Mathematical Models of Cobalt and Iron Ions Catalyzed Microwave Bacterial Deactivation

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Abstract: Time differences for *Enterococcus faecalis*, *Staphylococcus aureus,* and *Escherichia coli* survival during microwave irradiation (power 130 W) in the presence of aqueous cobalt and iron ions were investigated. Measured dependencies had "bell" shape forms with maximum bacterial viability between 1 - 2 min becoming insignificant at 3 minutes. The deactivation time for *E. faecalis*, *S. aureus* and *E.coli* in the presence of metal ions were smaller compared to a water control (4 -5 min). Although various sensitivities to the metal ions were observed, *S. aureus* and *E. coli* and were the most sensitive for cobalt and iron, respectively. The rapid reduction of viable bacteria during microwave treatment in the presence of metal ions could be explained by increased metal ion penetration into bacteria. Additionally, microwave irradiation may have increased the kinetic energy of the metal ions resulting in lower survival rates. The proposed mathematical model for microwave heating took into account the "growth" and "death" factors of the bacteria, forming second degree polynomial functions. Good relationships were found between the proposed mathematical models and the experimental data for bacterial deactivation (coefficient of correlation 0.91 - 0.99).

Keywords: Bacteria, iron, cobalt, theoretical model, microwave

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Introduction

Effective water disinfection from fecal coliforms is a problem whose solution, leads to the improvement of environmental health conditions. A resolution to this problem can reduce the levels of morbidity and mortality worldwide [1]. Presently, water disinfection of fecal coliforms commonly uses chemical and conventional radiant heating methods. Chemical methods use disinfecting compounds, such as hypochlorite, to disrupt normal cellular function [2] . Bacterial deactivation by conventional radiant heating is achieved through the thermal decay of biomolecules including proteins and DNA [3]. Both of these methods maintain fundamental disadvantages including harmful side effects and extensive disinfecting time.

To address this problem, the identification of rapid disinfection methods such as microwave heating was explored. Microwave heating is the use of microwave radiation (2.45GHz) to induce collisional deactivation resulting in thermal heating [4]. This process maintains several advantages over conventional radiant heating for bacterial deactivation including rapid heating rates and reduced energy requirements [5]. Additionally, microwave heating has been found to heat more uniformly compared to conventional radiant heating thereby increasing energy efficiency [6].

Bacterial deactivation using microwave irradiation showed both thermal and non-thermal effects. Goldblith and Wang [7] compared the deactivation of *Escherichia coli* and *Bacillus subtilis* spores using microwave irradiation versus conventional radiant heating. They

suggested that the deactivation of these bacteria resulted from the dielectric heating and did not maintain any secondary effect from the microwave irradiation. Jeng et al. [8] found no difference between conventional radiant and microwave heating for the disinfection of *B. subtilis* spores in automated temperature monitoring systems. Kazbekov and Vyacheslavov [9] found a series of biochemical processes that were altered in *E. coli* and *B. subtilis* under microwave irradiation which included thymidine and thymine uptake, leakage of potassium and hydrogen ions, and uptake of DNA. Fujikawa et al. [10] found no major differences in deactivation rates of *E. coli* in phosphate buffer between microwave and conventional radiant heating. Welt et al. [11] also found no difference between conventional and microwave deactivation of *Clostridium sporogenes* at 90°C, 100°C, and 110°C. Lechowich et al. [12], Vela et al. [13], Rosén [14] also found that the deactivation of several bacteria, including *Streptococcus faecalis* (later classified as *Enterococcus faecalis*) and *Saccharomyces cerevisiae*, under microwave irradiation resulted from the dielectric heating.

