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Thermal inactivation of bacteria—a new predictive model for the combined effect of three environmental factors: temperature, pH and water activity

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An empirical, linear-Arrhenius model for predicting the combined effect of three environmental factors on the thermal inactivation (i.e. death) rate of *Escherichia coli*, namely inactivation temperature, pH and water activity (a_w), is synthesised from published, and independent experimental data. For inactivation rates obtained both at isothermic condition, a constant temperature of 58°C, with a seven level pH range (pH 3 to pH 9), and a three level range of values of a_w (0.928 to 0.995); and anisothermic conditions (a range of inactivation temperature 52.05 to 63.10°C, with a four level pH range of between pH 3 to pH 9, and a three level range of a_w from 0.928 to 0.995), the model gave a very high degree of fit. The synthesised model is quadratic in form. For isothermic inactivation the model has three terms (pH, pH^2, and a_w^2) and explained 96.7% of the variance accounted for (%V) in the overall data (i.e. seven levels of pH and three levels of a_w). The model with four terms ($1/T$, pH, pH^2, and a_w^2) explained 80.7 %V in the overall anisothermic data, and between 90.1 and 95.3 %V at each level of a_w. Comparison with an existing model highlighted the advantages of the newly synthesised model including ease of formulation, and ease of use. The new model form should permit ready integration with equations describing the viscous and thermal properties in design for batch or continuous steriliser design. Findings suggest an additive linear-Arrhenius model is applicable to the combined influence of n environmental factors on the thermal inactivation rate. Copyright © 1996 Canadian Institute of Food Science and Technology. Published by Elsevier Science Ltd

Keywords: inactivation kinetics, modelling death kinetics, sterilisation kinetics, combined temperature, pH and water activity, thermal processing, food processing.

INTRODUCTION

Because of reliability and, generally, economy, thermal sterilisation has been widely used in the food industries for many years. A knowledge of thermal inactivation (death) kinetics of potential contaminating micro-organisms is therefore essential to proper steriliser design and operation. In addition to process temperature, the pH of operation was known to effect the rate of inactivation of thermally treated contaminants.

Davey *et al.* (1978) developed the first model for predicting the combined effect of both process temperature and pH on kinetics of thermal inactivation of bacteria. This empirical model they based on published data of Xezones & Hutchings (1965) for inactivation of *Clostridium botulinum* spores in a range of pH buffered foods, including spaghetti, tomato sauce and cheese, Macaroni creole, and Spanish rice, over a range of temperature of 110 to 118.3°C and pH 4.0 to pH 7.0. They illustrated their kinetic model with sample

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predictions for a flow-through tubular reactor in which equations describing the rheological and thermal properties of a fluid were combined with the model they derived for thermal inactivation of this thermally resistant spore former. The effect of pH was shown to be highly significant in influencing the necessary holding (sterilisation) time for a given design reduction in the number of viable spores of *Cl. botulinum* (both 10^{-12} and 10^{-14}) in these foods. They concluded that steriliser design and operation (and costs) were highly significantly influenced by pH.

Because other factors, especially the water activity (salt concentration) are known to influence thermal death rates of bacteria, our interest in the kinetics of inactivation of potential food contaminants has continued over many years. Recently we demonstrated a generalised and practical method for predicting the effect of combined process temperature and pH on thermal sterilisation of media containing bacterial contaminants using the established, temperature-only dependent sterilisation charts (Davey, 1993*a*).

Our particular interest has been the development and demonstration of predictive models for the combined effect of process variables on thermal inactivation (see, for example, Davey *et al.*, 1995; Davey, 1993*b*,*c*; Cerf, 1977) and, thermal denaturation of accompanying vitamin (Davey, 1989*a*; Coker *et al.*, 1993; Davey, 1993*b*,*c*), and also on bacterial growth. Some of this work has been reviewed recently by Davey *et al.* (1995), Davey, 1993*b*,*c*; McMeekin *et al.* (1993); Davey (1994), Davey & Daughtry (1995) and Cerf (1995). Because heat and mass transfer aspects of many food operations appear well understood, it is important that an adequate model of the influence of process variables on the kinetics of inactivation (and growth) of microbial contaminants is developed (Davey, 1992*a*). Much published information on the effect of environmental factors such as temperature, pH, and water activity (a_w) has been fragmentary, i.e. based on unrelated values of physical data selected from a range of published values, or values derived from published data, or limited experimental studies. One consequence is that they have little direct application to real processes. A comprehensive experimental testing of a continuous steriliser model together with publication of data appears not to have been reported since that of Davey & Wood (1984). Their studies, however, were limited to the effect of temperature only. A contributing factor to this relatively slow development is the multi-disciplinary nature of the problem.

