HiMedia) containing 10^5 spores was used for inoculation, followed by incubation at 55°C. Spore germination with

both organisms was checked in 5 different nutrient media.

Rejuvenation of lyophilized cultures

Both microwaved and autoclaved nutrient broth (140 ml medium in 500 ml bottle) were inoculated with suspension of test organism prepared from lyophilized powder. Contents from bottles were then distributed into sterile test tubes (4 ml in each tube), and incubated under static condition at appropriate temperature as described above. OD_{625} was measured at regular time intervals to follow bacterial growth.

During all the above experiments (performed in triplicate) uninoculated autoclaved and microwaved media served as sterility controls. The same were used as blank while measuring OD. To confirm sterility a loopful of content from these controls was streaked on sterile nutrient agar plate. Following incubation at 35ºC, absence of any growth was taken as confirmation of sterility.

Statistical analysis

Statistical analysis was performed using *t*-test with Microsoft® Excel. At $p<0.05$ the difference between microbial growth in autoclaved and microwaved media was considered significant.

Results and Discussion

Growth promotion

Results of growth promotion in microwaved and autoclaved media are presented in table 1-4. Cell density was derived by plotting OD of the experimental tube on a graph of OD_{625} vs. cell no. prepared using different McFarland turbidity standards (Hindler and Munro, 2010). Microwaved media supported better growth of bacteria as well as yeast than autoclaved media. Both amount as well as rate of growth were higher in microwaved media. With respect to percent increment in growth microwaved tryptone yeast extract broth proved most suitable medium for the growth of 4 different bacteria- *E. coli*, *S. aureus, S. flexneri,* and *S. pyogenes*. Microwaved LB broth was most suitable medium for the growth of *S. paratyphi A* and *P. oleovarans*. In four out of five media, organism which registered the highest growth increment was a gram-positive one.

Table 1. Growth of various bacteria in nutrient broth and LB broth

Organism	Nutrient broth			Luria Bertani broth		
	OD_{625} (Mean ± SD)		Growth increment in microwaved media (%)	OD_{625} (Mean ± SD)		Growth increment in microwaved media (%)
	Autoclaved	Microwaved		Autoclaved	Microwaved	
A. hydrophila	0.47 ± 0.00[a]	0.81 ± 0.01[a]	71.15	0.89 ± 0.01[a]	1.39 ± 0.01[a]	55.82
E. coli	0.34 ± 0.04	0.43 ± 0.01	25.00	0.45 ± 0.03[b]	0.64 ± 0.10[b]	42.16
P. oleovarans	1.46 ± 0.08	1.58 ± 0.38	8.56[*]	0.24 ± 0.00[a]	0.54 ± 0.01[a]	123.80
S. flexneri	0.62 ± 0.00	1.03 ± 0.00	65.75	0.68 ± 0.00[a]	0.74 ± 0.00[a]	8.33
S. parathyphi A	0.22 ± 0.01	0.29 ± 0.00	31.08	0.33 ± 0.00[a]	0.61 ± 0.00[a]	83.63
B. subtilis	0.40 ± 0.01	0.63 ± 0.00	57.03	0.51 ± 0.00	0.74 ± 0.00	45.98
S. aureus	0.32 ± 0.00	0.72 ± 0.02	124.61	0.45 ± 0.04[a]	0.56 ± 0.03[a]	23.95
S. epidermidis	0.48 ± 0.01[c]	0.62 ± 0.00[c]	29.07	0.88 ± 0.01[b]	1.36 ± 0.05[b]	53.67
S. pyogenes	0.42 ± 0.00	0.48 ± 0.00	13.25	0.38 ± 0.00	0.45 ± 0.01	16.53

[a]2x dilution [b]3x dilution [c]4x dilution; [*]$p>0.05$

Table 2. Growth of various bacteria in Mueller-Hinton broth and tryptone soya broth

