Mathematical models for *Enterococcus faecalis* recovery after microwave water disinfection

Earl Benjamin III, Aron Reznik, Ellis Benjamin, Saroj K. Pramanik, Louise Sowers and Arthur L. Williams

ABSTRACT

Microwave water disinfection is a rapid purification technique which can give billions of people access to clean drinking water. However, better understanding of bacterial recovery after microwave heating over time is necessary to determine parameters such as delayed bacterial growth rates and maximum bacterial yields. Mathematical models for *Enterococcus faecalis* recovery after microwave treatment in optimum growth conditions were developed for times up to 5 minutes using an optical absorbance method. Microwave times below 3 minutes (2,450 MHz, 130 W) showed that bacterial recovery maintained a time-dependent sigmoidal form which included a maximum value. At microwave times greater than three minutes, bacterial recovery, with a time-dependent exponential form, significantly decreased and did not reach the maximum value within the interval of observance (0–8 hours). No bacterial growth was found after 6 minutes of microwave treatment. The prepared mathematical models were produced by transforming the given variables to the logistic or exponential functions. We found that time-dependent maximum growth rates and lag times could be approximated with second order polynomial functions. The determined models can be used as a template to illustrate bacterial survival during water purification using microwave irradiation, in both commercial and industrial processes.

Key words | bacteria, *Enterococcus faecalis*, exponential function, logistic function, mathematical model, microwave treatment

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INTRODUCTION

Recent estimates suggest that more than 1.1 billion people do not have access to save drinking water (Bartram *et al.* 2001). Additionally, there are approximately 30 million surgical procedures in the United States which require sterile water for medical device cleaning (Mangram *et al.* 1999), where surgical infection is the most significant risk. Effective water disinfection is a problem whose solution results in the improvement of overall public health (Byamukama *et al.* 2000). Since the early days of water

disinfection, the presence of pathogenic bacteria in drinking water has been associated with fecal coliforms, especially Gram-positive *Enterococcus faecalis* (Cetinkaya *et al.* 2000; Malani *et al.* 2002; Tandon *et al.* 2007). Generally considered as a beneficial organism, *E. faecalis* has emerged as one of the major nosocomial pathogens (Hancock & Gilmore 2000).

Presently, water disinfection of fecal coliforms commonly uses chemical and/or conventional radiant

heating methods. Chemical methods use disinfecting compounds, such as hypochlorite, which disrupt normal cellular function (Fukuzaki 2006). Bacterial deactivation by conventional radiant heating is achieved through the thermal decay of biomolecules including proteins and DNA (Yura *et al.* 1993). Both of these methods maintain fundamental disadvantages including extensive disinfecting time and harmful side effects such as bioaccumulation. To address this problem, the identification of rapid methods such as microwave heating has been explored. Microwave heating is the use of microwave radiation to induce collisional deactivation resulting in thermal heating (Larhed *et al.* 2002). This process maintains several advantages over conventional radiant heating for bacterial deactivation including rapid heating rates and reduced energy requirement (Lidstrom *et al.* 2001). Additionally, microwave heating has been found to heat more uniformly compared with conventional radiant heating, thereby increasing energy efficiency (Datta & Hu 1992). These reasons led to the application of microwave radiation for the deactivation of *E. faecalis* (Lechowich *et al.* 1969; Sanborn *et al.* 1982; Benjamin *et al.* 2007).