The recent model development of Reichart (1994) is important, therefore, not least because of the extensive experimental data published for evidence of the influence of combined temperature, pH and water activity on thermal sterilisations, but also for the attempt to quantitatively model these data. Access to (raw) thermal sterilisation (and growth) data is increasingly restricted, even to researchers in the field, because of an increasing trend to "in confidence" commercial agreements. The model of Reichart (1994) for the thermal inactivation of *Escherichia coli* was developed on the basis of reaction kinetics.

In this paper, an alternative and new model, that is based in part on the earlier form of Davey *et al.* (1978) and, the more recent additive model for bacterial growth of Davey (Davey, 1994; Davey & Daughtry, 1995), is synthesised from the data of Reichart for the thermal inactivation of *E. coli* as influenced by combined temperature, pH and water activity. A comparison is made between the two model forms.

MATERIALS AND METHODS

Reichart (1994) reported experimental data for the thermal inactivation of *E. coli* at both isothermic (constant temperature) and, anisothermic (constantly varying temperature) conditions. At isothermic conditions the temperature was a constant 58°C, with a seven level pH range (pH 3 to 9) and, a three level range of values of a_w (0.928 to 0.995). At anisothermic conditions a range of temperature from 52.05 to 63.10°C, together with a four level range of pH (pH 3 to 9), and a three level range of a_w (0.928 to 0.995) was studied.

The isothermic model of Reichart (1994), involving the combined effect of pH and water activity, is given by:

$$k = Ba_w^{\rho_w}([H^+]^{\rho_H} + [OH^-]^{\rho_{OH}}) \quad (1)$$

where k is the specific inactivation rate (death rate constant) (t^{-1}), ρ the stoichiometric coefficient, $[H^+]$ and $[OH^-]$ are, respectively, the hydrogen and hydroxyl ion concentrations, a_w is the water activity, the subscript w is for water, and B (K) is a coefficient.

For the anisothermic studies, the combined effect of temperature, pH and a_w, is modelled by:

$$\frac{k}{T} = e^c e^{b/T} a_w^{\rho_w}([H^+]^{\rho_H} + [OH^-]^{\rho_{OH}}) \quad (2)$$

where T is the holding (inactivation) temperature (K), and C and b (K) are coefficients.

The particular strain of *E. coli* was B 200. Cells were cultured on agar slopes at 37°C. The pH of the heating menstrua was adjusted with NaOH and HCL solutions. Viable cell numbers were determined by the plate count method.

In the anisothermic studies the temperature of the inoculated heating medium was continuously heated by a heating spiral. The values of the specific inactivation rate (k) for the isothermic studies were calculated from regression equations of the linear section of survival curves. For the anisothermic studies, the values of k were calculated by numeric differentiation of plots of the measured temperature of the heating medium and the logarithm of the viable cell count, against time.

Data were presented in both tabulated and graphical form (see Reichart, 1994 for further experimental details).

An alternative model form derived by Davey *et al.* (1978), using published data of Xezones & Hutchings (1965) for *Cl. botulinum*, to predict the combined effect of temperature and pH on the thermal inactivation rate is given by:

$$\ln(k) = C_0 + C_1/T + C_2\text{pH} + C_3\text{pH}^2 \qquad (3)$$

where C_0 through to C_3 are coefficients for different media. The coefficients for these data were significant ($P \leq 0.05$) and there was no significant ($P > 0.05$) interaction terms (e.g. $1/T$*pH, $1/T$*pH^2 etc.). For these data there was no significant ($P > 0.05$) $1/T^2$ term, indicating no significant curvature due to temperature in the data for $\ln(k)$. No explanation for any physiological significance of the coefficients was given, although this is currently under study as reported by Davey *et al.* (1995).