Conversely, Shin and Pyun [15] compared the results of deactivation of *Lactobacillus plantarum* by conventional heating at 50°C versus continuous microwave heating and pulsed higher power microwave heating over a 30 minute period. 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 assisted the deactivation of these bacteria during microwave irradiation. Culkin and Fung [16] and Kozempel et al. [17] also suggest that a secondary non-thermal mechanism assisted in the deactivation of certain species of bacteria. Heddleson and Doores reported that non-thermal effects are likely to be due to the lack of precise measurements of the time-temperature history and its spatial variations [18].

In our recent paper dependencies between bacterial viability of *Enterococcus faecalis*, *Staphylococcus aureus*, and *Escherichia coli* versus microwave heating time and conventional radiant temperature, were found to have "bell" shape forms [19]. The proposed mathematical models for conventional and microwave heating took into account "growth" and "death" factors of bacteria whose, relationships formed second degree polynomial functions. These models were found to be consistent with experimental data maintaining correlation coefficients between 0.84 - 0.99.

Watanabe et al. showed that ionic strength was able to influence the bacterial survival during microwave irradiation [20]. They showed that 10-fold increase in *E. coli* deactivation rates in the presence of molar concentrations of NaCl and KCl. Transitional metals ions play an important role in the life processes of bacteria [21]. Several transitional metals, such as calcium, cobalt, copper, chromium, potassium, iron, magnesium, nickel, zinc, manganese, sodium, are essential for normal biochemical processes [22,23]. In small concentrations they serve as micronutrients and are used for redox-

processes, stabilize molecules through electrostatic interactions, are components of various enzymes, and regulate osmotic pressure [21]. However, other transitional metals such as silver, aluminum, cadmium, gold, mercury, lead, have no biological role and are potentially toxic to bacteria [24]. Toxicity of transitional metal ions depends on their concentration, form, and other factors, including water temperature [25, 26, 27].

Most scientific investigations with transitional metal ions were conducted with silver and copper ions in combination with other compounds [28, 29, 30]. Butkus et al. and Chambers et. al. reported that the addition of silver ions was effective against coliforms [31, 32]. Abad et al. [33] found that the existence of copper and silver ions in combination with low levels of chlorine leads to increased reduction of poliovirus. Landeen et al. showed the efficiency of copper and silver ions in combination with low level of chlorine for deactivation of *Legionella pneumophilla* [34]. Short et al. [35] established that copper ions reduced the number of *Staphylococcus aures*. Ragab-Depre [36] found that addition of a hydrogen peroxide-ascorbic acid-Cu(II) (60 min) to secondary effluents resulted in 99% reduction of *Enterobacteriaceal*, total and fecal coliforms and *Staphylococci*. Oganesov [37] proposed a mechanism for the deactivation of bacteria in water using silver ions. Myers and Nealson [38] studied the influence of manganese oxide on bacterial reduction and growth. Nealson and Saffarini [39] used iron and manganese for anaerobic respiration. Calomiris et al. [40] studied the effects of different metal ions such as 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 *Escherichia coli* in water. *Staphylococcus aureus* was sensitive to all of the metal ions mentioned above except the ion Al^{3+}. Nakahara et al. [41, 42] described the resistance of *staphylococcal* strains to several metal ions and antibiotics.

Unfortunately, the process of bacterial deactivation using only transitional metal ions takes several hours. On the other hand, the presence of metal ions can accelerate bacterial deactivation when coupled with other methods. For example, Butkus et al. [32] found that the addition of silver ions enhanced deactivation of coliphage MS-2 by UV disinfection. It is proposed that the presence of metal ions may increase the deactivation rate of bacteria during microwave treatment, thereby, reducing the time of treatment. In this work the effect of iron and cobalt ions in water on microwave deactivation of *E. faecalis, S. aureus* and *E. coli* was investigated.

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 a 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 heated in a Panasonic Inverter Microwave (model #NN-S543BF) at power 130W 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 the Presence of 1μM Cobalt and Iron Ions

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 Iron or Cobalt ion solution. The sample vials were then microwave heated 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 of a 50μL aliquot of the test solutions were calculated using the same method previously described for distilled water samples.