Most microwave disinfection studies were singly devoted to investigating bacterial deactivation with very few studies that determined bacterial growth after microwave treatment. The problem with these studies is that, without an understanding of bacterial recovery after water disinfection, prolonged storage of water supplies runs the risk of recontamination. The exposure of bacteria to microwave irradiation has resulted either in no observable effects on bacterial growth rate (Hamrick & Butler 1973) or, depending on the frequency and intensity of the microwave irradiation, either an increase or decrease in the growth rate (Flemming 1944; Webb & Dodds 1968; Webb & Booth 1969). Moore *et al.* (1979) found a 30 to 60% decrease in the ability of *Agrobacterium tumefaciens* to produce tumours on potato and turnip discs after treatment using low-level microwave irradiation with frequency of 10 GHz and an intensity of 0.58 mW cm^{-2} for 30–120 min. Dreyfuss & Chipley (1980) studied the effect of microwave irradiation on thermonuclease activity of *Staphylococcus aureus*. Cultures were exposed to microwave irradiation (at frequency of 2,450 MHz)

for 10, 20, 30 and 40 seconds and their thermo-nuclease activity was checked at an interval of 0–3 hours. Increased levels of activity with time post-radiation were observed when cells were exposed to microwave irradiation for 10 or 20 seconds. The data also indicated that little or no enzymatic activity was present after microwave irradiation for 30 or 40 seconds.

Shin & Pyun (1997) studied growth of *Lactobacillus plantarum* after exposure to continuous microwave irradiation (at frequency 2,450 MHz and power 650 W) in comparison with exposure to conventional heating at 50°C and pulsed microwave irradiation (with 500 pulses per second and maximum power into pulse of 6,000 W) for the same treatment time (30 minutes). They found that conventionally and continuous microwave irradiated cells showed lag periods of 10 and 22 hours, respectively, and reached the same maximum value after a longer lag time as unheated cells. Cells that were pulsed microwave irradiated had a lag time similar to that for continuous microwave irradiated cells but did not reach the same maximum level after 60 hours of incubation. In contrast to Shin & Pyun's (1997) results, Hossain & Dutta (2005) did not find differences between bacterial growth curves for *Escherichia coli* exposed to high-intensity microwave treatment (8.8 GHz pulsed at 1,000 Hz) or conventional heating (40 W kg^{-1}).

As can be seen in many of the papers mentioned above, no kinetic studies of bacterial recovery after microwave treatment were considered. Only Shin & Pyun (1997) estimated lag times for the growth of *L. plantarum* after exposure to continuous microwave irradiation, conventional heating, and pulsed microwave irradiation for similar experimental times. No mathematical models were prepared for bacterial growth cycles, thus, many important parameters such as the maximum bacterial number and bacterial recovery after microwave treatment for different microwave times were not determined (Perni *et al.* 2005). These important parameters can be used to indicate possible disinfection mechanisms and the effectiveness of microwave bacterial deactivation. Our work studied the kinetic growth of *E. faecalis*, a fecal coliform, at different microwave treatment times with modelling of this process to determine specific growth parameters.

MATERIALS AND METHODS

E. faecalis stock cultures

Stock cultures of *E. faecalis* (Ward Natural Scientific, Rochester, New York) were produced by growing in 100 ml of TSB (tryptic soy broth) overnight in a Labline Imperial III Incubator (model #305) at 37°C. Preliminary studies showed that 6–8 hours were required for *E. faecalis* to enter the stationary growth phase at 1.15–1.20 optical density (maximum value of bacterial growth). This time is in agreement with the results of Hancock & Perego (2004).

E. faecalis recovery after microwave disinfection

Aliquots of 1 ml of the stock culture of *E. faecalis* were placed into 24 vials containing 100 ml TSB at 25°C. These vials were divided into six experimental groups ($n = 4$) for microwave treatment at 1-minute intervals for times up to 5 minutes (our previous results (Benjamin *et al.* 2007) show total deactivation of *E. faecalis* after 6 min of microwave treatment). The flasks were heated in a Panasonic Inverter Microwave (model #NN-S543BF) at a power of 130 W for 1, 2, 3, 4 and 5 minutes. Results were compared with control samples, which were not micro-waved. After microwave treatment 1 ml aliquots were cultured in 100 ml TSB at 37°C for 8 hours, with no agitation, and tested for optical density (at 400 nm) at hourly intervals using a Beckman Life Science UV/VIS spectrophotometer to establish a growth curve.

