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MATHEMATICAL MODEL OF MANGANESE ION CATALYZED MICROWAVE DEACTIVATION OF *Enterococcus faecalis, Staphylococcus aureus* AND *Escherichia coli*

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Abstract – *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* survival was investigated using microwave irradiation (power 130 W) both in a water control and in the presence of a 1μM manganese ion solution. Measured survival dependencies had "bell' shape form with maximum bacterial viability between 1 - 2 min of microwave heating. Additional heating revealed bacteria survival decreasing up to 3 min of microwave heating when viability became insignificantly small. The total deactivation time of bacteria in the presence of manganese ions was significantly smaller then that of bacteria irradiated in the microwave without manganese ions present (4 -5 min). One possible explanation for the rapid reduction of bacterial survival during microwave irradiation in the presence of manganese ions is that increasing manganese ion penetration into bacteria along with microwave irradiation related to an increase of kinetic energy of ions, and damaging of bacteria by metal ions. The proposed mathematical model for microwave heating took into account "growth" and "death" factors of bacteria. It assumes that rates of bacterial growth and decay are linear functions of water temperature, and rate of bacterial decay that relates with metal concentration into water is also linear, which influenced the differential equation for the dependence between number of survival bacteria and temperature water. By using proportionality between the time of microwave heating and water temperature we derived the differential equation, between bacterial viability and time of microwave irradiation which was used as mathematical model for microwave heating in the presence of metal ions. This model had forms of second-degree polynomial functions. We received good relationships (with coefficient of correlation 0.92 - 0.99) between proposed mathematical model and experimental data for all bacterial deactivation.

Key words: Manganese, model, deactivation, microwave, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*

INTRODUCTION

Effective disinfection of water, especially from fecal coliforms, is an important step in reducing the occurrence of pathogenic conditions and improving overall public health. The World Health Organization reports that the deactivation of these bacteria is a vital step in reduction of water related diseases [3]. Microwave irradiation is one of the most useful methods for bacteria deactivation. Goldblith and Wang [15] conducted studies showing that the deactivation of *Escherichia coli* and *Bacillus subtilis* spores was accomplished by using microwave irradiation in comparison with conventional heating. They found that the deactivation of these bacteria was a result of the dielectric heating and not due to any secondary effect of the microwave irradiation. Lechowich et al.[19], Vela et al.[36], and Fujikawa et al.[13] also found that the deactivation of *Streptococcus faecalis* (later classified as *Enterococcus faecalis*) and *Saccharomyces cerevisiae* when using microwave irradiation resulted from the dielectric heating. Vaid and Bishop [35] studied destruction of bacterial endospores by microwave irradiation.

Conversely, Shin and Pyun [32] compared the results of deactivation of *Lactobacillus plantarum* by conventional heating at 50 °C versus continuous microwave heating and pulsed higher power microwave heating during 30 min.

Abbreviations: **CFU**, *Colony* Forming Unit; E. ***coli*** , *Escherichia coli;* ***E.faecalis***, *Enterococcus faecalis;* **min**, minute; **mL**, milliliters; ***S. aureus***, *Staphylococcus aureus*; **TSA**, Tryptic Soy Agar; **TSB**, Tryptic Soy Broth; **WHO**, World Health Organization.

They found greater reduction in bacterial survival for pulsed microwave heating relative to conventional and continuous microwave heating. These results suggested a secondary non-thermal mechanism that assisted in the deactivation of bacteria during higher power of microwave irradiation. Culkin and Fung [12] and Kozempel et al. [17] also suggest that a secondary non-thermal mechanism could assist in the deactivation of certain species of bacteria.

In our recent paper [4] we studied dependencies between bacterial viability and time of microwave heating of water for several fecal coliforms (*Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli*). We found that time dependencies had "bell" shape form with maximum at 1-2 min of microwave heating. Bacterial survival then decreased at 4-5 min of microwave heating and became insignificantly small. The proposed mathematical model for microwave heating accounts for both a "growth" and "death" factor of bacteria, and it had forms of second degree polynomial functions. We received good relationships (with coefficient correlation 0.84 - 0.99) between proposed mathematical model and experimental data.

