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Linear-Arrhenius models for bacterial growth and death and vitamin denaturations

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SUMMARY

The development of the quantitative, linear-Arrhenius model of Davey for predicting bacterial growth and death (inactivation) is reviewed. The applicability of the model to published data from independent researchers for both the growth phase and lag phase, involving combined environmental factors (T, a_w) is illustrated. Also illustrated is its applicability to thermal inactivation kinetics and vitamin denaturation (with combined T, pH). Integration of the model to produce complex models describing the thermal sterilization of liquid is demonstrated. Advantages of the model, including its simplicity and the fact that the coefficients to build the model can be obtained easily by relatively unsophisticated users, are highlighted in a comparison with other models.

INTRODUCTION

The relationship between a bacterium and its environment is a complex one. It is possible, however, to differentiate both a medium phase and a cellular phase. It is useful to classify the cellular phase according to the number of components used in its representation [3]. In reality, the cellular phase is multicomponent, that is, the cells exist as a heterogeneous mix of discrete entities in the medium. Alternatively, they may be represented by an 'average cell', that is, viewed as a single component in the media. Multicomponent models are termed *Structured*, whilst single component models are termed *Unstructured* [3,9]. Further, discrete heterogeneous cells constitute a *Segregated* view, and average cellular properties constitute an *Unsegregated* one. If cell heterogeneity does not significantly influence behavior, an 'average cell' approximation can be used to simplify a *Segregated* to an *Unsegregated* view [3]; however, the reality is a *Structured, Segregated* one, as is indicated in Fig. 1.

This idealized view (*Unstructured, Unsegregated*) is the one most widely used, especially by researchers in the general field of predictive microbiology. A number of these model approaches have been categorized by Davey [12,14], one of which is the linear-Arrhenius form.

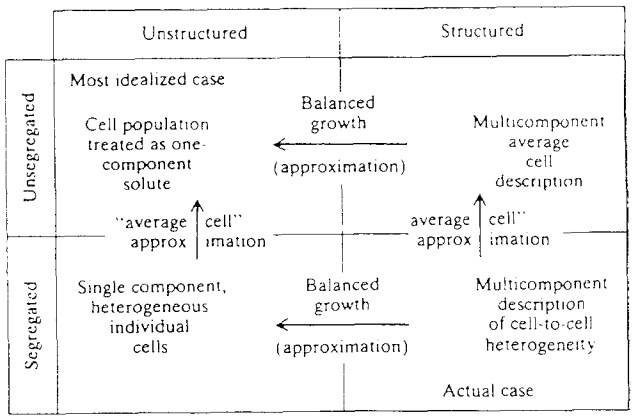

Fig. 1. Perspectives for cell population kinetic representations.

In this paper, the development of the linear-Arrhenius model of Davey is reviewed. Its application to bacterial growth (both growth and lag phase) involving combined environmental factors is illustrated. Also, its applicability to describe thermal inactivation kinetics and denaturation of water-soluble vitamins. Integration of the model with equations describing the continuous, isothermal sterilization of liquid is demonstrated. Advantages of the model are highlighted in a comparison with other models.

MATERIALS AND MODELS

Model development

In the late 1970s a study of continuous-process sterilization of liquid was undertaken [11]. Investigations were initially directed toward the combined influence of the residence-

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time distribution of bacterial cells and temperature. The holding time necessary for a desired reduction in the number of bacteria was determined by the length of sterilizer (all other variables being constant). It was clear from published data a major influence on bacterial kinetics, pH of the medium, was not accounted for.

Thermal inactivation (= death) kinetics are widely modeled by a first-order, or exponential, reaction [2,3] such that:

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

or, as it is usually written:

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

where t = time, N_0 = number of viable microorganisms at t = zero, N = number of viable microorganisms at $t = t$, and k = rate coefficient (with units of time^{-1}). The effect of temperature on the rate coefficient can be represented by the Arrhenius equation:

$$k = A \exp\left(\frac{-E}{RT}\right) \tag{3}$$

where T = absolute temperature, R = gas constant, E = activation energy, and A = frequency factor. Eqn. 3 reveals that a plot of ln k vs $1/T$ should yield a straight line.

The time required to reduce the number of viable microorganisms to 1/10th of the original number, the decimal reduction time D_T [2], is widely employed. It is easy to show that:

$$D_T = \frac{2.303}{k} \tag{4}$$

i.e., that the decimal reduction time is a form of k.