A widely used criterion for the goodness of fit of a model (Davey, 1993*b*, 1994; Davey & Daughtry, 1995 see also Davey, 1989*b*) is the per cent variance accounted for (%V) (Snedecor & Cochran, 1969). The %V is a measure of the difference between observed and predicted values and is given by:

$$\%V = 1 - \frac{(1 - r^2)(n - 1)}{(n - N - 1)} \qquad (4)$$

where n is the number of observations, N the number of terms (e.g. $1/T$, pH, pH^2, a_w^2) and r^2 is the multiple regression coefficient. The %V is considered a stringent test of goodness of fit where there are few data, or, a large number of terms in the model. From eqn 4 it can be seen that, as the number of terms increases the %V decreases. At $n \gg N$, %V approximates the value of r^2.

An alternative measure of the goodness of fit is the mean square error (MSE) (Davey, 1994). Ratkowsky *et al.* (1991) however have criticised the appropriateness of MSE after analyses of published data for bacterial growth responses (e.g. generation time and lag phase duration) in which they demonstrated that these responses became more variable as the mean magnitude increased.

To test the fit of the model form of eqn 3, regression analyses (Snedecor & Cochran, 1969) were carried out using MATHEMATICA® version 2.1 software on both the isothermic and anisothermic data of Reichart (1994).

RESULTS

Isothermic death of E. coli—modelling the combined effect of pH and a_w on the inactivation rate

For the isothermic sterilising temperature of 58°C, the model obtained for the inactivation rate (k) is:

$$\ln(k)_1 = -6.021 - 2.377\text{pH} + 0.1994\text{pH}^2 + 8.997a_w^2 \qquad (5)$$

Table 1. Statistical summary for isothermic destruction of *E. coli*

$\ln(k)_{s^{-1}} = -6.021 - 2.377\text{pH} + 0.1994\text{pH}^2 + 8.997a_w^2$

Coefficient	Estimate	Standard error	T statistic	P value
C_0	−6.021	0.6894	−8.733	0
C_1	−2.377	0.1208	−19.68	0
C_2	0.1994	0.0099	20.02	0
C_3	8.997	0.6525	13.79	0
%V = 96.7				
Source of variation	df	Sum of squares	Mean square	F ratio
Model	3	14.800	4.9335	197.26
Error	17	0.42517	0.025010	
Total	20	15.226		

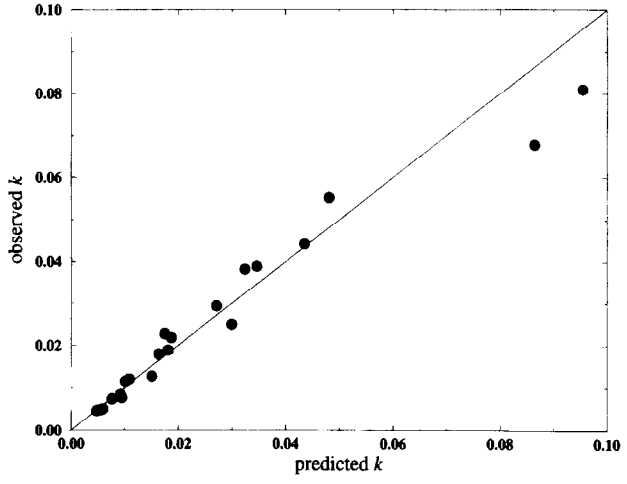

Fig. 1. Fit of the isothermic (T = 58°C) sterilisation model (Eq. (5)) to the overall observed values of the inactivation rate of *E. coli*.

As is shown, substitution for pH and a_w gives the value of the inactivation rate in s^{-1}. The difference between the observed value and predicted value of k, measured as %V (eqn 4) is 96.7%, indicating a very good fit to these data. Table 1 summarises the statistical evaluation of the isothermic data.

Figure 1 shows a comparison of the predicted value of the inactivation rate, from eqn 5, with the observed value of Reichart (1994), over all three values of a_w. A comparison of the predicted value of the inactivation rate with the observed value, at each of the seven pH, and three a_w values, is summarised in Figure 2.

Modelling the combined effect of T, pH and a_w on the anisothermic death of E. coli

The model obtained for the inactivation rate from the anisothermic data for all T, pH and a_w values, is:

$$\ln(k) = 86.49 - 0.3028 \times 10^5/T - 0.5470\text{pH} + 0.0494\text{pH}^2 + 3.067a_w^2 \qquad (6)$$