For increasing efficiency of microwave deactivation of bacteria, we can add definite (transitional) heavy metal ions into water. Transitional metal ions play important role in the life processes of bacteria. Several metals, such as calcium, cobalt, copper, chromium, potassium, iron, magnesium, nickel, zinc, manganese, sodium, are essential nutrients. In small concentrations, they act as micronutrients, which stimulate development of bacteria. These metals are used in biological processes such as redox-processes, to stabilize molecules through electrostatic interactions, as components of various enzymes, and for regulation of osmotic pressure [7]. Many other metals (for example, silver, aluminum, cadmium, gold, mercury, lead) may have no biological role, or be none essential, and can even be potentially toxic to bacteria [7]. Toxicity of transitional metal ions depends on their concentration into water, forms of compounds, and other factors, including water temperature [21, 26, 27]. K. Nageli [5] found that essential metal ions (for example, copper ions) in higher concentration into water also show oligodynamic actions: hampering of bacterial growth.

Most investigations with heavy metal ions were conducted with silver and copper ions in various forms coupled to other compounds. Previous research [8,11] found that the presence of silver ions effectively deactivated coliforms. Abad et al.[1] found that the existence of copper and silver ions into water in combination with low levels of chlorine leads to large reduction of poliovirus. Similar results were found in other papers [34, 37, 38]. Landeen et al.[18] showed efficiency of copper and silver ions in combination with low level of chlorine for deactivation of *Legionella pneumophilla*. Short et al.[33] found that compounds of copper ions reduced number of *Staphylococcus aureus*. Viability of *Escherichia coli* was decreased in presence of sodium chloride in dry sausage [14]. Ragab-Depre [31] found that treatment during 60 min of secondary effuents with hydrogen peroxide-ascorbic acid-Cu (II) resulted in 99% reduction of Enterobacteriaceal, total and fecal coliforms and staphylococci. Oganesov [28] proposed the device for deactivation of bacteria into water by using silver ions. Myers and Nealson [22] studied the influence of manganese oxide on bacterial reduction and growth. Nealson and Saffarini [25] used iron and manganese for anerobic respiration studies. Calomiris et al.[10] studied the influence of different metal ions Cu^{2+}, Pb^{2+}, Zn^{2+}, Al^{3+}, Sn^{2+}, and Cd^{2+} on *Escherichia coli*, *Staphylococcus aureus*, and several other bacteria. They found that the existence of Cu^{2+} and Zn^{2+} sufficiently decreased the number of bacteria *Escherichia coli* into water. *Staphylococcus aureus* were sensitive to all written above metal ions except the ion Al^{3+}[10]. Nakahara et al. [23,24] described distribution of resistance staphylococcal strains to several metal ions and antibiotics. Several authors [2,6,16,20,29,30] indicated that transitional (heavy) metal ions affect the antimicrobial activities of antibiotics.

Unfortunately, bacterial deactivation using only transitional (heavy) metal ions is time-consuming and requires several hours or even days. Other research suggests that the presence of metal ions into water can accelerate its bacterial deactivation when used with other methods. Butkus et al [9]. found that the presence of silver ions enhanced inactivation of coliphage MS-2 by UV disinfection. As we see from written above, investigations that were devoted to bacterial deactivation by using microwave irradiation did not use metal ions, which can help to decrease number of survival bacteria during microwave treatment, and reduce the time of treatment. This

observation became the purpose of our investigation.