Results

Effect of Existence of Cobalt Ions in Water 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 cobalt ions in water are summarized into Table 1 and Figs. 1 – 3 (square points). The results showed the existence of two distinct processes: increasing of number of viable bacteria with microwave time of during the first stage of heating, and passing through maximum value of bacterial viability then decreases in relationship to microwave time.

We can take into account two processes as previously described [19]: "growth factor" and "death factor" processes. For *E. faecalis* the "growth factor" was found in the first minute of microwave heating showing a 2.4% increase in bacterial viability. Above the optimal microwave time, *E. faecalis* viability decreased. The "death factor" occurred between the first and third minutes showing 102.4% decrease

in bacterial viability. The greatest drop in bacterial viability reduction (96.6%) was found between the second and third minutes of microwave heating.

Table 1: Bacterial survival of E. faecalis, S. aureus, and E. coli at various times using microwave heating in the presence and absence of cobalt ions.

Bacteria	Time (min)	Bacteria survival without cobalt ions, %	Bacteria survival with cobalt ions, %	Difference, %
E. faecalis	Control sample	100	100	0
	1	120.3	102.4	17.9
	2	112.0	100.4	11.6
	3	118.6	3.8	114.8
	4	32.0	0	32.0
S. aureus	Control sample	100	100	0
	1	138.7	54.2	84.5
	2	94.3	65.1	29.2
	3	73.2	0	73.2
	4	0.6	0	0.6
E. coli	Control sample	100	100	0
	1	116.7	125.0	-8.3
	2	108.7	116.0	-7.3
	3	24.9	0	24.9
	4	0.004	0	0.004

Table 1 shows the bacterial viabilities of *E. faecalis, S. aureus,* and *E. coli* (n=10) when microwaved for 4 minutes in the presence of cobalt ions compared to control samples (distilled water). The results showed notably lower bacterial viability in the presence of cobalt versus that of the control samples. Maximum differences were found between the first and third minutes of microwave heating.

Figure 1: *Effect of cobalt ions on microwave disinfection of E. faecalis.* Shows the effect that cobalt ions (solid line) have on the deactivation of *E. faecalis* (n = 10) during microwave heating compared to a distilled water control (dotted line). Cobalt ions reduced the time necessary for microwave disinfection of *E. faecalis.*

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Figure 2: *Effect of cobalt ions on microwave disinfection of E. coli.* Illustrates the effect that cobalt ions (solid line) have on the deactivation of *E. coli* (n = 10) during microwave heating compared to a distilled water control (dotted line). Cobalt ions had negligible effects on the microwave disinfection of *E. coli*.

Figure 3: *Effect of cobalt ions on microwave disinfection of S. aureus.* Demonstrates the effect that cobalt ions (solid line) have on the deactivation of *S. aureus* (n=10) during microwave heating compared to a distilled water control (dotted line). Cobalt ions appeared to enhance microwave radiation's disinfection ability for *S. aureus*.

E. coli also had a "growth factor" in the first minute of microwave irradiation (25.0% increase bacterial viability). After passing the maximum value, *E. coli* viability decreased. The largest drop in *E. coli* viability (116%) was found between the second and third minutes of microwave irradiation.

In contrast with other bacteria, *S. aureus* showed no major change in bacterial viability in the first minute of the microwave heating. After this initial minute, *S. aureus* viability then slowly decreased in the second minute with a 34.9% reduction. The third minute showed a 65.1% decrease in *S. aureus* viability. We can explain this fact by assuming that the "death factor" is influenced by the presence of cobalt ions which had a powerful effect on *S. aureus* compared to other test organism.

For comparison, in Table 1 and Figs. 1 – 3 we also show data from our previous paper [19] for microwave treatment for *E. faecalis*, *S. aureus* and *E. coli* in distilled water (triangular points). This data illustrates that the disinfection s in the presence of cobalt ions (3 min) is less than that of the distilled water control (4 - 5 min).