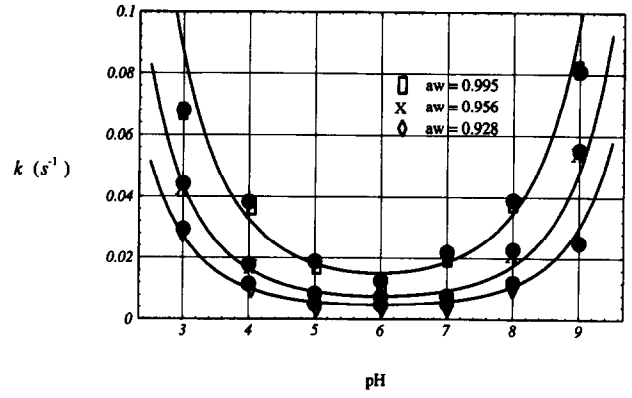

Fig. 2. Fit of the isothermic (T = 58°C) sterilisation model (Eq. (6)) to observed values of the inactivation rate of *E. coli*, at each of the seven pH, and three a_w, values.

Substitution for T (K) gives the value of the inactivation rate in s^{-1}. This model explained 80.7% of the $\%V$ in the anisothermic destruction data of *E. coli*. Table 2 provides a statistical summary for the anisothermic model fit.

Additionally, regression analyses were carried out on the anisothermic data at each of the three levels of a_w. The value of the coefficients for each model at each of the three values of the a_w, respectively, 0.995, 0.956 and 0.928 are summarised in Table 3. From Table 3 the resulting models, respectively, can be written:

$$\ln(k)_{a_w=0.995} = 83.65 - 0.2837 \times 10^5/T - 0.3668\text{pH} + 0.0268\text{pH}^2 \quad (7)$$

$$\ln(k)_{a_w=0.956} = 102.2 - 0.3437 \times 10^5/T - 0.9461\text{pH} + 0.0916\text{pH}^2 \quad (8)$$

$$\ln(k)_{a_w=0.928} = 119.6 - 0.3999 \times 10^5/T - 0.9942\text{pH} + 0.0868\text{pH}^2 \quad (9)$$

Substitution for T (K) and pH in equations 7–9 gives k in s^{-1}. Table 3 shows that with values of the $\%V$ of between 90.1% and 95.3%, these models give a very high degree of goodness of fit to the anisothermic inactivation data of *E. coli*.

Figures 3, 4 and 5, respectively, show the predicted value of the inactivation rate for anisothermic inactivation (eqn 6) against the observed value at each of the three values of a_w.

DISCUSSION

The isothermic model (eqn 5) is valid for the range of the values of the combined environmental factors of, respectively, pH 3 to 9, and 0.928 to 0.995 for water activity, at a constant sterilising temperature of 58°C, the model for the anisothermic death of *E. coli*, is valid for the range of values of the three combined environmental factors, of respectively, temperature, pH and a_w, of 52.05 to 63.10°C, pH 3 to 9, and 0.928 to 0.995. However, the very good fits obtained suggest these models could be extrapolated over a limited range of values of the environmental factors. Extrapolation must nevertheless be done with caution. The form of the synthesised model does not predict a limiting value.

The quadratic form of the synthesised model requires, as a minimum, a range of three levels for each environmental factor modelled, this requirement is, importantly, satisfied in the thermal inactivation data for *E. coli* of Reichart (1994). For isothermic conditions, a seven level pH, and three level a_w was studied. A minimum of six (maximum of 11) levels of sterilising temperature, at each of four levels of pH and three levels of a_w were studied to generate the anisothermic data. These data of Reichart represent a "monumental amount of work indicative of the type of time consuming, painstaking effort required to generate sufficient data for predictive purposes" (McMeekin *et al.*, 1993). The cost associated with generating bacterial death (and growth) data is undoubtedly a significant contributing factor in the trend to increasingly restricted access to data, mentioned earlier.

Table 2. Statistical summary for anisothermic destruction of *E. coli*

$$\ln(k)_{s^{-1}} = 86.49 - 0.3028 \times 10^5/T - 0.5470\text{pH} + 0.0494\text{pH}^2 + 3.067a_w^2$$

Coefficient	Estimate	Standard error	T statistic	P value
C_0	86.49	4.626	18.70	0
C_1	−0.3028	0.0155	−19.51	0
C_2	−0.5470	0.0982	−5.572	0
C_3	0.0494	0.0082	6.053	0
C_4	3.067	0.5771	5.314	0
$\%V$ = 80.7				
Source of variation	df	Sum of squares	Mean square	F ratio
Model	4	31.2689	7.81723	98.9762
Error	90	7.10828	0.0789808	
Total	94	38.3772		

