Growth of *Escherichia coli* O157:H7 at Fluctuating Incubation Temperatures

ABSTRACT

Temperature abuse of foods is often transitory and little information is available describing the response of the foodborne pathogen, *Escherichia coli* O157:H7, to nonisothermal and/or fluctuating temperature storage. Growth responses were determined for a mixture of three *E. coli* O157:H7 strains in brain heart infusion (BHI) broth as a function of temperature (static and fluctuating), initial pH (5, 6, and 7), and NaCl content (0.5, 1, 2, and 3%). Five 6-h "square-wave" fluctuating temperature regimes were used: 4 to 12, 4 to 19, 4 to 28, 8 to 19, and 12 to 28°C and compared with growth at 8, 10, 12, 19, and 28°C. The growth curves obtained from fitting the Gompertz equation for the fluctuating temperatures were compared to those obtained for the static temperatures. Increased NaCl concentration decreased growth temperature both for the fluctuating temperature growth curves and the static growth data. The cells grew or remained viable for up to 21 days under all conditions and fluctuating temperatures. Growth kinetics at fluctuating temperatures more closely approximated the higher temperature than the midpoint temperature of each cyclic range. The results indicate that transitory abuse could lead to more rapid growth than expected of *E. coli* O157:H7 in foods and that given sufficient time *E. coli* O157:H7 can grow at as low as 8°C.

Key words: *E. coli* O157:H7, growth kinetics, temperature abuse, fluctuating temperature

The bacterium, *Escherichia coli*, is normally considered nonpathogenic and can be isolated routinely from the intestinal tracts of warm- and cold-blooded animals, including humans. However, some *E. coli* strains are pathogenic, with specific strains such as O157:H7 causing severe diarrheal syndromes (*8*). In 1982 *E. coli* O157:H7 was identified as a life-threatening pathogen following its involvement in a food-related outbreak of gastroenteritis (*4*, *10*). This microorganism is now recognized as an important foodborne pathogen that is particularly associated with the consumption of undercooked ground beef (*4*).

The growth response of *E. coli* O157:H7 under temperature abuse conditions is an important food safety issue. As part of an effort to characterize the growth kinetics of *E. coli* O157:H7, our laboratory (*1–3*) and Sutherland et al. (*11*) reported on the effects of incubation temperature, initial pH, sodium chloride content, and sodium nitrite concentration. Both groups found that as incubation temperature decreased, as sodium chloride concentration increased, and as pH increased, the growth of O157:H7 decreased. Buchanan and Bagi (*1*) found that sodium nitrite became an effective inhibitor of O157:H7 growth at pH < 5.5 in BHI broth. The results were used to develop response surface models that can estimate the growth of O157:H7 strains in food systems. These studies employed static temperatures ranging from 4 to 42°C (*1–3*). However, abuse is often transitory and little information is available describing the response of pathogenic strains of O157:H7 to nonisothermal storage. The objective of this study was to evaluate the growth of the same three-strain mixtures of *E. coli* O157:H7 identified in the static temperature studies under conditions of transitory abuse. To simplify interpretations, the study was restricted to simple "square-wave" temperature profiles.

MATERIALS AND METHODS

Microorganisms

Three strains of *E. coli* O157:H7, 933, 45753-35, and A9218-C1, were obtained from the Microbial Food Safety Research Unit's culture collection. These are the same three strains used to develop the static temperature predictive models in earlier studies (*1–3*). The stock cultures of each strain were maintained in brain heart infusion broth (Difco Laboratories, Detroit MI), stored at 4°C, and transferred monthly.

Experimental design

A factorial design for the growth model was used to assess the effects of initial pH (5, 6, and 7), sodium chloride concentration (0.5, 1, 2, and 3%, wt/vol), and static (8, 10, 12, 19, and 28°C) and fluctuating (4 to 12, 4 to 19, 4 to 28, 8 to 19, and 12 to 28°C) incubation temperatures. The growth curves for those incubated at the fluctuating temperatures were started at the beginning of the higher temperature part of the cycle. Two replicate flasks of a single trial were examined for each combination tested.

Growth media

Brain heart infusion broth was rehydrated, supplemented with NaCl as required, and adjusted to the desired pH using 5N HCl or lN NaOH. The growth medium was dispensed in 50-ml portions to 250-

ml Erlenmeyer flasks, capped with a foam plug, and sterilized by autoclaving for 15 min at 121°C.