Therefore, the presence of cobalt ions into water increased the efficiency of the microwave deactivation of bacteria.

Effect of Existence of Iron Ions in Water 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 iron ions into water are summarized into Table 2 and Figs. 4 – 6 (square points). The results also showed the existence of both "growth" and "death" factors. In this circumstance, the "death" factor for *E. faecalis* is weaker, and *S. aureus* and *E. coli* are stronger in comparison with the influence of cobalt ions.

For *E. faecalis* the "growth factor" was found in the first minute of microwave heating showing a 4.7% increase in bacterial viability. The "death factor" for *E. faecalis* occurred between the first and fourth minutes leads to 104.7% of bacterial viability reduction. The greatest decline in bacterial viability reduction was found between the second and third minutes of microwave irradiation (63.7%). However, the influence of iron ions on *E. faecalis* was weaker when compared with the influence cobalt ions, and at the third minute results (38.2%).

Table 2: Bacterial survival of *E. faecalis*, *S. aureus*, and *E. coli* at various times using microwave heating in the presence and absence of iron ions.

Bacteria	Time (min)	Bacteria survival without cobalt ions, %	Bacteria survival with cobalt ions, %	Difference, %
	Control sample	100	100	0
E. faecalis	1	120.3	104.7	15.6
	2	112.0	101.9	10.1
	3	118.6	38.2	80.4
	4	32.0	0	32.0
	Control sample	100	100	0
S. aureus	1	138.7	98.8	39.9
	2	94.3	96.3	-2.0
	3	73.2	2.3	70.9
	4	0.6	0	0.6
	Control sample	100	100	0
E. coli	1	116.7	0.3	116.4
	2	108.7	0	108.7
	3	24.9	0	24.9
	4	0.004	0	0.004

Table 2 shows the bacterial viabilities of *E. faecalis*, *S. aureus*, and *E. coli* (n = 10) when micro-waved for 4 minutes in the presence of iron ions compared to control samples (distilled water).   The results showed that the presence of iron reduced the disinfection time for all test bacteria. The most significant decrease was *E coli* whose disinfection time reduced from four minutes to one minute.

Figure 4: *Effect of iron ions on microwave disinfection of E. faecalis.* Illustrates the effect that iron ions (solid line) have on the deactivation of *E. faecalis* (n = 10) during microwave heating compared to a distilled water control (dotted line).   Iron ions had a trivial effect on microwave disinfection of *E. faecalis.*

Figure 5: *Effect of iron ions on microwave disinfection of S. aureus.* Illustrates the effect that iron ions (solid line) have on the deactivation of *S. aureus* (n=10) during microwave heating compared to a distilled water control (dotted line). Similar to *E. faecalis*, iron ions had a negligible effect on microwave disinfection of *S. aureus.*

The number of viable *S. aureus* was constant during the first two minutes of microwave treatment with a rapid decrease (94%) during the third minute. *E. coli* viability showed a sharp decline during microwave heating in the presence of iron ions. During the first minute of microwave treatment bacterial viability for *E. coli* was reduced by 99.7%. Contrary to other data, *E. coli* disinfection denotes the strong influence of iron ions on micro-organisms.

Figure 6: *Effect of iron ions on microwave disinfection of E. coli.* Illustrates the effect that iron ions (solid line) have on the deactivation of *E. coli* (n = 10) during microwave heating compared to a distilled water control (dotted line). Unlike results from *E. faecalis* and *S. aureus*, iron ions reduced the disinfection time from four minutes for the distilled water control (dotted line) to under one minute for the iron enhanced group (solid line).

For comparison, Table 2 and Figs. 4 – 6, data for microwave treatment of *E. faecalis*, *S. aureus* and *E. coli* in distilled water (triangular points) was included from our previous paper [19]. This data showed that disinfection time in the presence of iron ions (1-3 min) is less than in distilled water (4 - 5 min). So, the presence of iron ions in water increased the efficiency of the microwave deactivation of bacteria.