Table 3. Fit of the anisothermic model for combined T and pH on the destruction rate of *E. coli* at each of three values of water activity

$$\ln(k)_{s^{-1}} = C_0 + C_1/T + C_2\text{pH} + C_3\text{pH}^2$$

Water activity	Coefficient				$\%V$
	C_0	$C_1 \times 10^{-5}$	C_2	C_3	
0.995	83.65	−0.2837	−0.3668	0.0268	95.3
0.956	102.2	−0.3437	−0.9461	0.0916	94.8
0.928	119.6	−0.4000	−0.9942	0.0868	90.1

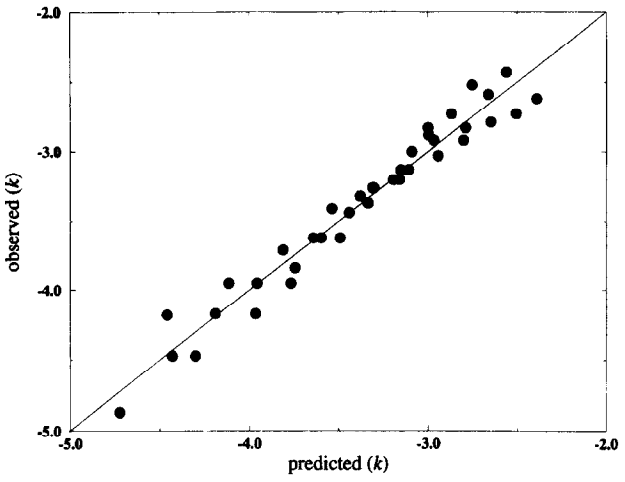

Fig. 3. Fit of the anisothermic model for the inactivation rate of *E. coli* (Eq. (7)) to the observed values, at a value of a_w = 0.995.

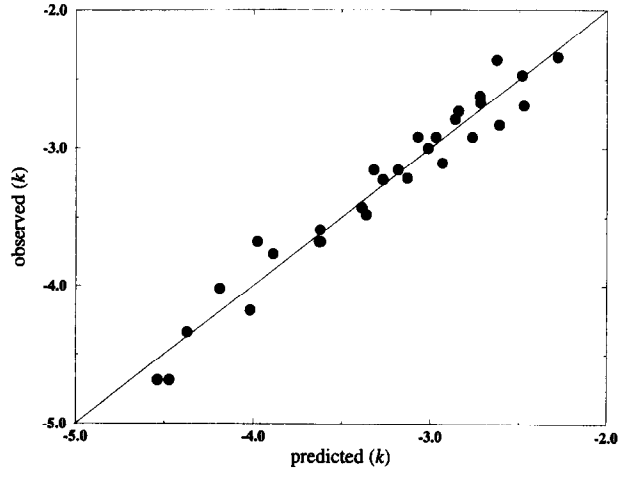

Fig. 4. Fit of the anisothermic model for the inactivation rate of *E. coli* (Eq. (8)) to the observed values, at a_w = 0.956.

The synthesised model form has been referred to as an additive, linear-Arrhenius (Davey, 1993*b*; Davey *et al.*, 1995), or, Davey, model (McMeekin *et al.*, 1993; Buchanan, 1993; Skinner *et al.*, 1994). This is because it is Arrhenius in form, in $1/T$ (K), with the other environmental factors apparently "added in" and because the model form is described as linear in statistical terminology (Davey, 1992*b*, see also Baranyi & Roberts, 1992; Whiting & Buchanan, 1993). The synthesised model therefore reflects an Arrhenius form, modified, i.e. shifted on an axis, by the effect of the other environmental factors.

It is perhaps significant that for all available inactivation data, those of Reichart (1994), Davey *et al.* (1995) and, of Xezones & Hutchings (1965), as analysed by Davey *et al.*, 1978), a $1/T^2$ term in the models, has not proven statistically significant, although this term is used widely with bacterial growth models (McMeekin *et al.*, 1993; Davey, 1993*b*). This implies that little, or more strictly no, curvature is apparent when inactivation data