Culture techniques

Test cultures were prepared and sampled using the techniques described by Buchanan and Klawitter (*3*). Overnight cultures (18 to 24 h at 37°C) were grown separately in BHI. A 1.0-ml portion of each strain was added to 7.0-ml of 0.1% peptone water and then diluted to achieve a level of approximately 10^5 colony-forming units (CFU)/ml. Each test flask was inoculated with 0.5 ml of the mixture to obtain an initial inoculum of approximately 10^3 CFU/ml. For isothermal cultures, the flasks were incubated at the appropriate temperatures on rotary shakers at 150 rpm (Model 3520, Lab-Line Instrumentation, Inc., Melrose Park, IL). Samples were removed at appropriate intervals, diluted with 0.1% peptone water, surface plated in duplicate on brain heart infusion agar (BHIA)(Difco) using a Spiral Plater (Spiral Systems, Inc., Cincinnati, OH), and incubated for 20 to 24 h at 37°C before enumeration.

For nonisothermal incubation, a computer-controlled incubator (Hotpack, Philadelphia, PA) was programmed to change temperature with a "square wave" cycle every six hours. Temperature changes were monitored using a PC thermocouple expansion board (Cyber Research, Inc., Branford, CT) fitted with thermocouple wires (Omega Engineering, Inc., Stamford, CT). A thermocouple was placed in the culture flask containing 50 ml of the growth media. This flask was located at the center of the rotary shaker (150 rpm). Samples were removed three times during each 6 h phase of the cycle and processed as described above.

Curve fitting

The Gompertz equation (*5*) in conjunction with Abacus, a nonlinear regression program employing a Gauss-Newton iteration procedure (ABACUS, USDA, ERRC, Philadelphia, PA), was used to fit the growth curves for the data obtained at the static and fluctuating growth temperatures (Table 1). During curve fitting, the Gompertz A and C parameters were fixed using the observed population density values. The Gompertz parameters (A, B, C, M) were subsequently used to calculate exponential growth rates (EGR), generation times (GT), lag phase duration (LPD), maximum cell population density (MPD), and time to a 1,000-fold increase in population density ($T_{1,000}$).

RESULTS AND DISCUSSION

Static temperature

The growth data for static incubation temperatures (8 to 28°C) are presented in Table 2. Growth was defined as at least 1-log-unit increase in CFU/ml. Growth of *E. coli* O157:H7 was observed at 8°C at pH 5,6,7 and 0.5% NaCl in BHI. Calculated $T_{1,000}$ values were 1558, 329.8, and 631.7 h, respectively. With 1% NaCl, growth was limited to the pH 6 cultures having a $T_{1,000}$ value of 380.9 h. Buchanan et al. (*2*, *3*) reported no growth at 8°C for the mixture of the three strains used. However, experiments were conducted for a longer period of time in this study. At 10°C and initial pH values of 5 and 7, growth was limited to media with 0.5 and 1% NaCl. Growth occurred over the entire NaCl range in media with an initial pH of 6. For conditions at 8 and 10°C that did not support growth, the cells remained viable for more than 21 days. The observed GT and $T_{1,000}$ values for the static temperature cultures were in general agreement with those predicted by the models of Buchanan and Klawitter (*3*).

TABLE 1. *Equations for Gompertz's parameters and derived kinetic values*

The Gompertz equation

$L(t) = A + C \exp(-\exp(-B(t - M)))$, where

L(t) = log count of bacteria (log CFU/ml) at time (t) (in hours);

A = asymptotic log count bacteria as time decreases indefinitely (initial count) bacteria, (log CFU/ml);

C = asymptotic amount of bacteria growth that occurs as T increases (number of log cycles of growth (log CFU/ml);

B = relative growth rate at M, [log (CFU/ml)/h], where

M = the time at which the absolute growth rate is maximal (h).

Derived growth kinetic values

Exponential growth rate (EGR), the time cells divide at a constant rate, is BC/2.71818[(log CFU/ml)/h].

Generation time (GT), the time cells take to double in number, is log (2)2.71828/BC; in hours.

Lag phase duration (LPD), the time before cells start to grow at a constant rate, is M–l/B; in hours.

Maximum population density (MPD), the final count at the end of growth study, is A + C; [log (CFU/ml)/h].

Time one-thousand ($T_{1,000}$), the time to obtain a 3-log unit increase in cell numbers, is 10.1155GT+LPD; in hours.

Fluctuating temperature

The "square-wave" temperature cycle was described by Powers et al. (*9*) as having the shortest time over which changes in temperature occur, thereby minimizing the gradual adaptation of bacteria to changing temperature and giving the most distinct transition in growth rates. With 50 ml of broth in a 250-ml flask, the time to obtain a 1°C-change in temperature was found to be 3 ± 0.5 min, regardless of the initial temperature differences. This time compares well with those (4 ± .5 min) reported by Zwietering et al. (*12*, *13*), who obtained their "square-wave" results by manually moving the growth vessels between temperatures on a heating block; Li and Torres (*6*) manually moved their growth vessels between incubators.