MATERIALS AND METHODS

Microwave Deactivation of E. faecalis, S. aureus and E. coli in Distilled Water

Stock cultures of *E. faecalis, S. aureus* and *E. coli* (Ward Natural Scientific, Rochester, NY) were created by growing them in TSB (Tryptic Soy Broth) overnight in a Labline Imperial III Incubator (model # 305) at temperature of 37°C. An aliquot of 10µL of stock culture was then placed into 250 mL sample vials containing 100 mL of distilled deionized water purified by a Barnsted e-Pure System (model # D4641). The sample vials were then microwave irradiated in a Panasonic Inverter Microwave (model # NN-S543BF) at power 130 W for 1, 2, 3, 4, and 5 minutes. Results were compared with control samples which were not microwave irradiated.

Fifty microliters of the test solutions were plated in exponential spiral fashion on to 10 TSA (Tryptic Soy Agar) plates for each sample group using a Spiral Biotech Autoplate 4000 (model # AP 4000). The plates were then incubated overnight in a Labline Imperial III Incubator (model # 305). Finally the colony forming units were counted using a Spiral Biotech Q-count (model # 510) auto-plate reader set for a 50µL exponential spiral pate setting. The CFUs (colony forming units) were then calculated and graphed using Grapad Prism®.

Microwave Deactivation of E. faecalis, S. aureus and E. coli in 1µM Manganese

Stock cultures of *E. faecalis, S. aureus* and *E. coli* (Ward Natural Scientific, Rochester, NY) were created by growing them in TSB (Tryptic Soy Broth) overnight in a Labline Imperial III Incubator (model # 305) at temperature of 37°C. An aliquot of 10µL of stock culture was then placed into 250 mL sample vials containing 100 mL a 1.0 x 10^{-6} M of Manganese ion solution. The sample vials were then microwave irradiated in a Panasonic Inverter Microwave (model # NN-S543BF) at power 130 W for 1, 2, 3, 4, and 5 minutes. Results were compared with control samples, which were not microwave irradiated. The CFUs into an aliquot of 50µL of the test solutions were calculated by the same method as for the control samples in distilled water.

RESULTS AND DISCUSSION

Effect of Manganese Ion on microwave deactivation of E. faecalis, S. aureus and E. coli

Experimental results for microwave irradiation of *E. faecalis, S. aureus,* and *E. coli* in the presence of manganese ions (triangle points, dotted lines) were summarized in RESULTS AND DISCUSSION Table 1 and Figs. 1 - 3.

The results showed the existence of two distinct processes: increasing of number of survival bacteria with time of microwave irradiation on the first stage of heating, passing through maximum value of survival bacteria with following decreasing of number of survival bacteria with time of microwave irradiation.

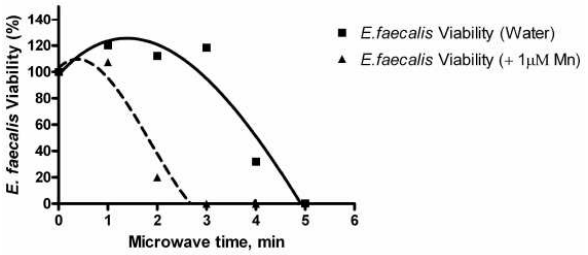

Figure 1. Effect of Manganese Ions on Microwave Disinfection of *E. faecalis.*

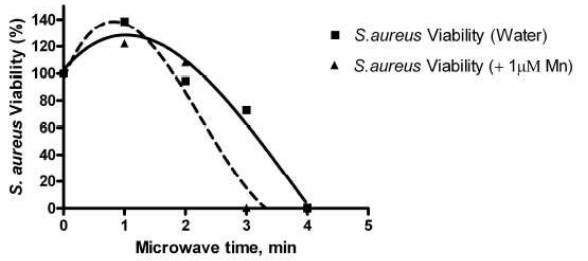

Figure 2. Effect of Manganese Ions on Microwave Disinfection of *S. aureus*

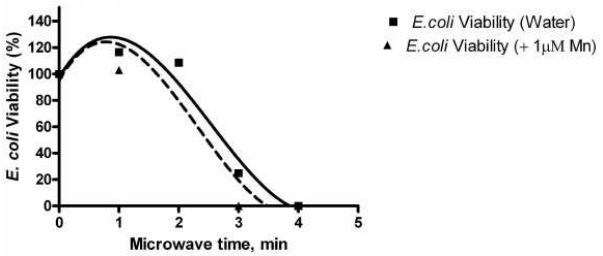

Figure 3. Effect of Manganese Ions on Microwave Disinfection of *E. coli*

Table 1. Bacteria survival of *E. faecalis*, *S. aureus*, *E. coli* at various times using microwave heating in the presence of Manganese ions