Organism	Mueller-Hinton broth			Tryptone soya broth		
	OD_{625} (Mean ± SD)		Growth increment in microwaved media (%)	OD_{625} (Mean ± SD)		Growth increment in microwaved media (%)
	Autoclaved	Microwaved		Autoclaved	Microwaved	
A. hydrophila	0.84 ± 0.00	0.96 ± 0.00	13.60	0.58 ± 0.01[b]	0.97 ± 0.00[b]	66.09
E. coli	0.38 ± 0.00[a]	0.55 ± 0.00[a]	43.48	0.59 ± 0.00[b]	0.82 ± 0.00[b]	38.27
P. oleovarans	0.79 ± 0.20[a]	1.08 ± 0.00[a]	36.99	0.71 ± 0.01[b]	0.82 ± 0.00[b]	20.70
S. flexneri	0.30 ± 0.00	0.39 ± 0.00	27.77	0.57 ± 0.00	0.84 ± 0.00	45.92
S. parathyphi A	0.44 ± 0.00	0.58 ± 0.00	33.10	0.48 ± 0.00	0.67 ± 0.00	38.19
B. subtilis	0.26 ± 0.01[a]	0.53 ± 0.02[a]	105.0	0.66 ± 0.00	1.03 ± 0.00	55.87
S. aureus	0.29 ± 0.00	0.45 ± 0.01	50.50	1.13 ± 0.00	1.31 ± 0.00	16.81
S. epidermidis	0.16 ± 0.00	0.14 ± 0.00	-13.10[*]	0.72 ± 0.02	1.36 ± 0.00	87.77
S. pyogenes	0.29 ± 0.00	0.32 ± 0.01	8.69	0.79 ± 0.00	0.82 ± 0.00	3.53

[a]2x dilution [b]3x dilution; [*]p>0.05

Table 3. Growth of various bacteria in Tryptone yeast extract broth

Organism	Tryptone yeast extract broth		
	OD_{625} (Mean ± SD)		Growth increment in microwaved media (%)
	Autoclaved	Microwaved	
A. hydrophila	0.53 ± 0.00	0.89 ± 0.00	53.51
E. coli	0.54 ± 0.02	0.86 ± 0.01	57.96
P. oleovarans	0.47 ± 0.02[a]	0.66 ± 0.00[a]	39.53
S. flexneri	0.32 ± 0.00	0.54 ± 0.01	67.69
S. parathyphi A	0.20 ± 0.00	0.31 ± 0.01	50.23
B. subtilis	0.23 ± 0.00	0.37 ± 0.00	60.85
S. aureus	0.47 ± 0.00	1.11 ± 0.00	134.17
S. epidermidis	0.18 ± 0.00	0.28 ± 0.00	56.35
S. pyogenes	0.15 ± 0.04	0.25 ± 0.02	61.14

[a]2x dilution

Table 4. Growth of yeast in different media

Media	Organism	OD_{625} (Mean ± SD)		Growth increment in microwaved media (%)
		Autoclaved	Microwaved	
Yeast malt broth	*C. albicans*	0.29 ± 0.00	0.49 ± 0.00	69.65
	S. cerevisiae	0.69 ± 0.07 [c]	1.10 ± 0.07 [c]	76.78
Potato dextrose broth	*C. albicans*	0.29 ± 0.00 [a]	0.32 ± 0.00 [a]	12.06
	S. cerevisiae	0.69 ± 0.01 [a]	1.31 ± 0.00 [a]	88.69
YPD broth	*C. albicans*	0.52 ± 0.00 [b]	0.81 ± 0.00 [b]	55.53
	S. cerevisiae	0.51 ± 0.01	0.66 ± 0.00	28.34

[a]2x dilution [b]5x dilution [c]6x dilution

Table 5. Generation time of different bacteria in nutrient broth, LB broth, and Mueller-Hinton broth

Organism	Generation time (g) (h)					
	Nutrient broth		Luria Bertani broth		Mueller-Hinton broth	
	Autoclaved	Microwaved	Autoclaved	Microwaved	Autoclaved	Microwaved
A. hydrophila	8.36	7.71	3.19	3.14	4.86	3.19
E. coli	10.37	7.71	7.00	5.28	4.97	4.73
P. oleovarans	4.89	3.74	8.13	6.54	2.00	1.57
S. flexneri	13.08	9.70	21.50	20.06	6.71	6.66
S. parathyphi A	1.33	1.09	15.05	9.40	15.05	10.03
B. subtilis	5.89	4.09	1.89	1.24	10.03	7.50
S. aureus	13.68	8.85	15.84	12.04	6.68	6.68
S. epidermidis	6.14	4.42	3.50	3.30	9.12	15.05
S. pyogenes	6.02	5.37	10.37	5.57	21.5	10.30