Representative growth curves for the growth of *E. coli* O157:H7 under nonisothermal incubation are presented in Figure 1. Step-like growth curves were obtained at all the fluctuating temperatures and were more pronounced at the 2 and 3% NaCl concentrations. Li and Torres (*6*) and Nielson and Zeuthen (*7*) reported similarly shaped curves for growth of *Bacillus thermosphacta* in a liquid medium and for *B. thermosphacta* and *Serratia liquefaciens* in sausage.

The overall effects of fluctuating temperatures and growth conditions were estimated by generating growth curves using the Gompertz equation (Table 3). The B and M values of the Gompertz equation were used to calculate growth curves. The observed and calculated growth curves were compared and a representative plot is illustrated in Figure 2, which provided a means to compare the impact of the changing environmental conditions. Under fluctuating conditions where no growth occurred, the cells remained viable for more than 21 days. At fluctuating temperature conditions where growth occurred, GT and $T_{1,000}$ increased with greater percentages of NaCl and decreased pH as was observed with static conditions.

TABLE 2. *Growth kinetics of* Escherichia coli *O157:H7 cultured at static temperatures between 8 and 28°C in conjunction with initial pH values of 5, 6, and 7 and NaCl levels of 0.5, 1, 2, and 3 % NaCl*

pH	Temp (°C)	% NaCl	A	C	B	M	EGR	GT	LPD	M	$T_{1,000}$
5	8	0.5	4.02	2.02	0.0028	451.9	0.002	144.7	94.8	6	1558.1
		1	4.18	NG[a]	-[b]	-	-	-	-	-	-
		2	4.03	NG	-	-	-	-	-	-	-
		3	3.97	NG	-	-	-	-	-	-	-
	10	0.5	3.24	6.04	0.006	247.5	0.013	22.6	80.8	9.3	309.2
		1	3.25	6.35	0.0041	306.7	0.01	31.4	62.8	9.6	380.7
		2	3.25	NG	-	-	-	-	-	-	-
		3	3.25	NG	-	-	-	-	-	-	-
	12	0.5	3.08	6.57	0.127	74	0.307	1	66.1	9.7	76
		1	3.04	6.58	0.024	82.3	0.058	5.2	40.6	9.6	93
		2	3.05	NG	-	-	-	-	-	-	-
		3	3.1	NG	-	-	-	-	-	-	-
	19	0.5	3.65	6.28	0.089	22.7	0.206	1.5	11.5	9.9	26.3
		1	3.63	6.1	0.102	14.3	0.229	1.3	4.5	9.7	17.8
		2	3.56	6.12	0.068	20.6	0.153	2	5.9	9.7	25.8
		3	3.2	6.73	0.062	22.1	0.154	2	6	9.9	25.8
	28	0.5	3.7	5.6	0.232	7.5	0.478	0.6	3.2	9.3	9.6
		1	3.46	5.55	0.511	8.4	1.043	0.3	6.4	9	9.4
		2	3.83	5.14	0.296	8.2	0.56	0.5	4.8	9	10.3
		3	3.74	5.2	0.171	17.9	0.327	0.9	12.1	8.9	21.4
6	8	0.5	2.86	6.68	0.0063	291.9	0.015	19.4	133.2	9.5	329.8
		1	3.04	6.75	0.0057	341.2	0.014	21.3	165.8	9.8	380.9
		2	2.86	NG	-	-	-	-	-	-	-
		3	2.86	NG	-	-	-	-	-	-	-
	10	0.5	3.2	6.4	0.013	96	0.031	9.8	19.1	9.6	118.6
		1	3.22	6.31	0.013	117.6	0.03	10	40.7	9.5	141.6
		2	3.2	6.44	0.013	213.6	0.031	9.8	136.7	9.6	235.5
		3	3.25	3.62	0.022	251.5	0.029	10.3	206	6.9	310
	12	0.5	2.87	6.77	0.043	56.9	0.107	2.8	33.6	9.6	62.1
		1	2.88	6.33	0.018	76.1	0.042	7.2	20.5	9.2	93.2
		2	2.85	6.68	0.087	93.4	0.214	1.4	81.9	9.5	96.1
		3	2.95	6.54	0.011	125.9	0.026	11.4	35	9.5	150
	19	0.5	3.19	6.76	0.132	11.15	0.328	0.9	3.6	10	12.8
		1	3.15	6.23	0.179	11.03	0.41	0.7	5.4	9.4	12.9
		2	3.1	6.3	0.125	14.67	0.29	1	6.7	9.4	17.2
		3	3.03	6.53	0.092	15.48	0.221	1.4	4.6	9.6	18.4
	28	0.5	3.29	6.03	0.249	6.33	0.552	0.5	2.3	9.3	7.8
		1	3.19	6.21	0.268	6.7	0.612	0.5	3	9.4	7.9
		2	3.18	5.99	0.144	10.55	0.317	0.9	3.6	9.2	13.2
		3	3.26	6.53	0.055	23.06	0.132	2.3	4.9	9.8	27.9
7	8	0.5	3.23	5.01	0.01	566.5	0.018	16.3	466.5	8.2	631.7
		1	3.28	NG	-	-	-	-	-	-	-
		2	3.17	NG	-	-	-	-	-	-	-
		3	3.19	NG	-	-	-	-	-	-	-
	10	0.5	3.15	6.35	0.016	136.79	0.037	8.1	74.3	9.5	155.8
		1	3.19	6.23	0.013	130.41	0.03	10.1	53.5	9.4	155.7
		2	3.23	NG	-	-	-	-	-	-	-
		3	3.17	NG	-	-	-	-	-	-	-
	12	0.5	2.85	6.88	0.037	52.92	0.094	3.2	25.9	9.7	58.4
		1	2.96	6.77	0.042	71.2	0.105	2.9	47.4	9.7	76.5
		2	2.96	6.59	0.018	86.63	0.044	6.9	31.1	9.6	100.8
		3	2.83	6.51	0.012	128.83	0.029	10.5	45.5	9.3	151.4
	19	0.5	3.22	6.78	0.081	14.8	0.202	1.5	2.5	10	17.5
		1	3.17	6.69	0.09	15.5	0.222	1.4	4.4	9.9	18.1
		2	3.14	6.57	0.052	36.23	0.126	2.4	17	9.7	41.2
		3	3.14	6.87	0.032	67.88	0.081	3.7	36.6	10	74.3
	28	0.5	3.33	6.18	0.285	5.7	0.648	0.5	2.2	9.5	6.9
		1	3.33	6.14	0.265	6.47	0.599	0.5	2.7	9.5	7.8
		2	3.3	6.2	0.282	10.04	0.643	0.5	6.5	9.5	11.2
		3	3.21	6.32	0.195	11.61	0.453	0.7	6.5	9.5	13.2