Bacteria	Time (min)	Bacteria survival, (- metal ions %)	Bacteria survival, % (+ metal ions %)	Difference, %
E. faecalis	0	100	100	0
	1	120.3	107.4	12.9
	2	112.0	20.1	91.9
	3	118.6	0	118.6
	4	32.0	0	32.0
S. aureus	0	100	100	0
	1	138.7	122.2	16.5
	2	94.3	108.4	-14.1
	3	73.2	0.6	72.6
	4	0.6	0	0.6
E. coli	0	100	100	0
	1	116.7	103.0	13.7
	2	108.7	109.0	-0.3
	3	24.9	0	24.9
	4	0.004	0	0.004

We can take into account two processes as described before in the paper [4]: "growth factor" and "death factor" processes. For *E. faecalis* the "growth factor" was found at interval until the first minute of microwave heating showing a 7.4% increase in bacterial viability. After passing the maximum value *E. faecalis* viability decreased. The "death factor" that occurs between the first and third minutes leads to 107.4% of bacterial viability reduction. The largest rate of bacterial viability reduction was found between the first and second minutes of microwave irradiation (87.3% per minute). *S. aureus* showed the "growth factor" at interval until the first minute of microwave irradiation (a 22.2% of bacterial viability growth). After passing the maximum value *S. aureus* viability decreased but slower in compare with *E. faecalis*. Between the first and second minute of microwave irradiation *S. aureus* bacterial viability decreased by 13.4%. The largest reduction of *S. aureus* bacterial survival was found between the second and third minutes of microwave irradiation where there was a 107.8% of bacterial viability reduction.

For *E. coli* the "growth factor" was found within the first two minutes of microwave irradiation where bacterial viability was increased by 9%. The "death factor" is responsible for the following reduction of 109.0% of survival bacteria into the interval from two to three minutes of microwave irradiation. For comparison, in Table 1 and Figs. 1 – 3 we also show data from our companion paper [4] for microwave treatment of the same bacteria into water without the presence of manganese ions (square points, solid lines).

Results show that dependencies between bacterial survival and time of microwave irradiation have similar “bell” shape form for both experiments with and without manganese ions, however, the numbers of survival bacteria at the same times of microwave heating usually were smaller for experiments with manganese ions into water as compared with experiments conducted with distilled water. Also, the graphs for time dependencies were shifted to lower values for microwave treatment for the manganese enriched groups relative to the water control group. After initial increasing of the number of viable bacteria, dependencies passed through maximum value at time 1 –2 min of microwave irradiation with following reduction to insignificant level at 3 min. In the experiments without metal ions into water viability of bacteria depends on bacterial forms had significant value until 4 – 5 min of microwave treatment. The faster reduction of bacterial survival during microwave treatment in the presence of metal ions can be explained by increasing of kinetic energy of metal ions with increasing temperature of the water due to transformation of vibrational energy from microwave to heat energy of the water. Metal ions with higher energy have larger possibility to enter into bacterial cell. On first stage of penetration into cell, essential metal ions such as manganese can improve stability of bacteria. But with following increasing kinetic energy of ions they damage cell membranes of bacteria, disrupt cellular functions, and destroy the structure of DNA [7].

Mathematical Treatment of Microwave Deactivation of E. faecalis, S. aureus and E. coli with and without 1μM Manganese Ion

For preparation of mathematical model for bacterial deactivation in the presence of metal ions, we have used the assumptions of existence of two processes. Growth of bacteria by improving conditions of their reproduction on initial stage of microwave heating, and following reduction of survival bacteria by increasing of “death” factor with increasing the time of microwave irradiation.