Table 6. Generation time of different bacteria in tryptone soya broth and tryptone yeast extract broth

Organism	Generation time (g) (h)			
	Tryptone soya broth		Tryptone yeast extract broth	
	Autoclaved	Microwaved	Autoclaved	Microwaved
A. hydrophila	1.00	0.59	12.75	9.83
E. coli	12.54	9.40	13.43	10.90
P. oleovarans	9.70	9.40	10.10	7.50
S. flexneri	11.14	6.02	7.50	6.02
S. parathyphi A	1.12	0.84	11.99	11.62
B. subtilis	1.06	0.59	6.89	6.84
S. aureus	3.44	1.60	1.21	0.30
S. epidermidis	1.71	0.83	3.17	0.88
S. pyogenes	5.20	5.01	60.20	25.08

Table 7. Generation time of yeast in in different media

Media	Organism	Generation time (g) (h)	
		Autoclaved	Microwaved
Yeast malt broth	*C. albicans*	6.54	5.28
	S. cerevisiae	5.18	4.77
Potato dextrose broth	*C. albicans*	2.59	0.75
	S. cerevisiae	3.71	3.42
YPD broth	*C. albicans*	7.71	5.57
	S. cerevisiae	3.42	3.40

Table 8. Generation time for spores in different media

Spores	Media	Generation time (g) (h)	
		A	MW
B. coagulans	Nutrient broth	6.27	5.18
	LB broth	13.68	9.12
	Mueller-Hinton broth	15.84	10.03
	Tryptone soya broth	2.70	2.19
	Tryptone yeast extract	8.85	6.54
B. stearothermophilus	Nutrient broth	12.54	12.18
	LB broth	7.34	21.5
	Mueller-Hinton broth	8.85	5.57
	Tryptone soya broth	3.73	0.92
	Tryptone yeast extract	16.72	15.05

Growth curve

Generation time of different microorganisms in autoclaved and microwaved media was estimated from growth curve experiments (Table 5-7). All organisms registered lesser generation time (i.e. faster growth) and achieved higher cell densities in microwaved media (Figure 1-2), except *S. epidermidis* in Mueller-Hinton broth. Still shorter generation time is obviously attainable if constant shaking is provided during incubation. Tryptone soya broth allowed 6 of the test bacteria to achieve a growth rate faster than in any other media.

Spore germination

Autoclaved and microwaved media were tested for their ability to support germination of *B. coagulans* and *B. stearothemophilus* spores. Microwaved media supported better germination of both the spores. After germination organisms registered faster growth (Table 8) and higher cell density in microwaved media except *B. stearothermophilus* in LB broth. Both organisms registered their least generation time in tryptone soya broth.

Rejuvenation of lyophilized cultures

Microwaved nutrient broth supported better rejuvenation of lyophilized bacterial cultures. It allowed the test organisms- *E. coli, P. aeruginosa,* and *S. aureus* to reach 93.54%, 7.01%, and 81.81% higher cell density at faster rate (Table 9) than that in autoclaved broth.