[a] NG, no growth.

[b] -, undefined, no growth.

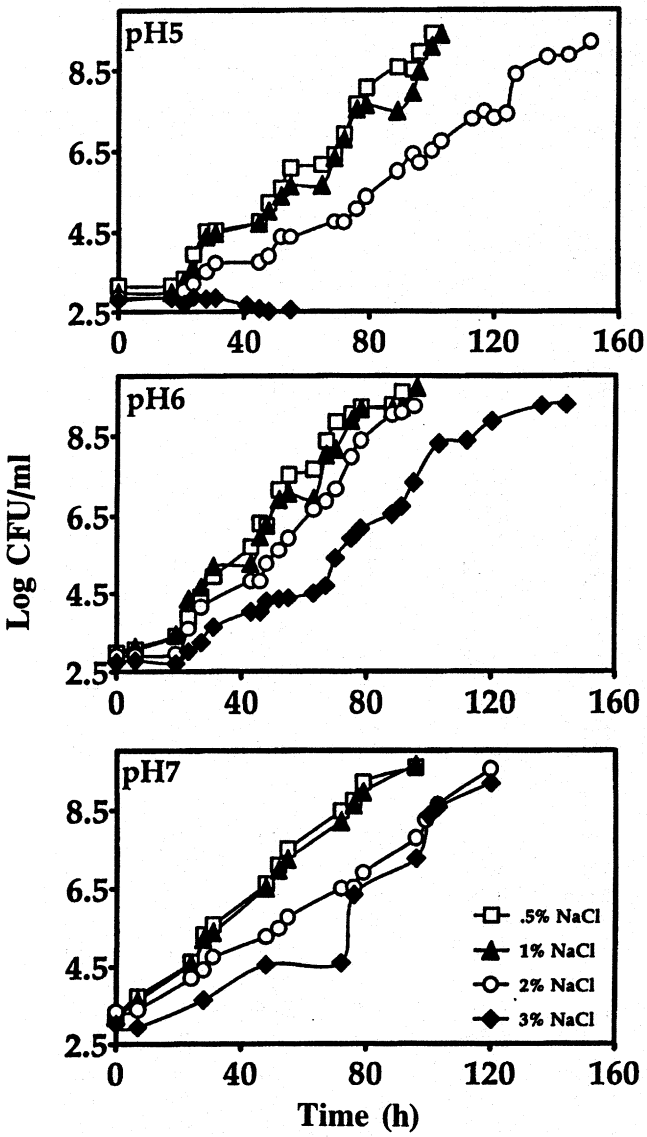

Figure 1. *Growth curves of* Escherichia coli *O157:H7 in BIH broth at fluctuating temperatures of 4 to 19°C in conjunction with initial pH values of 5, 6, and 7 and NaCl levels of 0.5, 1, 2, and 3%.*