RESULTS AND DISCUSSION

E. faecalis recovery after microwave treatment

Experimental results for *E. faecalis* growth curves are summarized in Table 1 and Figure 1 with untreated samples as experimental controls. For comparison the tables and figures were compared to control samples which did not receive microwave treatment.

Growth curves for recovered *E. faecalis* (Figure 1) showed sigmoidal forms for microwave times between 1 and 5 minutes, reaching a maximum value (1.26 units) after 5 hours of observation. No differences were found for growth curves after 1 or 2 min of microwave treatment in comparison with control samples. This fact can be explained by the insignificant influence of microwave irradiation on bacterial deactivation at these irradiation times. Bacterial growth is unaffected by small increases in temperature during the first stage of microwave treatment (Benjamin *et al.* 2007). After 3 min of microwave treatment the growth curve retains a sigmoidal form but the number of recovered bacteria decreases reaching a maximum (1.09 units) after 7 hours of observation (Figure 1). This observation can be explained by the increasing number of injured bacteria during microwave treatment, leading to a lower level of recovered bacteria, thereby delaying the start of logarithmic growth of the bacteria. Above 4 minutes of microwave treatment the bacterial growth cycle signifi-cantly changes, acquiring an exponential form and does not reach maximum values for the whole observation period.

Table 1 | Optical density at 400 nm (rel. units) for recovered *E. faecalis* for different times of microwave treatment

Time of bacterial recovery (hours)	Optical density (rel. units)					
	Control	1 min	2 min	3 min	4 min	5 min
0	0.071 ± 0.001	0.079 ± 0.003	0.079 ± 0.004	0.04 ± 0.005	0.045 ± 0.003	0.038 ± 0.001
1	0.147 ± 0.002	0.163 ± 0.003	0.164 ± 0.005	0.068 ± 0.003	0.04 ± 0.003	0.032 ± 0.001
2	0.377 ± 0.003	0.435 ± 0.006	0.447 ± 0.009	0.13 ± 0.002	0.036 ± 0.003	0.027 ± 0.001
3	0.871 ± 0.005	0.931 ± 0.004	0.945 ± 0.009	0.381 ± 0.04	0.048 ± 0.007	0.033 ± 0.001
4	1.158 ± 0.003	1.186 ± 0.002	1.186 ± 0.005	0.751 ± 0.05	0.095 ± 0.023	0.041 ± 0.001
5	1.252 ± 0.002	1.253 ± 0.003	1.249 ± 0.002	1.008 ± 0.017	0.198 ± 0.07	0.043 ± 0.004
6	1.251 ± 0.002	1.248 ± 0.004	1.245 ± 0.003	1.076 ± 0.006	0.341 ± 0.119	0.075 ± 0.026
7	1.253 ± 0.002	1.244 ± 0.004	1.246 ± 0.001	1.095 ± 0.004	0.50 ± 0.118	0.081 ± 0.03
8	1.26 ± 0.004	1.259 ± 0.003	1.262 ± 0.001	1.089 ± 0.005	0.719 ± 0.079	0.144 ± 0.068
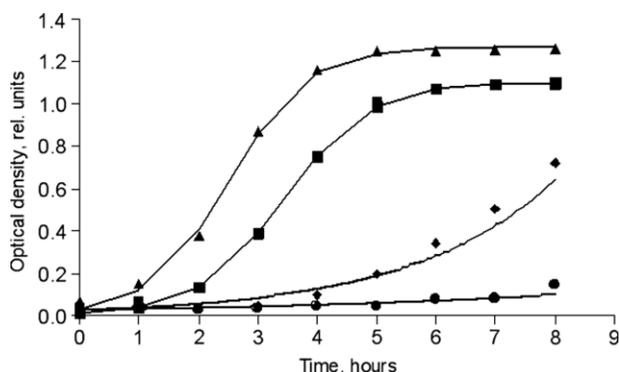
Figure 1 | Effect of microwave treatment on *E. faecalis* reproduction. Experimental points: control sample without microwave treatment (▲), after microwave treatment for 3 min (■), 4 min (◆) and 5 min (●).