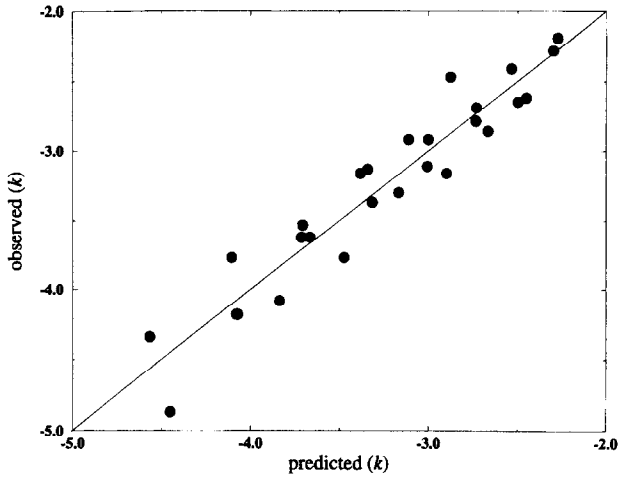

Fig. 5. Fit of the anisothermic model for the inactivation rate of *E. coli* (Eq. (9)) to the observed values, at a_w = 0.928.

is plotted as $\ln(k)$ vs $1/T$ (K). Because values of k are essentially secondary data, that is they are obtained from survival curves, this is not to say that there is no curvature, e.g. shoulder or tail (Cerf, 1977; Casolari, 1994), in the raw survivor data (plotted as the number of survivors against reciprocal of the inactivation temperature). Inactivation is, usually, a very much faster process than is bacterial growth, where typically growth is over hours or even days. The shorter exposure (holding) times involved in inactivations compared with times in bacterial growth, together with the entrenched view of a log–linear (i.e. first order) process with inactivation temperature, may mask any non-linear temperature effects on the derived values of k. Davey *et al.* (1995) and also Casolari (1994) have pointed to the need for such a reassessment. It is not considered sufficient that, in the many cases, model predictions in inactivation are conservative if based on log–linear assumptions.

It is evident the synthesised model provides a high degree of accurate fit to the thermal inactivation data of *E. coli*. Readers should note as an important feature of the model the consistent sign on the coefficients for the anisothermic studies in equations 7–9: C_0 and C_3 have a positive sign, and the remaining two coefficients have a negative one.

Table 4 summarises, for the anisothermic data, the value of the residuals, i.e. the experimentally observed value minus the predicted value of the inactivation rate (k), for the range of inactivation temperature, pH and a_w. From these tabulated values it can be seen that at a_w = 0.956, the model under predicts the value of the inactivation rate at values of pH in the range 3 to 5, with sterilising temperatures spanning the range 55 to 60°C. This is not readily apparent from Figure 4 (fit of the model at a_w = 0.956). These tabulated values serve to highlight that the model, when applied in this region of sterilising temperature and pH, should be done with (limited) qualification.

Table 4. Value of the residual (experimentally observed value minus the predicted value) of the inactivation rate (k) for the anisothermic destruction of *E. coli*