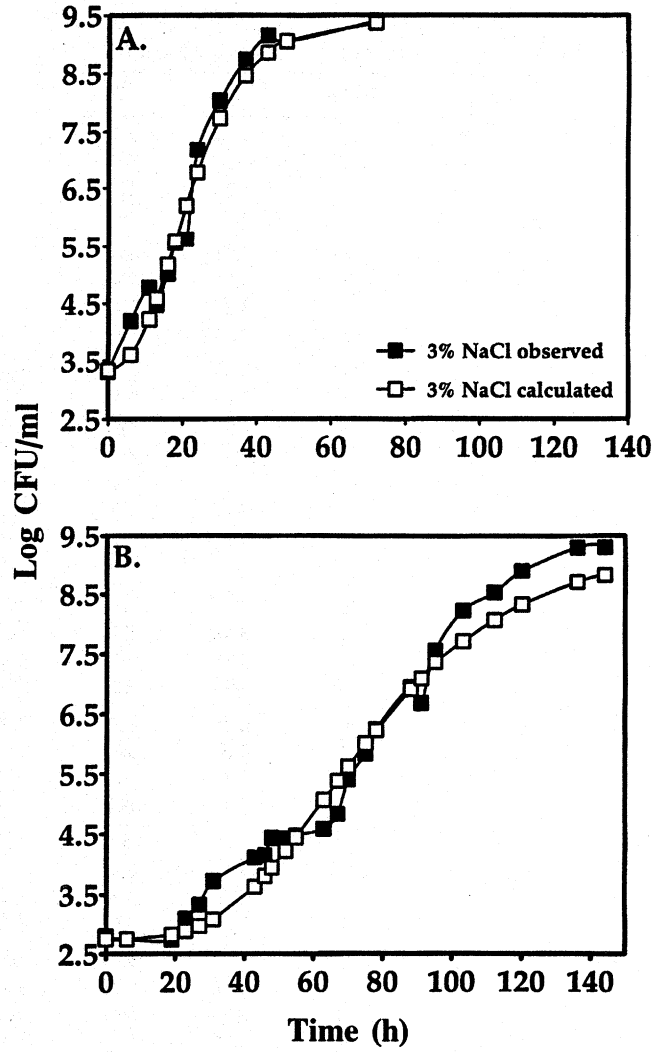

Figure 2. *Observed and calculated growth curves for* Escherichia coli *O157:H7 grown under fluctuating temperatures conditions A, 12 to 28°C, pH 6, 3% NaCl; B, 4 to 19°C, pH 6, 3% NaCl.*

The GT and LPD obtained from the Gompertz equation were compared for the static high and low, fluctuating temperatures, and the midpoint temperature calculated with the predictive model (Table 4). Generally the GTs and LPDs for growth with the fluctuating temperatures were much closer to those for growth at the comparable higher static temperatures than those for growth at the lower static temperatures. The calculated GTs and LPDs using the midpoint temperature were longer than those observed in the fluctuating temperature regime. Growth kinetics at the fluctuating temperatures were more closely approximated by the higher temperature than the midpoint temperature for each cycle (Table 4). Estimation for the fluctuating temperature regimes based on the midpoint temperature kinetics should not be applied.

The experimental procedure started with incubation at the higher temperature (19 and 28°C) then cycled to the lower, since *E. coli* does not grow at 4°C. Cells started at the most favorable conditions could complete the lag phase before the low-temperature portion of the cycle began, for example, 1% NaCl, pH 6, 28 to 4°C. For this reason the $T_{1,000}$ values tended to be more standard and consistent over the range of experimental conditions (Table 4).

Zwietering et al. (*12*) reported that shifts in temperature near the minimum growth temperature exposed the bacteria to stress and that more experimental work and data points are needed to predict growth under fluctuating conditions. This exposure to stress may explain the few inconsistent GTs and LPDs obtained, as pH 5, 0.5, and 1% NaCl for 12 to 28°C. Zwietering et al. (*13*) found the predictive lag times were the same for fluctuating regimes of high to low or for the reverse order.

With the step-like curves observed for the slower fluctuating growth regimes, the Gompertz equation reasonably estimates the growth kinetics, but may need modifications to increase accuracy. Li and Torres (*6*) tested several formulas to obtain better estimates of the growth kinetics. They reported that the use of the Gompertz equation was a reliable estimate of growth at the fluctuating temperature but stated that no single model could estimate the growth of all microorganisms for given temperatures and growth conditions. Li and Torres (*6*) suggested the use of separate estimations for the lag and log phases to predict bacterial spoilage at fluctuating temperatures. Zwietering et al. (*12, 13*) also concluded that a modified Gompertz equation is better at predicting bacterial growth at fluctuating temperatures and that the use of several models is necessary to estimate growth.