Discussion

Results show that differences between bacterial survival and microwave time have similar "bell" shape forms for both with and excluding metal ions in most circumstances. Lower survival rates were found for *E. faecalis*, *S. aureus* and *E. coli* in the presence of metal ions under microwave heating when compared to their distilled water controls.   The results suggest that metal ion's ability to enhance bacterial deactivation primarily occurs above temperatures needed for optimal growth. Microwave disinfection times for all three bacteria were also lowered in the presence of metal ions such as cobalt and iron. This shift found a reduction of bacterial viability becoming insignificantly small at the third minute of microwave heating.   Experimental controls of distilled water found bacterial viability requiring 4 – 5 min of microwave irradiation for significant reduction. The rapid reduction of viable bacteria during microwave irradiation in the presence of aqueous metal ions indicates an increase of kinetic energy of the solution. Metal ions with higher kinetic energy have a greater possibility to enter into the bacteria. Initially, the addition of metal ions is seen as a source of nutrition. As the kinetic energy of the metal ion increases, there is an increase in ion penetration. Following metal ion penetration, they begin to damage the cell membranes of bacteria, disrupt cellular functions, and destroy the structure of DNA [24].

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For preparation of mathematical model for bacterial deactivation in the presence of metal ions, we infer 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 [19] we found that rate of change (dN/dT) of bacterial survival with water temperature (T) is described by formula:

$$\frac{dN}{dT} = \beta - \delta \qquad (1)$$

where, β and δ - growth and decay of viable bacteria 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 :

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

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

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

$$\frac{dN}{dT} = \beta - \delta_1 - \delta_2 \qquad (3)$$

where, $\delta_1 = a_1 T + b_1$ - 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. If metal ions concentration is permanent, we can decide that $\delta_2(M)$ is constant. Then after taking integral from (3) we will receive:

$$N(T) = A_1 T^2 + B_1 T + C_1 \qquad (4)$$

where A_1, B_1, C_1 - coefficients that depend on bacteria forms, type and concentration of metal ions. Also, we can use relationship between microwave time t and temperature T of water [19], and find dependence between number N of viable bacteria and microwave time t:

$$N(t) = A_2 t^2 + B_2 t + C_2 \qquad (5)$$

where A_2, B_2, C_2 - coefficients that depend on bacteria forms, type, and concentration of metal ions. Therefore, we can expect that relationship between number N of viable bacteria and microwave time t will be given by second order polynomial function (5).

In accordance with this theory, we carry out approximation of experimental results with polynomial function (5). Figs. 1 – 6 show the results of this approximation (solid curves). For comparison with previous results, we also provided theoretical curves for water without metal ions (dotted 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 3.

Table 3: Coefficients of mathematical models (equation 5)

Metal Ion	Bacteria	A_2	B_2	C_2	Coefficient of correlation
Cobalt	*E. faecalis*	-24.75	45.19	95.5	.97
	S. aureus	-4.825	-14.44	93.4	.91
	E. coli	-35.25	74.85	96.4	.98
Iron	*E. faecalis*	-10.42	15.12	101.3	.97
	S. aureus	-23.20	40.4	95.5	.97
	E. coli	0	-99.7	100	.99

Table 3 shows the coefficients for the theoretical mathematical models for *E. faecalis*, *S.aureus*, and *E. coli*. The mathematical models show between a 0.99 and a 0.91 correlation coefficient when compared to experimental data.

Comparison of mathematical model with experimental results shows good correlation (coefficient of correlation 0.91 - 0.99) for most results that confirm the assumptions accepted in this model. An exception is the influence of iron ions on microwave bacteria deactivation. For this influence, we recommend to use the linear model. Existence of mathematical model for microwave heating of water with metal ions allows for the determination of viable bacterial levels at predetermined times.

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