Table 9. Generation time of different bacteria in nutrient broth after revival from lyophilized form

Organism	Generation time (g) (h)	
	A	MW
E. coli	7.71	5.90
P. aeruginosa	6.84	5.18
S. aureus	4.77	3.62

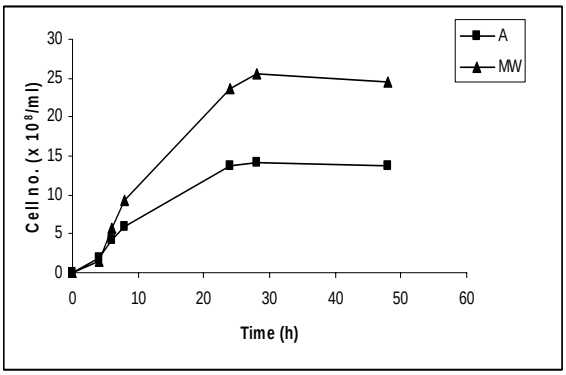

Figure 1. Growth curve of *E. coli* in microwaved and autoclaved nutrient broth

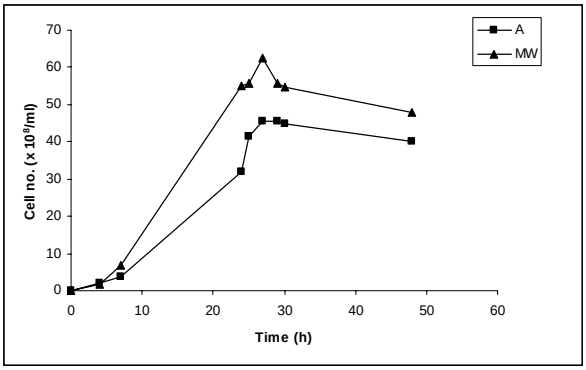

Figure 2. Growth curve of *S. cerevisiae* in microwaved and autoclaved yeast malt broth

Here we show that growth media sterilized by microwave irradiation promotes faster growth of various bacteria as well as yeasts. We found that broth made by microwaved method can be stored safely for up to 72 h, without any indication of contamination. Thus, the use of microwave irradiation is an effective method of sterilization of liquid growth media of varying composition. Microwaves have been known to be effective at killing microorganisms (Najdovski *et al*. 1991; Zakaira *et al*. 1992; Wu, 1996; Madigan *et al*. 2009), but a common sterilization protocol applicable for biological media of varying matrix is still not available.

Better retention of nutrient quality in microwaved growth media due to shorter heat exposure than in autoclave seems to be the major reason for better microbial growth in microwaved media in our experiments. Due to longer 'heating-holding-cooling' cycle in autoclave, some degree of nutrient quality deterioration becomes unavoidable. Media undergoes evaporation during MW treatment, as a result of which media become somewhat nutrient concentrated. To check whether this factor has played any major role behind superior performance of microwaved media, we made *E. coli* grow in an nutrient broth which was brought to initial volume by addition of sterile distilled water after MW treatment, thus nullifying the effect of evaporation on nutrient composition. Simultaneously a control was set wherein *E. coli* was grown in microwaved media with no volume adjustment. Growth of *E. coli* in both the media did not differ significantly (p>0.05; data not shown), indicating no important role being played by evaporation during MW treatment. Simultaneously along with this same organism was grown in autoclaved nutrient broth, and growth in it was lesser than that in microwaved media prepared in either way. Autoclaved nutrient broth with more amount of ingredients (so as to attain equivalence to microwaved media with no

volume adjustment) was also inoculated with *E. coli*, but it supported no higher growth than normal autoclaved broth. Similar results were obtained with *A. hydrophila*.

It is difficult to ascertain whether better nutrient retention due to lesser heat exposure can solely account for improved bacterial growth in microwaved media. One of the other possible reasons leading to better microbial growth in microwaved media may be the 'MW specific athermal effects'. Despite existence of non-thermal effects of MW being controversial (Welt *et al*. 1994; Wayland *et al*. 1997), possibility of some role being played by it can not be rejected fully. Non-thermal effect was suggested to have an important role in the inactivation of microorganisms in suspension (Jeng *et al*. 1987). Microbial destruction during microwave exposure has also been shown to occur at lower temperatures and shorter time periods in comparison to conventional heating methods (Banik *et al*. 2003).

As different components of microbiological media can interact with each other under the influence of heat, sometimes their heat induced interactions may result in formation of undesirable products (viz. Amadori products), some of which may be inhibitory to microorganisms. Inhibition of *A. actimomycetemcomitans* due to Maillard reaction products in autoclaved medium was overcome by preparing the medium with microwaves (Bhattacharjee *et al*. 2009). Due to lesser heat exposure during MW treatment there is a reduced probability of unwanted interactions among different medium components.

