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Sterilization of Culture Media for Microorganisms Using a Microwave Oven Instead of Autoclave

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ABSTRACT

Different culture media in different volumes were exposed to microwave (MW) irradiation for 2, 3 and 4 minutes to investigate the ability of MW to destroy microorganisms and compared with media sterilized by conventional autoclaving method. MW sterilized media were screened for microbial growth.

Exposure of different microorganisms to microwave irradiation resulted in destruction of all microorganisms within 3 minutes. Using MW for irradiation is a practical, easy, rapid and energy saving way to sterilize different types of culture media with no effects on the quality of culture media and microbial growth after sterilization. It can be used as alternative apparatus instead of autoclave in microbiology laboratories for preparing different sizes in rapid and routine experiments especially in the conditions of the weak electricity current and interruption.

Keywords: Microwave, sterilization, irradiation, culture media.

4,3,2

3

INTRODUCTION

Sterilizing the growth medium is essential before its use in microbiology laboratory. This process of growth medium sterilization is commonly performed by autoclave.

The disadvantage of autoclave sterilization process is that it takes a long period of time. During the autoclaving process, the temperature is 121°C and the pressure is 15psi, for 15 minutes. After the sterilization process, it still needs several hours to wait until the pressure inside the apparatus drops again up to 1 atm to permit opening the apparatus. (Bhowmik, 2011) In this study microwave oven was used instead of autoclave as alternative apparatus to sterilize culture media.

Microwave energy is a type of high frequency radio wave, familiar to many as 'Microwave ovens'. It causes water molecules to vibrate at extremely high speed producing friction which in turn produces heat. (Spencers *et al.*, 1985)

Microwave sterilization of plastic tissue culture vessels was found to be effective, rapid and relatively inexpensive to inactivate different test viruses, certain bacteria and fungi (Sanborn *et al.*, 1982). Moreover, microwave (MW) radiation has been used for effectively disinfecting gauze pieces and hospital white coats, aseptic packaging of food (Bhattacharjee *et al.*, 2009). Emergency sterilization of media using a microwave oven has also been reported by Hengen. (Hengen, 1997).

The aim of this study is to evaluate the ability of microwave oven in sterilizing 15, 100, 200 and 300 ml of both liquid and solid culture media for different durations and compare the growth of different microorganisms on culture media sterilized by microwave to standard autoclave methods.

MATERIALS AND METHODS

1. Microwave oven

A domestic type of microwave oven (Gosonic- Model No. GOM-423) operating at 50Hz was used for sterilization of media.

2. Microorganisms

The test organisms, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Penicillium sp.* were obtained from College of Science/ department of Biology.

3. Culture media preparation

Different volumes (15, 100, 200 and 300 ml) of nutrient agar (NA), nutrient broth (NB), blood base agar to determine hemolysis, and potato dextrose agar (PDA) were used in the study. Prepared cultured media were inoculated with 1ml of overnight culture of sporulated, non sporulated bacteria and fungal spores separately to make sure that the media contain vegetative bacteria, spores and fungal spores before sterilization.

Two sets of culture media were prepared according to manufacturers instruction. One set of the culture media was sterilized using autoclave for 15 minutes at 15 lb (121°C) used as control and the second set was divided into three groups, they were heated by exposing to microwaves for 2, 3 and 4 minutes. 5% blood agar was prepared by adding 5 ml of blood to 95 ml blood base agar. Plates of two sets were poured in the same way as the conventional method and were allowed to solidify. Nutrient broth was distributed in amounts of 10 ml in screw cap containers; each of which consisted of three plates/tubes incubated for 24 hours in the temperature of 37°C.

A set of tubes was incubated along with the inoculated media without heating by microwave or autoclaving served as control.

4. Inoculation

After incubation, in order to observe any differences between the microbial growth on both sets of culture media, plates with no growth were inoculated with tested bacteria using streak plate technique and incubated overnight at 37°C.

For nutrient broth three to four colonies of test bacteria were touched with wire loop and then emulsified sterile nutrient broth and incubated overnight at 37°C.

A fungal disc was cut by using sterile cork borer and placed on the surface of the culture media and incubated at room temperature for 48h.

RESULTS AND DISCUSSION

Culture media that inoculated with *E. coli*, *P. aeruginosa*, *S. aureus* and *Penicillium* showed no growth after 2 minutes exposing to microwave oven radiation while number of colonies of *B. cereus* formed after 2 min heating by microwave. All volumes of culture media used were sterilized after 3 minutes and no effect of media volume was observed on sterilization process. The chamber sizes of microwave ovens vary. Thus, the number of flasks per load may also vary. To prepare a large volumes of culture media three flasks containing 300 ml of culture media per load can be helpful.