n	a_w	pH	T (°C)	k pred. (s^{-1})	Residual	n	a_w	pH	T (°C)	k pred. (s^{-1})	Residual
1	0.995	3	54.70	0.0179	0.0036	49		5	60.20	0.0479	−0.0171
2		3	55.65	0.0233	0.0034	50		5	60.95	0.0587	−0.0188
3		3	56.25	0.0276	0.0068	51		5	61.60	0.0701	−0.0255
4		3	56.95	0.0335	0.0102	52		5	62.15	0.0813	−0.0273
5		3	57.55	0.0396	0.0165	53		5	62.65	0.0930	−0.0340
6		3	58.05	0.0455	0.0197	54		5	63.10	0.1049	−0.0370
7		3	58.05	0.0515	0.0290	55		7	56.00	0.0165	−0.0034
8		5	53.75	0.0101	0.0014	56		7	57.25	0.0233	0.0019
9		5	55.05	0.0146	0.0047	57		7	59.35	0.0416	0.0008
10		5	56.25	0.0204	0.0064	58		7	60.15	0.0517	0.0022
11		5	57.25	0.0269	0.0093	59		7	60.90	0.0635	0.0018
12		5	58.10	0.0340	0.0068	60		7	61.60	0.0767	0.0177
13		5	58.85	0.0418	0.0121	61		9	52.45	0.0098	−0.0006
14		5	59.50	0.0500	0.0040	62		9	53.85	0.0146	0.0007
15		5	60.10	0.0589	0.0025	63		9	55.10	0.0208	0.0044
16		5	60.65	0.0684	−0.0032	64		9	56.20	0.0283	0.0113
17		5	61.10	0.0773	−0.0045	65		9	57.15	0.0368	0.0171
18		7	53.50	0.0103	0.0050	66		9	57.95	0.0460	0.0233
19		7	54.80	0.0148	0.0044	67		9	58.70	0.0565	0.0281
20		7	55.95	0.0205	0.0040	68		9	59.35	0.0676	0.0288
21		7	57.00	0.0275	0.0056	69	0.928	3	55.05	0.0133	−0.0056
22		7	57.90	0.0352	0.0032	70		3	56.05	0.0176	−0.0023
23		7	58.65	0.0433	0.0004	71		3	57.05	0.0232	0.0036
24		7	59.30	0.0518	−0.0035	72		3	57.95	0.0298	0.0126
25		7	59.90	0.0610	−0.0020	73		3	58.70	0.0367	0.0173
26		7	60.40	0.0700	0.0051	74		3	59.35	0.0438	0.0408
27		7	60.80	0.0780	0.0101	75		5	58.35	0.0246	−0.0076
28		9	52.05	0.0111	−0.0034	76		5	59.35	0.0323	−0.0093
29		9	53.15	0.0151	−0.0037	77		5	60.20	0.0408	−0.0039
30		9	54.05	0.0195	−0.0041	78		5	60.95	0.0500	−0.0075
31		9	54.90	0.0248	−0.0094	79		5	61.60	0.0596	−0.0023
32		9	55.65	0.0306	−0.0114	80		5	62.20	0.0701	0.0028
33		9	56.30	0.0367	−0.0099	81		5	62.70	0.0801	0.0318
34		9	56.90	0.0434	−0.0113	82		7	56.10	0.0144	−0.0014
35		9	57.40	0.0499	−0.0115	83		7	57.35	0.0204	0.0027
36		9	57.85	0.0565	−0.0157	84		7	58.45	0.0276	0.0017
37		9	58.25	0.0631	−0.0133	85		7	59.45	0.0364	0.0074
38		9	58.60	0.0694	−0.0104	86		7	60.40	0.0471	0.0068
39	0.956	3	55.10	0.0158	−0.0067	87		7	61.15	0.0578	0.0101
40		3	56.20	0.0216	−0.0036	88		7	61.70	0.0670	0.0228
41		3	57.15	0.0281	−0.0050	89		9	55.30	0.0187	−0.0034
42		3	58.00	0.0355	−0.0079	90		9	56.40	0.0254	0.0014
43		3	58.75	0.0437	−0.0113	91		9	57.35	0.0331	0.0013
44		3	59.40	0.0522	−0.0098	92		9	58.20	0.0419	0.0027
45		3	59.95	0.0607	−0.0109	93		9	58.95	0.0515	0.0099
46		3	60.45	0.0695	−0.0081	94		9	59.60	0.0615	0.0091
47		3	60.90	0.0786	−0.0058	95		9	60.15	0.0715	0.0308
48		5	59.35	0.0380	−0.0128						

Table 5. Comparison of the fit of the Reichart, and newly synthesised Davey, models for the thermal inactivation of *E. coli* as effected by combined *T*, pH and a_w

Model	Isothermic data		Anisothermic data	
	Reichart	Davey	Reichart	Davey
Terms (N)	3	3	4	4
$\%V$	95.6[a]	96.7	81.1[b]	80.7

[a] Calculated from Table 4 of Reichart (1994).
[b] Calculated from Table 10 of Reichart (1994).

The synthesised model is empirical because, in common with many microbiological models, it has as yet no theoretical foundation. However, our findings here, taken together with those of Davey *et al.* (1995) and Davey *et al.* (1978), suggest the model is of a general, or perhaps, universal form. Further investigation with robust inactivation data is needed to confirm this. A possible form, suggested on the basis of this and other studies in bacterial growth (Davey, 1993*b*, 1994; McMeekin *et al.*, 1993) is:

$$\ln(k) = C_0 + C_1/T + \sum_{i=2}^{j}(C_{2i-1}F_i + C_{2i}F_i^2) \qquad (10)$$

where, in addition to inactivation temperature T, j environmental factors (F), pH, a_w etc., act in combination to effect cell destruction. The absolute value (ABS) of each of the resulting coefficients however might not be greater than zero; see, for example, eqns 5 and 6 for a_w.