Bacterial species differ in their susceptibility to MW inactivation. Microwaves can kill bacterial spores more effectively in presence of water than when dry (Najdovski *et al*. 1991). Bacterial spores have similar resistance to MW as to conventional heating (Sasaki *et al*. 1998). However, Celandroni *et al*. (2004) have shown that the effect exerted by microwaves on spores is different from that of autoclave. Contrary to conventional heating, microwaves promote formation of stable complexes between dipicolinic acid and other spore components, thereby preventing the release of dipicolinic acid from spores. The conventional heated spores exhibited a cortex layer that was up to 10 times wider than that of untreated spores i.e. an extremely relaxed cortex. This relaxation of cortex was not observed during MW treatment, cortex maintained its original width even though exposure time and sample temperature for conventional heat and MW was same. For much of the time during MW exposure, media remain at highest reachable temperature, strengthening the probability of reliable sterilization despite shorter treatment time. Superheating is a well recognized phenomenon for homogenous suspensions subjected to microwaves (Bond *et al*. 1991; Barghust *et al*. 1992), which can raise the temperature of water-based media above normal boiling point of 100^{o}C, and thus can effect microbial killing better than that occurring under normal boiling.

Though reports of MW sterilization for one or another particular medium have appeared in literature in past, suitability of MW sterilization for a wide variety of media and growth of different microorganisms in MW sterilized media has not been reported. Iacoviello and Rubin (2001) reported sterile preparation of antibiotic-selective LB agar plates using a microwave oven. They reported that plates prepared with a microwave oven could be stored for short-term (1-10 days at 4^{o}C) without contamination, and showed that sterilization of media by autoclave could be replaced with short round of heating the media in a microwave oven. Efficacy of the antibiotic present in the media was not compromised by the MW heating process. With respect to microorganism contamination, plates prepared with the microwave protocol were shown to have the same shelf life as those

prepared by the traditional autoclave method. Liquid media that is made fresh from powder and then boiled for several min could be used for growing bacterial cells without getting into too much trouble, given that most gram-negative bacteria would not survive the boiling treatment (Hengen, 1997). Faster growth and greater viability of *A. actinomycetemcomitans* were reported in both liquid and solid growth media if they were sterilized by MW radiation rather than by autoclaving. One difference between autoclaved and microwaved media was that the autoclaved media were darker brown in colour, which was suggested to be due to the Maillard reaction products (Amadori products). Such products are formed by autoclaving a mixture of lysine and glucose, and can inhibit growth of certain microorganisms (Bhattacharjee *et al.* 2009). MW sterilization thus can be recommended for any media containing such ingredients (e.g., lysine and glucose) whose interaction under the influence of heat can generate Amadori or other inhibitory products. Bhattacharjee *et al*. (2009) found that broth or plates made by MW method can be stored at room temperature for more than a month, indicating it to be an effective method for sterilization of liquid or solid growth media. Besides, it has the advantage that there can be better control of sterilization time whereas autoclaving time cannot be controlled easily since it is always accompanied by extra heating cycles during the conditioning and exhaust cycles.

Our experiments clearly indicate suitability of MW sterilization for liquid nutrient media. It can be an attractive alternative to conventional autoclaving, especially when media are needed for immediate use or for such organisms which are not able to grow well in autoclaved media, and also when high biomass yield is of particular importance. It will be useful to test its suitability for solid media of varying compositions. Presence of agar in solid media may cause bumping of contents inside vessel during MW heating. If pressurized MW vessels (which can be operated at pressure equivalent to that in autoclave) of sufficiently large volume can be made available at reasonable cost, then MW sterilization is likely to find wide acceptance. Such apparatus will avoid evaporation as opposed to open-vessel operations. If the challenges of scale-up (in terms of volume, multiple vessels at a time), real-time temperature monitoring and control, and availability of a wide range of MW-compatible materials can be met, then MW sterilization may find wider acceptance in microbiology laboratories.