TABLE 3. *Growth kinetics of* Escherichia coli *O157:H7 cultured at fluctuating temperature regimes in conjunction with initial pH values of 5, 6, and 7 and NaCl levels of 0.5, 1, 2, and 3%*

pH	Temp (°C)	% NaCl	A	C	B	M	EGR	GT	LPD	M	$T_{1,000}$
5	4 to 12	0.5	3.25	NG[a]	-[b]	-	-	-	-	-	-
		1	3.27	NG	-	-	-	-	-	-	-
		2	3.19	NG	-	-	-	-	-	-	-
		3	3.14	NG	-	-	-	-	-	-	-
	4 to 19	0.5	3	7.11	0.047	39.45	0.123	2.4	18.2	10.1	42.9
		1	2.97	6.76	0.044	37.9	0.109	2.8	15.2	9.7	43
		2	2.9	6.33	0.049	47.69	0.114	2.6	27.3	9.2	54
		3	2.91	NG	-	-	-	-	-	-	-
	4 to 28	0.5	3.23	6.18	0.074	18.98	0.168	1.8	5.5	9.4	23.6
		1	3.17	6.05	0.094	25.07	0.209	1.4	14.4	9.2	29
		2	3.31	6.06	0.34	39.42	0.758	0.4	36.5	9.4	40.5
		3	3.16	6.1	0.164	86.27	0.368	0.8	80.2	9.3	88.4
	8 to 19	0.5	3.08	6.01	0.522	31.21	1.154	0.3	29.3	9.1	31.9
		1	3.12	6.43	0.265	34.43	0.627	0.5	30.7	9.6	35.5
		2	3.19	6.27	0.045	36.61	0.104	2.9	14.4	9.5	43.7
		3	3.21	6.06	0.038	46.77	0.085	3.6	20.5	9.3	56.4
	12 to 28	0.5	3.22	6.31	0.109	12.49	0.253	1.2	3.3	9.5	15.3
		1	3.27	6.12	0.086	18.81	0.194	1.6	7.2	9.4	22.9
		2	3.19	6.29	0.055	24.83	0.127	2.4	6.6	9.5	30.6
		3	3.21	-	-	-	-	-	-	-	-
6	4 to 12	0.5	3.03	6.45	0.099	122.13	0.235	1.3	112	9.5	125
		1	3.08	6.27	0.097	128.53	0.224	1.3	118.2	9.4	131.8
		2	3.14	5.96	4.13	378.18	9.055	0	377.9	9.1	378.3
		3	3.1	5.83	0.066	409.63	0.142	2.1	394.5	8.9	416
	4 to 19	0.5	3	7.11	0.264	39.45	0.691	0.4	35.7	10.1	40.1
		1	2.97	6.76	0.044	37.9	0.109	2.8	15.2	9.7	43
		2	2.9	6.33	0.049	47.69	0.114	2.6	27.3	9.2	54
		3	2.75	6.56	0.033	62.94	0.08	3.8	32.6	9.3	70.9
	4 to 28	0.5	3.29	6.38	0.516	13.57	1.211	0.2	11.6	9.7	14.1
		1	3.14	6.28	0.092	13.73	0.213	1.4	2.9	9.4	17.2
		2	3.21	6.07	0.085	17.45	0.19	1.6	5.7	9.3	21.7
		3	3.11	5.89	0.096	25.27	0.208	1.4	14.9	9	29.5
	8 to 19	0.5	2.99	6.37	0.058	34.42	0.136	2.2	17.2	9.4	39.6
		1	3.04	6.26	0.06	33.2	0.138	2.2	16.5	9.3	38.6
		2	3.1	6.19	0.041	45.75	0.093	3.2	21.4	9.3	54
		3	3.06	6.01	0.028	81.77	0.062	4.9	46.1	9.1	95.2
	12 to 28	0.5	3.26	6.4	0.129	12.51	0.304	1	4.8	9.7	14.8
		1	3.23	6.27	0.136	13.47	0.314	1	6.1	9.5	15.8
		2	3.28	6.03	0.12	15.34	0.266	1.1	7	9.3	18.4
		3	3.32	6.15	0.094	17.73	0.213	1.4	7.1	9.5	21.4
7	4 to 12	0.5	3.15	6.39	0.013	216.49	0.031	9.8	139.6	9.5	239.2
		1	3.11	6.16	0.011	199.55	0.025	12.1	108.6	9.3	230.8
		2	3.07	6.38	0.008	209.06	0.019	16	84.1	9.5	246.2
		3	3	5.86	0.006	366.64	0.013	23.3	200	8.9	435.4
	4 to 19	0.5	3.24	6.35	0.043	32.67	0.1	3	9.4	9.6	39.7
		1	3.21	6.45	0.04	34.26	0.095	3.2	9.3	9.7	41.3
		2	23.36	6.19	0.028	52.48	0.064	4.7	16.8	9.6	64.5
		3	3.03	6.16	0.046	67.86	0.104	2.9	46.1	9.2	75.3
	4 to 28	0.5	2.89	6.65	0.147	18.59	0.36	0.8	11.8	9.5	20.3
		1	3.24	6.15	0.109	15.43	0.247	1.2	6.3	9.4	18.6
		2	3.31	6.27	0.214	25	0.494	0.6	20.3	9.6	26.5
		3	3.1	5.62	0.12	28.51	0.248	1.2	20.2	8.7	32.4
	8 to 19	0.5	3.14	6.21	0.486	33.68	1.11	0.3	31.6	9.4	34.4
		1	3.05	6.46	0.057	33.04	0.135	2.2	15.5	9.5	38
		2	3.17	6.2	0.043	35.64	0.098	3.1	12.4	9.4	43.4
		3	2.95	6.36	0.045	39.08	0.105	2.9	16.9	9.3	45.8
	12 to 28	0.5	2.84	7.18	0.114	12.02	0.301	1	3.2	10	13.4
		1	2.89	6.81	0.124	13.21	0.311	1	5.1	9.7	14.9
		2	2.72	6.67	0.101	13.94	0.248	1.2	4	9.4	16.3
		3	2.84	6.66	0.086	22.67	0.211	1.4	11	9.5	25.5