The published decimal reduction time data of Xezones and Hutchings [34], plotted as ln k vs $1/T$ for different pHs, revealed the dependence of the rate coefficient on pH. Fig. 2 shows these data for *Clostridium botulinum* inactivation in spaghetti, tomato sauce and cheese. Coincidently, a similar dependence of the rate coefficient on pH for thiamine denaturation is apparent in a plot of the data of Feliciotti and Esselen [20], Fig. 3. Comparison of Fig. 2 and Fig. 3 shows the heat resistance of the spores decreases by a factor of about 4.5 in going from pH 7 to pH 4, whilst thiamine denaturation increases by a factor of nearly 22 for a similar change in pH. These trends imply that maximum sterilization with minimum vitamin damage could be achieved by processing at low pH.

This combined effect of temperature and pH on the rate coefficient was explained in the development of an Arrhenius model of the form:

$$\ln k = C_0 + C_1/T + C_2\,\mathrm{pH} + C_3\,\mathrm{pH}^2 \tag{5}$$

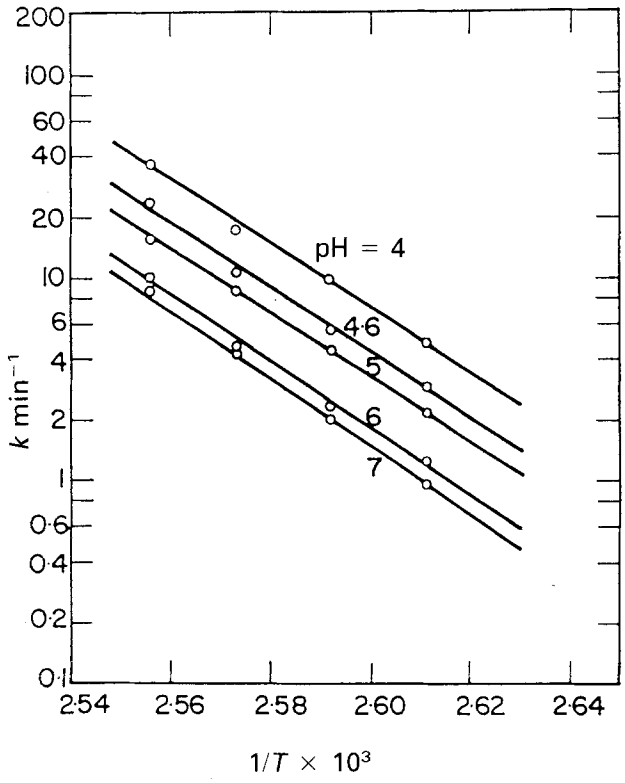

Fig. 2. Plot of the rate coefficient for *Cl. botulinum* inactivation in spaghetti, tomato sauce and cheese against $1/T$ for different pHs.

Figs 4 and 5 show, respectively, a comparison of the predictions [16] of Eqn. 5 (with appropriate values of the model constants, C_0–C_3) against the published data for the rate coefficient for *Cl. botulinum* inactivation, and thiamine denaturation, against pH for different temperatures.

Meat is readily spoiled by microorganism growth because of its high moisture and nutrient content. Since chilling is used to preserve fresh meat, a mathematical model of the air chilling of freshly slaughtered carcass meats was developed (CSIRO Division of Food Processing, Meat Research Laboratory, Brisbane, unpublished data).

During chilling, the temperature of the meat surfaces decrease. Initially, the surface water activity (a_w) decreases also, but the meat surface 're-wets' during the later part of a conventional chilling cycle. It was important to account for these changes in a_w in an integrated form to establish the potential growth of bacteria of a public hygiene significance.

In observing published data from independent researchers spanning 50 years, the same dependence of the rate coefficient on combined temperature and the variable a_w was noted [12]. A $1/T^2$ term accounted for observed 'curvature' in an Arrhenius plot. The model developed for combined temperature–a_w for these growth data is:

$$\ln k = C_0 + C_1/T + C_2/T^2 + C_3\,a_w + C_4\,a_w^2 \tag{6}$$

A comparison of the model predictions of Eqn 6 (with appropriate values for the constants, C_0–C_4) against the