All tested bacteria including *B. cereus* showed no growth on culture media exposed to microwave radiation for 3 and 4 minutes. This attributed to resistance of *B. cereus* spores which needed more time than vegetative cells to be killed. This result is in agreement with that reported by Sanborn and others (1982) who tested spore suspensions of two *Bacillus* species and have found these species to be much more resistant to microwave radiation and killed in 180 seconds. They also reported that spores of *B. cereus* were germinated on cooked rice after 10 minutes heating at 80 or 90°C, while in present study heating by microwave radiation for 3 minutes was enough to kill all spores present in both liquid and solid culture media.

Tubes that used as control showed normal growth of different microorganism including inoculated bacteria (Fig. 1) and fungi while autoclaved set of culture media was sterile. Most plates did not show any growth indicating that microwave sterilizes the media in the same way as autoclave. The inoculated plates showed slight difference in growth pattern as shown in (Table 1) that demonstrated the effect of microwave on the visible growth colony of these bacteria on solid media at different time periods (2 min, 3 min, 4 min and 0 min) and showed that microwave oven succeed to sterilize all of the media at 3 minutes.

Table 1: Effect of Microwave at different times on the growth of some bacteria on solid media

Types of Bacteria	2 Minute	3Minute	4Minute	Autoclaving
<i>E. coli</i>	–	–	–	–
<i>S. aureus</i>	–	–	–	–
<i>P. aeruginosa</i>	–	–	–	–
<i>B. cereus</i>	+	–	–	–

The effect of microwave radiation on the growth of bacteria in liquid media at different time periods (2 min, 3 min, 4 min and 0 min) was explained in (Table 2). Incubated tubes did not show any growth except *B. cereus* which showed growth at 2 minutes.

Table 2: Effect of Microwave at different times on the growth of some bacteria in liquid media

Types of Bacteria	2 Minute	3 Minute	4 Minute	0 Minute (Control)	Autoclaving
<i>E. coli</i>	–	–	–	+	–
<i>S. aureus</i>	–	–	–	+	–
<i>P. aeruginosa</i>	–	–	–	+	–
<i>B. cereus</i>	+	–	–	+	–

(–) = No growth, (+) = Showed growth

It was observed that the exposure of microorganisms to a microwave radiation could kill them, many studies were established the effect of microwave radiation that used for disinfection or sterilization purposes. In a study by Park *et al.*, (2006), cultures of *E. coli* and *B. cereus* were exposed to the microwave in a home microwave oven, they were completely destroyed after two and four minutes, respectively. When toothbrushes contaminated with *Streptococcus mutans* heated by microwave oven for five minutes they completely decontaminated (Belanger-Ginquere and Belanger, 2011). Another study showed that microwaves lead to reducing the number of bacteria on dentures (Glass *et al.*, 2011).

The effect of the microwave radiation on fungus *Penicillium* on the Potato dextrose agar (PDA) medium at 2, 3, 4 and 0 minutes showed that shown in (Table 3). All plates of PDA that heated by MW for different duration of times and inoculated with *Penicillium* showed no growth as shown in (Table 3). After sterilization by microwave and inoculation the plates were inoculated with the fungus and incubated for more than 48 h at room temperature they showed high growth rate which indicate that the microwave enhance the growth of *Penicillium*.

Table 3: Effect of Microwave at different times on the growth of *Penicillium* on PDA

Time	Growth
2 Minute	-
3 Minute	-
4 Minute	-
0 Minute (Control)	+
Autoclaving	-

(-) = No growth, (+) = Showed growth

A study by Jankovic *et al.*, (2014) on the effects of microwave radiation on microbial cultures for 1, 2, 3 or 4 minutes, showed that the killing effect was observed after an exposure of two minutes or longer and *Candida albicans* was more susceptible to microwave radiation than bacterial cultures. This result is in agreement with our result that the best time for sterilization by microwave is 3 minutes.

There were differences in color between the microwave-sterilized media and the autoclaved media. Microwave sterilized media were lighter than the autoclaved one which was suggested to be due to the Maillard reaction products as shown in Fig. (2-4). Maillard reactions are group of various complicated non-enzymatic reactions between free amino groups of protein and carbonyl groups of reducing sugars. This reaction results in the darkening of the autoclaved media's color due to the production of melanoidines. Such products are formed by autoclaving a mixture of lysine and glucose, and can prevent growth of microorganisms (Bhattacharjee *et al.*, 2009). MW sterilization so 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 (Kothari *et al.*, 2011).

S. aureus, *B. cereus* and *P. aeruginosa* produced Beta hemolysis on blood agar medium sterilized by both microwave and autoclave. In this study the zoon around the colony seemed to be clearer on MW sterilized medium than autoclaved. This may be due to the dark-brown color of the autoclaved blood base agar when comparing with MW sterilized one which was lighter.

Most media were still sterile after being kept in the incubator for 24 hours and then in the refrigerator for one month which is consistent with a study by Bhattacharjee *et al.*, (2009) who

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.