[a] NG, no growth.

[b] -, undefined, no growth.

TABLE 4. *Comparison of GT, LPD and $T_{1,000}$ values obtained for growth at static and fluctuating temperatures with the calculated midpoint temperature*

		pH 5			pH 6			pH 7		
NaCl%	Temp (°C)	GT	LPD	$T_{1,000}$	GT	LPD	$T_{1,000}$	GT	LPD	$T_{1,000}$
0.5	4	-[c]	-	-	-	-	-	-	-	-
	12	1.0	66.1	76.0	2.8	33.6	62.1	3.2	25.9	58.4
	F[a]	-	-	-	1.3	112.0	125.0	9.8	139.6	239.2
	C[b]	NR[d]		NR						
	4	-	-	-	-	-	-	-	-	-
	19	1.5	11.5	26.3	.9	3.6	12.8	1.5	2.5	17.5
	F	2.4	18.2	42.9	.4	35.7	40.1	3.0	9.4	39.7
	C	6.8	46.9	115.7	3.8	30.5	68.9	3.6	30.9	67.3
	4	-	-	-	-	-	-	-	-	-
	28	.6	3.2	9.6	.5	2.3	7.8	.5	2.2	6.9
	F	1.8	5.5	23.6	.2	11.6	14.1	.8	11.8	20.3
	C	2.6	22.7	49.0	1.7	15.2	32.4	1.8	15.4	33.6
	8	144.7	94.8	1558.1	19.4	133.2	329.8	16.3	466.5	631.7
	19	1.5	11.5	26.3	.9	3.6	12.8	1.5	2.5	17.5
	F	.3	29.3	31.9	2.2	17.2	39.6	.3	31.6	33.4
	C	4.3	34.4	77.9	2.6	22.6	48.9	2.6	22.9	49.2
	12	1.0	66.1	76.0	2.8	33.6	62.1	3.2	25.9	58.4
	28	.6	3.2	9.6	.5	2.3	7.8	.5	2.2	6.9
	F	1.2	3.3	15.3	1.0	4.8	14.8	1.0	3.2	13.4
	C	1.4	11.4	25.6	1.0	7.8	17.9	1.1	7.9	19.0
1.0	4	-	-	-	-	-	-	-	-	-
	12	5.2	40.6	93.0	7.2	20.5	93.2	2.9	47.4	76.5
	F	-	-	-	1.3	118.2	131.8	12.1	108.6	230.8
	C	NR		NR						
	4	-	-	-	-	-	-	-	-	-
	19	1.3	4.5	17.8	.7	5.4	12.9	1.4	4.4	18.1
	F	2.8	15.2	43.0	2.8	15.2	43.0	3.2	9.3	41.3
	C	9.1	58.6	150.7	4.3	38.4	81.9	3.7	40.1	77.5
	4	-	-	-	-	-	-	-	-	-
	28	.3	6.44	9.4	.5	3.0	7.9	.5	2.7	7.8
	F	1.4	14.4	29.0	1.4	2.9	17.2	1.2	6.3	18.6
	C	3.6	27.8	61.2	1.8	18.6	36.9	1.7	19.1	36.3
	8	-	-	-	-	-	380.9	-	-	-
	19	1.3	4.5	17.8	.7	5.4	12.9	1.4	4.4	18.1
	F	.5	30.7	35.5	2.2	16.5	38.6.	3	31.6	38.0
	C	5.6	42.6	99.2	2.9	28.1	57.4	2.6	29.3	55.6
	12	5.2	40.6	93.0	7.2	20.5	93.2	2.9	47.4	76.5
	28	.3	6.4	9.4	.5	3.0	7.9	.5	2.7	7.8
	F	1.6	7.2	22.9	1.0	6.1	15.8	1.0	5.1	14.9
	C	1.6	13.7	29.9	1.0	9.3	19.4	1.0	9.41	9.5
2.0	4	-	-	-	-	-	-	-	-	-
	12	-	-	-	1.4	81.9	96.1	6.9	31.1	100.8
	F	-	-	-	.03	377.9	378.3	16.0	84.0	246.2
	C	NR		NR						
	4	-	-	-	-	-	-	-	-	-
	19	2.0	5.9	25.8	1.0	6.7	17.2	2.4	17.0	41.2
	F	2.6	27.3	54.6	2.6	27.3	54.0	4.7	16.8	64.5
	C	20.0	72.2	274.5	7.6	48.8	125.7	11.7	52.9	110.8
	4	-	-	-	-	-	-	-	-	-
	28	.5	4.8	10.3	.9	3.6	13.2	.5	6.5	11.2
	F	.4	36.5	40.5	1.6	5.7	21.7.	6	20.3	26.5
	C	6.4	34.9	99.6	2.8	23.4	51.7	2.2	25.4	47.7
	18	-	-	-	-	-	-	-	-	-
	19	2.0	5.9	25.8	1.0	6.7	17.2	2.4	17.0	41.2
	F	2.9	14.4	43.7	3.2	21.4	54.0	3.1	12.4	43.4
	C	11.7	52.9	171.3	4.7	35.6	83.1	3.6	39.6	76.0
	12	-	-	-	1.4	81.9	96.1	6.9	31.1	100.8
	28	.5	4.8	10.3	.9	3.6	13.2	.5	6.5	11.2