In our previous work [4] we found that rate of change dN/dT of survival bacteria with water temperature, T, is described by formula

$$dN/dT = \beta - \delta \qquad (1)$$

where β and δ - growth and decay of bacterial number with changing water temperature on 1 C related with “birth factor” and “death factor”, respectively. β and δ depend on temperature, and in the first approximation, we can use linear functions

$$\beta = a_0 T + b_0 \text{ and } \delta = a_1 T + b_1 \qquad (2)$$

where a_0, b_0, a_1, b_1 - coefficients that depend on forms of bacteria.

If water contains also metal ions with concentration, M, we have to change the formula (1) and include the term that gives influence of metal ions on bacterial death. After this transformation formula (1) will be

$$dN/dT = \beta - \delta_1 - \delta_2 \qquad (3)$$

where, $\delta_1 = a_1T + b_1$ - decay of bacteria number that relates with changing water temperature on 1 C, $\delta_2 (M)$ - decay of bacteria number that relates with changing metal ions concentration. In the first approximation, we can also use linear function

$$\delta_2 = a_2M + b_2 \qquad (4)$$

If we put formulas (2) and (4) into expression (3) we will receive

$$dN/dT = (a_0 - a_1)\,T - a_2M + b_0 - b_1 - b_2 \qquad (5)$$

If metal ions concentration is permanent than we can simplify the expression (5)

$$dN/dT = AT + B \qquad (6)$$

where $A = (a_0 - a_1)$ and $B = - a_2M + b_0 - b_1 - b_2$ are coefficients that depend on bacterial forms, type and concentration of metal ions.

If we take integral from (6) we will receive

$$N(T) = A_1T^2 + B_1T + C_1 \qquad (7)$$

where A_1, B_1, C_1 - coefficients that depend on bacterial forms, type and concentration of metal ions.

Also, we can use relationship between microwave time, t , and temperature, T, of water [4] to find dependence between number, N, of survival bacteria and microwave time t

$$N(t) = A_2t^2 + B_2t + C_2 \qquad (8)$$

where A_2, B_2, C_2 - coefficients that depend on bacterial forms, type and concentration of metal ions.

Therefore, we can expect that relationship between number, N , of survival bacteria and microwave time, t ,will be given by second order polynomial function (8).

In accordance with this theory, we carry out approximation of experimental results with polynomial function (8). Figs. 1 – 3 shows the results of this approximation (dotted curves). For comparison with previous results, we also gave theoretical curves for water without metal ions (solid curves). The values of the coefficients A_2, B_2, C_2 that gave the best correlation with experimental results were calculated by using the least squares method and placed into Table 2.

Table 2. Coefficients of mathematical model (equation 8)

Bacteria	A_2	B_2	C_2	Coefficient of correlation
E. faecalis	-6.875	-18.11	108.1	0.92
S. aureus	-32.65	66.57	97.1	0.99
E. coli	-28.00	54.6	94.1	0.95

Comparison of mathematical model with experimental results shows good correlation (coefficient of correlation 0.92 - 0.99) that confirm the assumptions accepted in this model.

Existence of mathematical model for microwave heating of water with metal ions allows the determination of bacterial survival levels at predetermined times.