The number of recovered bacteria also significantly decreased to 0.72 units (4 minutes) and 0.14 units (5 minutes) after 8 hours of observation. This can also be explained by the increased number of injured bacteria with increased microwave time and temperature.

Mathematical treatment of *E. faecalis* disinfection

When considering the kinetics of bacteria recovery after microwave heating one must first understand bacterial growth patterns. Researchers depict bacteria growth by assuming that growth rates at given times are proportional to the number of bacteria at this time (Chick 1908). This assumption leads to the differential equation:

$$\frac{dN}{dt} = kN \tag{1}$$

where N, t and k are number of bacteria at a specific time, time and growth constant, respectively. But this equation predicts unlimited exponential growth of bacteria with increasing time (t):

$$N(t) = N_0 \exp(kt) \tag{2}$$

where the initial number of bacteria (N_0) at time $t = 0$. This is in contrast with experimental data that showed the limitation of bacterial numbers with increasing time. Bacterial growth usually starts at a value of zero and then accelerates to a maximum growth rate (μ_m) over a certain period of time, resulting in a lag time (λ). In addition, the growth curves contain a final phase in which the rate of growth decreases, finally reaching zero.

For an explanation of this fact we suppose that coefficient (k) depends on 'growth' and 'death' rates of bacteria:

$$k = \beta - \delta \tag{3}$$

where β and δ are 'growth' and 'death' rates, respectively. It is assumed that rate of growth is greater than or equal to death rate ($\beta \geq \delta$).

After substitution of Equation (3) into (1) we obtain:

$$\frac{dN}{dt} = (\beta - \delta)N \tag{4}$$

In the first approximation, we can use linear functions for both rates:

$$\beta = a_0 - a_1 N \quad \text{and} \quad \delta = b_0 - b_1 N \tag{5}$$

where a_0, a_1, b_0 and b_1 are coefficients describing bacterial form, strain and type of heating. By using Equations (5), differential Equation (4) will be:

$$\frac{dN}{dt} = (A - BN)N \tag{6}$$

where $A = a_0 - b_0$, $B = a_1 - b_1$ are coefficients describing bacterial form, strain and type of heating.

Differential Equation (6) is a form of logistic equation (Korn & Korn 1977). Solution of this equation with the initial-value condition $N(0) = N_0$ will be:

$$N(t) = \frac{N_0}{\frac{BN_0}{A} + \left(1 - \frac{BN_0}{A}\right)\exp(-At)} \tag{7}$$

If time (t) approaches infinity ($t \to \infty$) then $N(t) \to N_M$, where N_M is maximum bacterial number. To find this maximum we can take the limit of Equation (7).

$$N_M = \lim_{t \to \infty} N(t) = \frac{A}{B} \tag{8}$$

By using Equation (8), we can rewrite Equation (7):

$$N(t) = \frac{N_M}{1 + \left(\frac{N_M}{N_0} - 1\right)\exp(-At)} \tag{9}$$

or

$$N(t) = \frac{C_1}{1 + C_2 \exp(-At)} \tag{10}$$

where $C_1 = N_M$ and $C_2 = (N_M/N_0 - 1)$ are coefficients.

Therefore, we can expect that the relationship between N (bacterial number) in the sample and t (time) after microwave irradiation will be given by logistic function (10).

Unfortunately, all curves did not reach their maximum value during the observation time (8 hours after microwave treatment). For these curves, we use exponential approximation where:

$$N(t) = C_1 \exp(C_2 t) \tag{11}$$

In accordance with this theory, we carry out an approximation of experimental data with the logistic (Equation (10)) or exponential function (Equation (11)). The values of the coefficients C_1, C_2 and A that gave the best correlation with experimental results are shown in Table 2. Figure 1 shows a comparison of experimental data at different microwave times for *E. faecalis* versus theoretical calculated curves. These results show a good correlation between experimental data and the theoretical curves.