Because it was observed that the heating of microorganisms to a certain temperature by microwaves could kill them, many studies were performed attempting to establish the minimal dose of microwave radiation that could be used for disinfection or sterilization purposes (Jankovic *et al.*, 2014). A study have been carried out to screen the effect of microwaves on killing several bacterial specie in food (Pagan *et al.*, 1998) and on bacterial cell directly (Vaid and Bishop, 1998; Woo *et al.*, 2000). But research on the effect of microwave on microorganism in culture media was carried out by Baqai and Saleem, (1992) in Pakistan and Prijana *et al.*, (2016) in Padjadjaran.

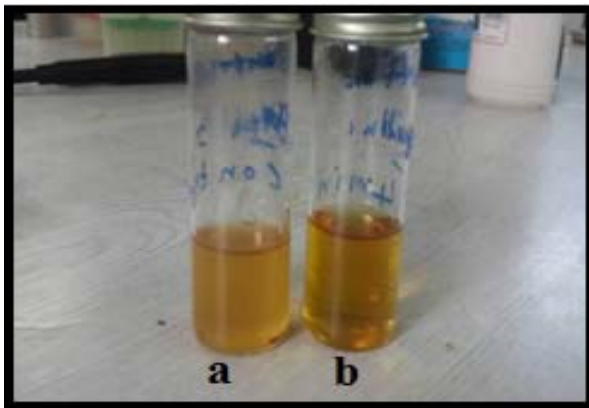


Fig. 1: Growth of bacteria in control tube (a) while no growth in MW sterilized medium (b)

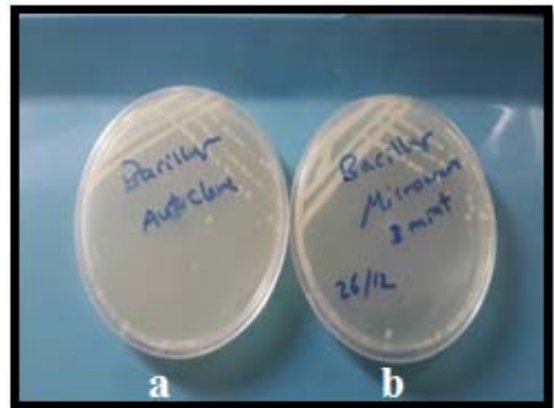


Fig. 2: *B. cereus* growth on medium sterilized by autoclave (a) and MW (b)

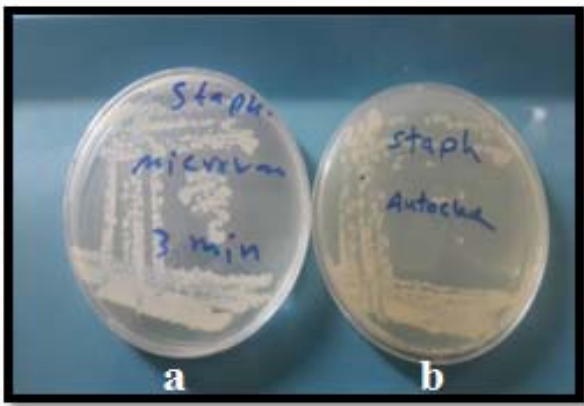


Fig. 3: *S. aureus* growth on medium sterilized by autoclave (a) and MW (b)

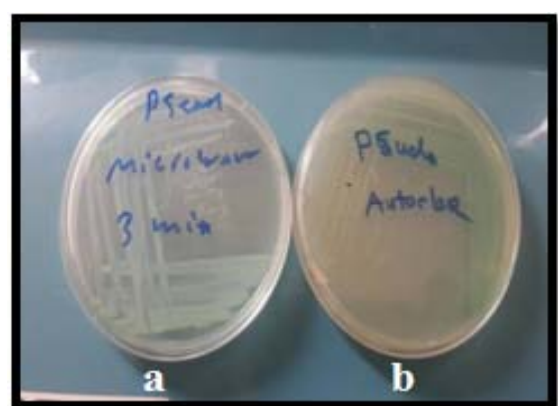


Fig. 4: *P. aeruginosa* growth on medium sterilized by autoclave (a) and MW (b)

Routine use of microwave oven should be recommended as it is time saving. Media prepared in microwave oven takes many minutes while the whole process of autoclaving takes more than 1.5 hours. Besides this, sterilization by microwave takes less space and can be used anywhere, especially in few time electric areas and the running cost is also very low, then MW sterilization may be more acceptant in microbiology laboratories.

Understanding the mechanism of effect of microwaves on microorganisms is very important for the possible future use of microwave technology, not much is known about the mechanisms because previous studies have been limited in scope and number.

CONCLUSION

In conclusion, results have shown that 3 minutes heating by microwave oven irradiation is a practical, easy, rapid and economical (energy saving) way to sterilize different sizes of different types of culture media with no effects on the quality of culture media and microbial growth after incubation. Microwave oven also takes less space and can be used anywhere as alternative apparatus instead of autoclave whether for microbiology laboratory experiments for students or research studies in microbiology laboratory.

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