NaCl	% Temp (°C)	pH 5			pH 6			pH 7		
		GT	LPD	$T_{1,000}$	GT	LPD	$T_{1,000}$	GT	LPD	$T_{1,000}$
	F	2.4	6.6	30.6	1.1	7.0	18.4	1.2	4.0	16.3
	C	2.9	17.4	46.7	1.3	11.6	24.8	1.1	12.1	23.2
3.0	4	-	-	-	-	-	-	-	-	-
	12	-	-	-	11.4	35.0	150.0	10.5	45.5	151.4
	F	-	-	-	2.1	394.5	416.0	23.3	200.0	435.4
	C	NR		NR						
	4	-	-	-	-	-	-	-	-	-
	19	2.0	6.0	25.8	1.4	4.6	18.4	3.7	36.6	74.3
	F	-	-	-	3.8	32.6	70.9	2.9	46.1	75.3
	C	43.1	72.3	508.3	14.5	51.4	198.1	10.0	64.3	165.5
	4	-	-	-	-	-	-	-	-	-
	28	.9	12.0	21.4	2.3	4.9	27.9	.7	6.5	13.2
	F	.8	80.2	88.4	1.4	14.9	29.5	1.2	20.2	32.4
	C	12.8	38.2	167.8	4.8	26.3	74.9	3.6	30.7	67.1
	8	-	-	-	-	-	-	-	-	-
	19	2.0	6.0	25.8	1.4	4.6	18.4	3.7	36.6	74.3
	F	3.6	20.5	56.4	4.9	46.1	95.2	2.9	16.9	45.8
	C	24.2	55.2	300.0	8.6	38.7	125.7	6.2	46.9	109.6
	12	-	-	-	11.4	35.0	150.0	10.5	45.5	151.4
	28	.9	12.0	21.4	2.3	4.9	27.9	.7	6.5	13.2
	F	-	-	-	1.4	7.1	21.4	1.4	11.0	25.5
	C	5.5	20.6	76.2	2.2	13.8	36.1	1.8	15.0	33.2

[a] F, fluctuating temperature regime.
[b] C, calculated using midpoint temperature with predictive model.
[c] -, no growth observed.
[d] NR, no result presented, as beyond the valid region of predictive model.

CONCLUSIONS

The pathogenic strains of *E. coli* O157:H7 are a concern for the food-service industry, particularly where temperature abuse may occur. This work shows that the use of mid-point temperatures in a fluctuating regime in a predictive model gives an incorrect estimate for actual growth. The use of the higher temperature in a abuse situation would be the safest in determining growth by the predictive models for *E. coli* O157:H7, since the general pattern was closest to that of the higher static temperature and this provides a conservative (safe) bias. Analysis of the data obtained during this study will continue to determine a predictive model for these growth conditions. This research also showed that given sufficient storage time at 8°C, *E. coli* O157:H7 can grow in BHI broth. Extra care should be used to avoid temperature abuse when handling foods that may be contaminated with *E. coli* O157:H7.

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