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REFERENCES

1. Abad, F.X., Pinto R.M., Diez, J.M., and Bosch, A., Disinfection of human enteric viruses in water by copper and silver in combination with low levels of chlorine. Appl. Environ. Microbiol., 1994, **60**: 2377- 2383.
2. Alkaysi, H.N., Abdel-Hay, M.H., Sheikh Salem, M., Gharabeh, A.M., and Nawas, T.E., Chemical and microbiological investigations of metel ion interaction with norfloxacin, Int. J. Pharm., 1992, **87:** 73-77
3. Bartram J., Osseiran N., and Schlein L., Water for Health, Taking Charge, World Health Organization Publishing, Geneva, 2001, pp. 5-7.
4. Benjamin E.; Reznik A.; Benjamin E.; Williams A.L., Mathematical Models for Conventional and Microwave Thermal Deactivation of *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli*, J. Cell. Mol. Biol.(in press)
5. Beveridge, T.J., Metal ions and bacteria, John Wiley & Sons, New York, 1989,
6. Blazer, J., and Luthy, R., Comparative study of antagonistic effects of low pH and cation supplementation on in vitro activity of quinolones and amynoglycocides against *Pseudomonas aeruginosa*. J. Antimicrob. Chemother., 1988, **22**: 15-22.
7. Bruins, M.R., Kapil, S., and Oehme, F.W., Microbial resistance to metals in the environment, *Exotoxicol. Environ. Safety*. 2000, **45**: 198-207.
8. Butkus, M.A., Edling, L., and Labare, M.P., The efficacy of silver as bacterial agent: advantages, limitations, and considerations for future use. *J. Water Supply Res. Technol., AQUA*, 2003, **52:** 407-415.
9. Butkus, M.A., Labare, M.P., Starke, J.A., Moon, K., and Talbot, M., Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection. *Appl. Environ. Microbiol.*, 2004, **70**: 2848-2853.
10. Caromiris, J.J., Armstrong, J.L., and Seidler, R.J., Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. *Appl. Environ. Microbiol.*, 1984, **47**: 1238-1242.
11. Chambers, C.W., Proctor, M., and Kabler, P.W., Bacterial effect of low concentration of silver. *J. Am.Water Works Assoc.*, 1962, **54**: 208-216
12. Culkin, K.A., and Fung, D.Y.C., Destruction of *Escherichia coli* and *Salmonella typhimurium* in