Table 5 provides a direct comparison of the fit of the Reichart, and newly synthesised Davey, models. Both model forms contain an equal number of coefficients for the isothermic and anisothermic data, and appear to give the same high degree of accurate fit, as is seen in the comparison of values of $\%V$. They both can be described as parsimonious, thus fulfilling an important criteria of a good model in predictive microbiological process modelling (Davey, 1993*c*) or predictive microbiology (McMeekin *et al.*, 1993). The value of the model coefficients of the new model can be obtained relatively more easily, and by unsophisticated users, than for the Reichart form however. An extension of the Reichart model so as to include further environmental factors acting in combination would seem to be a more difficult proposition than that for the new model where these could, based on present knowledge, be readily added in as implied through eqn 10. The value of these model coefficients in the new model form are determined by (simple) linear regression. The Reichart model, in contrast to the linear-Arrhenius, does nevertheless represent an attempt to develop a model based on an (flexible) understanding of current knowledge.

Figure 6 presents a three-dimensional plot of the surface of the anisothermal inactivation model of *E. coli* (eqn 6) over the range of experimental inactivation temperature, pH and a_w. This figure helps one to visualise the synthesised form and illustrates the smooth nature of the predicted surface of the model. Equations 7–10 will have a similar surface. Figure 6 highlights clearly the increasing value of k (greater rate of destruction of *E. coli*) with increasing temperature and, value of the a_w, and the quadratic (curving) effect of pH. This quadratic dependence of the inactivation rate on pH indicates a minimum value of k with pH (at a given T and a_w). This value is obtained from setting the first derivative of the model equation to zero to obtain: $pH_{kmin} = -C_3/2C_4 = 5.5$ for these data.

CONCLUSIONS

An additive, linear-Arrhenius form provides an accurate model for the prediction of the combined effect of three environmental factors on the thermal inactivation of *E. coli*: sterilising temperature, pH and water activity.

The model is advantageous over an existing one in that it is easier to formulate (i.e. to obtain values of the

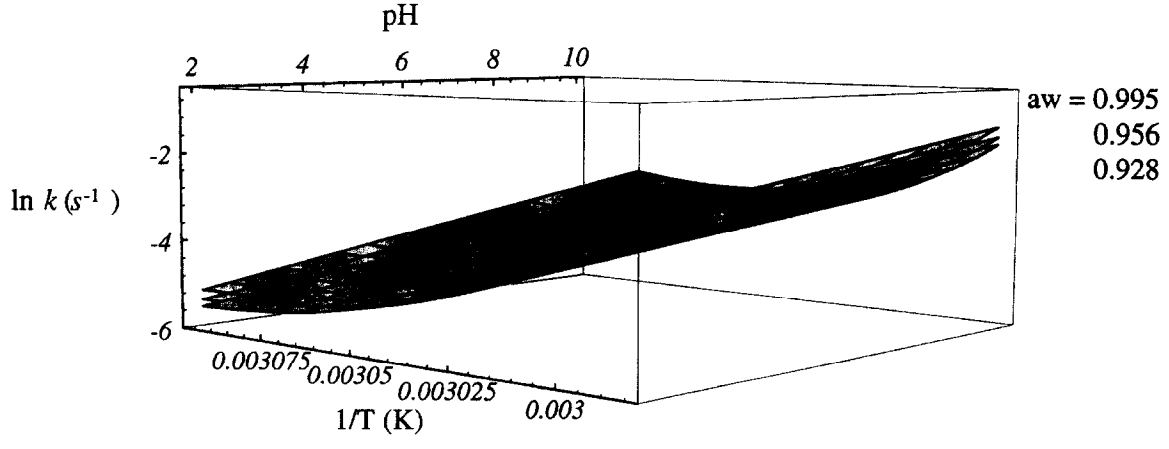

Fig. 6. Predicted surface of the thermal inactivation rate model (Eq. (6)) for the anisothermic death of *E. coli* at three values of water activity (a_w).

coefficients of the model for different media) and to use. Its simpler form appears to be more readily integrated with equations describing the viscous and thermal properties in a steriliser operation.

A universality of the model form is suggested when findings from this study are taken together with those from earlier studies of the influence of combined temperature and pH on thermal inactivation. It seems, at this stage, that a sort of additivity principle is apparent in which the effect of environmental factors on bacterial inactivation can be summed in the way demonstrated in the model. There are as yet insufficient, published and independent data to test this.

The model should be of practical use in assessing a range of inactivation conditions and could be extended to include a number of environmental factors where reliable data are available.

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