Calculated coefficients for time dependence of bacterial growth help us estimate the maximum growth rate (μ_m) and lag time (λ) for given bacteria and time of microwave treatment. Maximum growth rate (μ_m) is defined as the slope of the tangent line to the growth

curve at the inflection point. Lag time (λ) is defined as the time at the point of intersection of the time axis and this tangent line. By using the method that was reported by Zwietering *et al.* (1990), which was devoted to bacterial growth after conventional heating, we can rewrite Equation (10) as:

$$N(t) = \frac{C_1}{1 + \exp\left[\frac{4\mu_m}{C_1}(\lambda - t) + 2\right]} \tag{12}$$

where $\mu_m = C_1 A/4$ and $\lambda = \ln C_2 - 2/A$.

Calculated values of maximum growth rates (μ_m) and lag times (λ) for given bacteria and microwave time are listed in Table 2. Values of μ_m and λ for data approximated with the exponential function (Equation (11)) were calculated for the maximum time of observation (8 hours). As can be seen in Table 2, increasing microwave time leads to a decrease in maximum growth rates (μ_m): from 0.48 units h^{-1} (without microwave treatment) to 0.03 units h^{-1} (microwave treatment for 5 min). Figure 2 shows the dependence between maximum growth rate (μ_m) and time (t) of microwave treatment. For an explanation of this dependence, we can use the formula of Ratkowsky *et al.* (1982) to substantiate the equation proposed by Zwietering *et al.* (1990) (Equation (12)) that was proposed for conventional heating experiments:

$$\sqrt{\mu_m} = b(T - T_m) \tag{13}$$

where T, T_m and b are water temperature, maximum temperature for bacteria recovery and coefficient that describes bacterial type, respectively. By assuming a

Table 2 │ Calculated coefficients of Equations (10) or (12) and values for the maximum growth rate (μ_m) and lag times (λ)

Time of microwave treatment min	C_1	C_2	A	μ_m	λ
Control	1.2653	43.7333	1.5129	0.48	1.18
1	1.2615	37.4484	1.5397	0.49	1.05
2	1.2603	36.5321	1.5472	0.49	1.03
3	1.1039	116.363	1.3823	0.38	2
4	1.3695	138.391	0.6283	0.21	4.73
5*	0.0124	0.2878		0.03	4.97

*Uses exponential approximation.
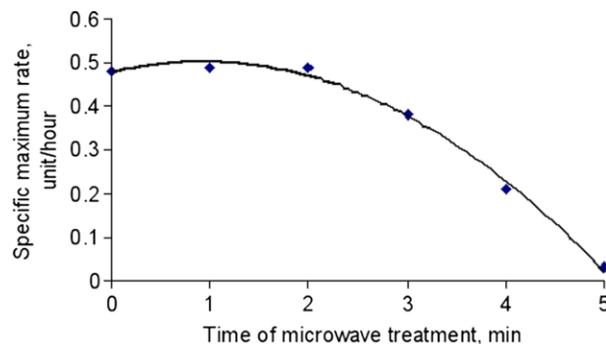
Figure 2 │ Effect of microwave treatment time on specific maximum rate of *E. faecalis* growth.