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References

Baghurst, D.R., Mingos, D.M.P. 1992. Superheating effects associated with microwave dielectric heating. *J. Chem. Soc., Chem. Commun.,* 674-677.

Banik, S., Bandyopadhyay, S., Ganguly S. 2003. Bioeffects of microwave-a brief review. *Bioresour. Technol.* **87**: 155-159.

Bhattacharjee, M.K., Sugawara, K., Ayandeji, O. T., 2009. Microwave sterilization of growth medium alleviates inhibition of *Aggregatibacter actinomycetemcomitans* by Maillard reaction products. *J. Microbiol. Meth.,* **78**: 227-230.

Bond, G., Moyes, R.B., Pollington S.P., Whan D.A., 1991. The superheating of liquids by microwave radiation. *Chemistry and Industry.*

Celandroni, F., Longo, I., Tosoratti, N., Giannessi, F., Ghelhardi, E., Salvetti, S.,Baggiani, A., Senesi, S. 2004. Effect of microwave radiation on *Bacillus subtilis* spores. *J. Appl. Microbiol.,* **97**: 1220-1227.

Hengen, P., 1997. Emergency sterilization using microwaves. *Trends Biochem. Sci.,* **22**: 68-69.

Hindler, J.F., and Munro, S. 2010. Antimicrobial susceptibility testing. In *Clinical microbiology procedures handbook,* Ed. L. Garcia, USA: ASM press, pp: 5.14.1.1-5.14.1.4. http://vanguardia.udea.edu.co/cursos/Bacteriologia/Hand%20Book/sections/05.14.01.pdf (last accesssed 4 May, 2010)

Iacoviello, M.P., Rubin, S.A. 2001. Sterile preparation of antibiotic selective LB agar plates using a microwave oven. *BioTechniques.,* **30**: 963-965.

Jeng, D.K.H., Kaczmarek, K.A., Woodworth, A.G., Balasky, G. 1987. Mechanism of microwave sterilization in the dry state. *Appl. Environ. Microbiol.,* **53**: 2133-2137.

Madigan, M.T., Martinko J., Dunlap, P., Clark, P. 2009. *Brock Biology of Microorganisms*, San Franscisco: Pearson Education.

Najdovski, L., Draga, A.Z., Kotnik, V. 1991. The killing activity of microwaves on some non-sporogenic and sporogenic medically important bacterial strains. *J. Hosp. Infect.,* **19**: 239-247.

Sasaki, K., Honda, W., Ohsawa, S., Miyake, Y., Kawashima, Y. 1998. A study of microwave sterilizer for injection ampules (No.5): evaluation of sterilization effect on the head space of ampules. *J. Pharm. Sci. Technol., Jpn.,* **58**: 136-146.

Sasaki, K., Honda, W., Shimizu, K., Lizima, K., Ehara, T., Okuzawa K., Miyake Y. 1995. Microwave continuous sterilization of injection ampoules. *PDA J. Pharm. Sci. Technol.,* **50**: 172-179.

Sasaki, K., Mori, Y., Honda, W., Miyake, Y. 1998. Selection of biological indicator for validating microwave heating sterilization. *PDA A Pharm. Sci. Technol.,* **52**: 60-65.

Wayland, J.R., Brannen, J.P., Morris, M.E. 1977. On the interdependence of thermal and electromagnetic effects in the response of *Bacillus subtilis* spores to microwave exposure. *Radiat. Res.,* **71**: 251-258.

Welt, B., Tong, C., Rossen, J., Lund, D. 1994. Effect of microwave radiation on inactivation of *Clostridium sporogenes* (PA 3679) spores. *Appl. Environ. Microbiol.,* **60**: 482-488.

Wu, Q. 1996. Effect of high power microwave on indicator bacteria for sterilization. *IEEE Trans. Biomed. Eng.,* **43**: 752-754.

Xi, X., Wu, D., Wang, G., Wang, W. 2002. Research and development on microwave sterilization. *J. Biomed. Eng.,* **19**: 334-336.

Zakaria, Z., Neas, E.D. 1992. Process for rapid sterilization of biological media. US patent. No. 5,108,701