microwave-cooked soups. *J. Milk Food Technol.*, 1975, **38**: 8-15.
13. Fujikawa, H., Ushioda, H., and Kudo, Y., Kinetics of *Escherichia coli* destruction by microwave irradiation. *Appl. Environ. Microbiol.*, 1992, **58**: 920-924.
14. Glass, K.A., Loeffelholz, J.M., Ford, J.P., and Doyle, M.P., Fate of *E. coli* 0157:H7 as affected by pH or sodium chloride and in fermented dry sausage. *Appl. Environ. Microbiol.*, 1992, **58**: 2513-2516.
15. Goldblith, S.A., and Wang, D.I.C., Effect of microwaves on *Escherichia coli* and *Bacillus subtilis*. *Appl. Microbiol*. 1967, **15**: 1371-1375.
16. Gudmundsson, A., Erlendsdottir, H., Gottfredsson, M., and Gudmundsson, S., Impact of pH and cationic supplementation on in vitro postantibiotic effect. *Antimicrob. Agents Chemother*., 1991, **35**: 2617-2624
17. Kozempel, M.F., Annous, B.A., Cook, R.D., Scullen, O.J., and Whiting, R.C., Inactivation of microorganisms with microwaves at reduced temperatures. *J. Food Protection.* 1998, **61**: 582-585.
18. Landeen, L.K., Yahya, M.T., and Gerba, C.P., Efficacy of copper and silver ions and reduced levels of free chlorine in inactivation of *Legionella pneumophilla*. *Appl. Environ. Microbiol.*, 1989, **55**: 3045-3050.
19. Lechowich, R.V., Beuchat, L.R., Fox, K.I., and Webster, F.H., Procedure for Evaluating the effects of 2,450- megahertz microwaves upon *Streptococcus faecalis* and *Saccharomyces cerevisiae*. *Appl. Microbiol.*, 1969, **17**: 106-110.
20. Marques, A.M., Congregado, F., and Simon-Pijol, D.M., Antibiotic and heavy metal resistance of *Pseudomonas aeruginosa* isolated from soils. *J. Appl. Bacteriol.*, 1979, **47**: 347-350.
21. Meargeay, M., Neis, D., Schlegel, H.D., Gerits, J., Charles, P., and van Gijsegem, F., *Alcaligenes eutrophus* CH34 is a facultative chemolithtroph with plasmid-bone resistance to heavy metals. *J. Bacteriol.*, 1985, **162**: 328-334.
22. Myers, C.R., and Nealson, K.H., Bacterial reduction and growth with manganese oxide as the sole electron accepter, *Science*, 1988, **240:** 1319-1321.
23. Nakahara, H., Ishikawa, T., Sarai, Y., and Kondo, I., Distribution of resistance to metals and antibiotics of staphylococcal strains in Japan. *Zentralbl. Bakteriol. Mikrobiol.*, 1977, **237**: 470-476.
24. Nakahara, H., Ishikawa T., Sarai, Y., Kondo, I., Kozulue, H., and Silver, S., Linkage of mercury, cadmium, and arsenate and drug resistance in clinical isolates of *Pseudomonas aeruginosa. Appl. Environ. Microbiol*., 1977, **33**: 975-976.
25. Nealson, K.H., and Saffarini, D., Iron and Manganese in anaerobic respiration: environmental significance, physiology, and regulation. *Ann. Rev. Microbiol.*, 1994, **48**: 311-343.
26. Nies, D.H., Microbial heavy-metal resistance, *Appl. Microbiol. Biotechnol.* 1999, **51**: 730-750.
27. Nies, D.H., and Silver, S., Ion efflux systems involved in bacterial metal resistance. *J. Industrial Microbiol.*, 1995, **14**: 186-199.
28. Oganesov, V.E., Device for water deactivation by using silver ions, Russian Patent N 2131399, 1998.
29. Pelletier, C., Prognon, P., Bourlioux, P., Roles of divalent cations and pH in mechanism of action of nitroxolone against *E. coli* strains. *Antimicrob. Agents. Chemother*., 1995, **39**: 707-713.
30. Perez-Giraldo, C., Hurtado, C., Moran, F.J., and Blanko, M.T., The influence of magnesium on ofloxacin activity against different growth phases of *E. coli*. *J. Antimicrob. Chemother.*, 1990, **25**: 1021-1026.
31. Radab-Depre, N.J., Water disinfection withhydrogen peroxide-ascorbic acid-copper(II) system. *Appl. Environ. Microbiol.*, 1982, **44:** 555-560
32. Shin, J.K., and Pyun, Y.R., Inactivation of *Lactobacillus plantarum* by pulsed-microwave irradiation. *J. Food Science*. 1997, **62**: 163-166.
33. Short, B.R.D., Vargas, M.A., Thomas, J.C., O'Hanlon, S., and Enright, M., In vitro activity of a novel compound the metal ion chelating agent AQ^{+}, against clinical isolates of *Staphylococcus aureus*. *J. Antimicrob*. *Chemotherapy*, 2006, **57**: 104- 109.
34. Thurman, R.B., and Gerba, C.P., The molecular mechanism of copper and silver ion disinfection of bacteria and viruses. *Crit. Rev. Environ. Control*, 1989, **18**: 295-315.
35. Vaid A., and Bishop, A.H., The destruction by microwave radiation of bacterial endospores and amplification of released DNA. *J. Appl. Microbiology*, 1998, **85**: 115-122.
36. Vela, G.R., and Wu, J.F., Mechanism of lethal action of 2,450-MHz radiation on microorganisms. *Appl. Environ. Microbiol.*, 1979, **37**: 550-553
37. Yahya, M.T., Straub, T.M., and Gerba, C.P., Inactivation of coliphage MS-2 and poliovirus by copper, silver and chlorine. *Can. J. Microbiol*, 1992, **38**: 480-485.
38. Yahya, M.T., Straub, T.M., Gerba, C.P., and Margolin, A.B., Inactivation of coliphage MS-2 and poliovirus in copper, galvanized and plastic domestic water pipes. *Int. J. Environ. Health Res.*, 1991, **1**: 76-86.