proportionality between water temperature (T), microwave irradiation and time (t) (Benjamin *et al.* 2007) (Equation (13)) we can rewrite it in the form:

$$\sqrt{\mu_m} = b_1 t + b_0 \tag{14}$$

where b_0 and b_1 are coefficients describing bacterial type and microwave heating. After squaring both sides of Equation (14) we obtain:

$$\mu_m = B_2 t^2 + B_1 t + B_0 \tag{15}$$

where B_0, B_1 and B_2 are coefficients that describe bacterial type of and microwave heating, respectively. Therefore, we can expect that the relationship between maximum growth rate (μ_m) and time (t) of microwave treatment will be given by the second order polynomial function (Equation (15)). In concert with this treatment, we carried out an approximation of experimental relationship between μ_m and t with polynomial function (Equation (15)). Coefficients that give the best approximation are $B_0 = 0.4782$, $B_1 = 0.0541$ and $B_2 = -0.2911$. In Figure 2 for comparison, we show a theoretical curve that was developed from Equation (15) using the values of coefficients listed above. Comparison of the correlation coefficients of the theoretical curve and experimental results is 0.98. We have to note that the proposed formula for the relationship between the maximum growth rate (μ_m) and time (t) of microwave treatment has a limited time interval. It can be used for $0 \leq t \leq t_m$, where t_m is the maximum time value that can be found by taking $\mu_m = 0$ in Equation (15) and solving it to obtain the quadratic equation. In our case, for given coefficients B_0, B_1 and B_2 we can find that $t_m = 5.1$ minutes.

As can be seen from Table 2, if time of microwave treatment is greater than the lag time (λ), then the lag time increases: from 1.18 min (without microwave treatment) to 4.97 min (microwave treatment for 5 min). This tendency can be explained by the increasing number of injured bacteria with increasing time of microwave treatment. In Figure 3 we plot the dependence between lag time (λ) and time (t) of microwave treatment. For an explanation of this dependence, we used the formula of
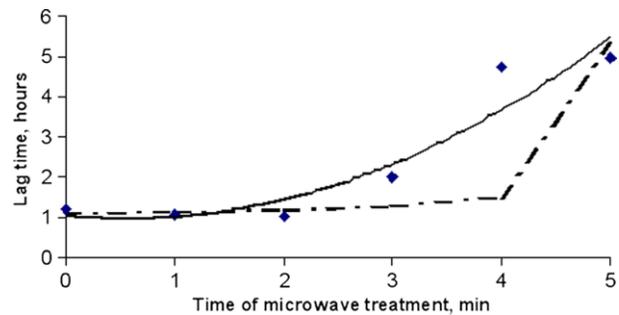
Figure 3 | Effect of microwave treatment time on lag time of *E. faecalis* growth. Broken line: theoretical curve produced using Equation (17); solid line: growth curve developed using Equation (18).

Zwietering *et al.* (1991), which is proposed for conventional heating experiments.

$$\ln \lambda = \frac{p}{T - q} \tag{16}$$

The variables for Equation (16) are water temperature (T), and coefficients describing bacterial type (p and q). By using the assumption of proportionality between water temperature (T) and microwave heating time (t) (Benjamin *et al.* 2007), Equation (16) can be rewritten in the form:

$$\ln \lambda = \frac{P}{t - Q} \tag{17}$$

where P and Q are coefficients describing bacterial type and microwave heating. In accordance with this consideration, we carry out an approximation for the experimental relationship of (λ) and (t) as a polynomial function (Equation (18)). Coefficients that give the best approximation are $P = -1.23$ and $Q = 5.70$. In Figure 3 for comparison, we show a theoretical curve that was produced using Equation (17) using the values of coefficients listed above.

Better correlation with experimental results was found when we approximated this dependence as a second-order polynomial function:

$$\lambda = D_2 t^2 + D_1 t + D_0 \tag{18}$$

where D_0, D_1 and D_2 are coefficients describing bacterial type and microwave heating. Coefficients that give the best approximation are $D_0 = 1.0468$, $D_1 = -0.2628$ and $D_2 = 0.2295$. In Figure 3 we also approximated a growth curve that was developed using Equation (18) using the values of coefficients listed above for comparison.

Table 3 | Calculated coefficients of Equation (10) for data from Shin & Pyun (1997), and for the maximum growth rate (μ_m) and lag times (λ)

Type of treatment	C_1	C_2	A	μ_m	λ
No treatment (control sample)	45.8	29.747	0.278	3.18	5.01
Conventional heating	48.5	881.418	0.239	3.6	20
Continuous microwave treatment	46.9	222,448.671	0.326	3.82	31.63
Pulsed microwave treatment	46	1,184.304	0.151	1.74	33.62

The correlation coefficient between the theoretical curve and experimental results is 0.92.

To examine this model, data from Shin & Pyun (1997) were accurately predicted using our equation. This paper showed recovery of *L. plantarum* after conventional heating, continuous microwave treatment and pulsed microwave treatment (Shin & Pyun 1997). Theoretical models calculated from Equation (10) were used to predict growth curves cited in this paper (Shin & Pyun 1997). The values of the coefficients C_1, C_2 and A that gave the best correlation with experimental results for *L. plantarum* growth are shown in Table 3. In Figure 4 for comparison we plot experimental data from Shin & Pyun (1997) and theoretical curves that were calculated by using Equation (10) with coefficients C_1, C_2 and A from Table 3. We obtained good agreement between theoretical curves and experimental data. Using the method reported above, we were able to calculate the maximum growth rate (μ_m) and
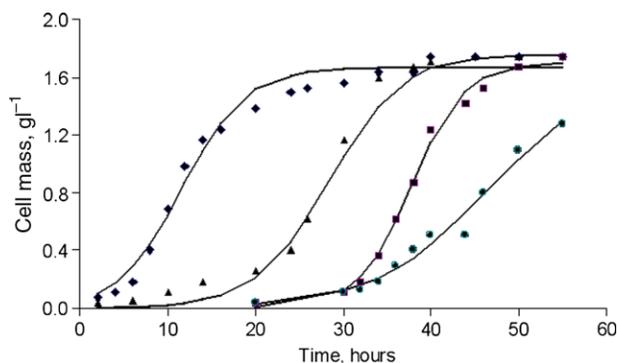
Figure 4 | Comparison of theoretical curves (solid curves) with experimental results (points) from Shin & Pyun (1997) for growth of *Lactobacillus plantarum* without treatment (◆), after conventional heating (▲), after continuous microwave treatment (■) and after pulsed microwave treatment (●).

lag time λ) for bacterial growth after all treatments. Calculated values of maximum growth rate (μ_m) and lag time (λ) for the data from Shin & Pyun (1997) are also given in Table 3. The results show that maximum growth rate (μ_m) for conventional heating and continuous microwave treatment are similar for unheated bacteria *L. plantarum*; however, the pulsed microwave treatment value of μ_m is 50% lower. Calculated lag times (λ) from growth curves of *L. plantarum* showed the smallest value for unheated bacteria and the largest for bacteria after pulsed microwave treatment. This is in agreement with the experimental results of Shin & Pyun (1997). However, quantitative values of lag times calculated in our work are larger compared with Shin & Pyun (1997). We can explain differences in lag times by minor differences in environmental factors including microwave and incubation temperature. These results can be applied for mathematical modelling of bacterial growth after microwave treatment for other bacteria and the prediction of several growth parameters for recovered bacteria.

CONCLUSION

Technological advances have increased microwave output while reducing overall power requirements, making microwave irradiation a viable water disinfection method. The creation of mathematical models for microwave water disinfection and recovery after microwave treatment, which accurately predict bacterial deactivation and growth patterns, will support the application of this method. This study presents mathematical models for bacterial recovery after microwave treatment. These models show good correlation with experimental data conducted as a part of this examination as well as findings reported by Shin & Pyun (1997) for various bacterial strains. Together with mathematical models for microwave bacterial deactivation developed in our previous paper (Benjamin *et al.* 2007) these new models describe the entire bacterial cycle: deactivation during microwave treatment along with bacterial recovery. These models are essential if microwave disinfection is to be used as a water disinfection method in response to needs in underdeveloped and rural locations.

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