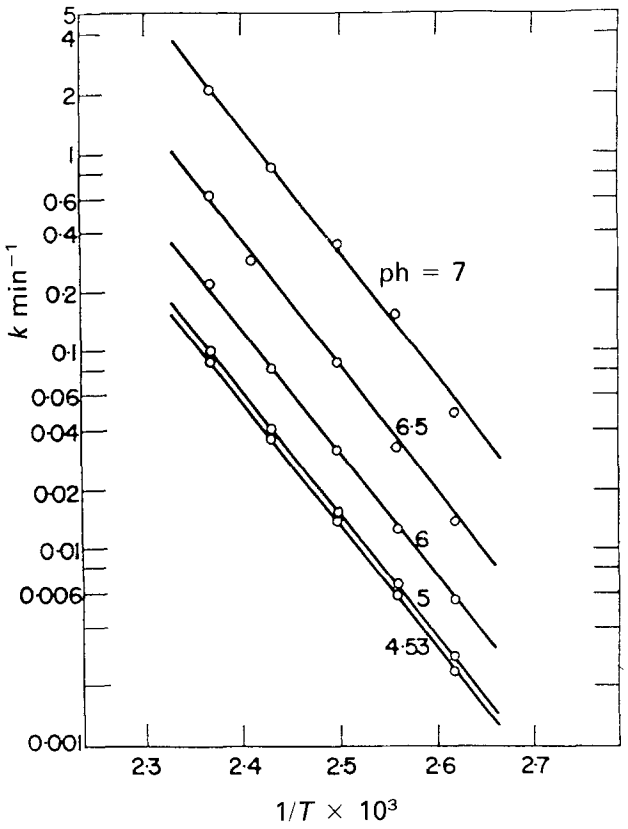

Fig. 3. Plot of the rate coefficient for thiamine denaturation against $1/T$ for different pHs.

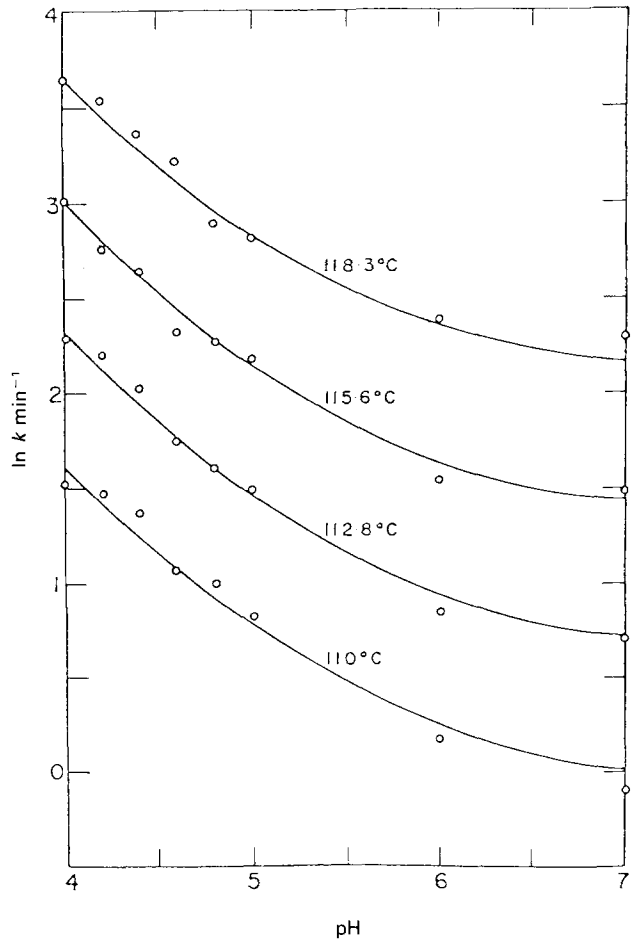

Fig. 4. Plot of the rate coefficient for *Cl. botulinum* inactivation in spaghetti, tomato sauce and cheese against pH for different temperatures.

experimental data of Broughall et al. [6] for different water activities is shown in Fig. 6.

A measure of 'goodness of fit' of the model, the percent variance accounted for (%V), was adopted [12,13]. This is given by [32]:

$$\%V = 1 - \frac{(1-r^2)(n-1)}{(n-N_T-1)} \tag{7}$$

where r^2 = the multiple regression coefficient, n = the number of data, and N_T = the number of terms in the model. (N_T = 4 for Eqn 6, namely, 1/T; $1/T^2$; a_w, and a_w^2.) It is seen that the %V is more appropriate than is r^2 for few data where a high value of r^2 might give the impression of too good a fit. Eqn 7 reveals that at $n \gg N_T$, $\%V \approx r^2$.

The hypothesis that the model might also explain the lag phase of bacterial growth was examined [14]. It is important to realize that any model simulation of chilling done only with the growth phase component would be over-conservative. Growth would not begin immediately. For lag phase data the model developed is given by:

$$\ln(1/\text{lag time}) = C_0 + C_1/T + C_2/T^2 + C_3 a_w \tag{8}$$

It should be noted that it is the reciprocal of the lag time which is used. This is done to maintain consistent units (of time^{-1}) in handling both growth phase and lag phase data [14].

The model explained a wide range of independently published sets of lag phase data [14]. An illustration is given in Table 1 which compares predicted with published experimental values of the lag time data of Mackey and Kerridge [24] with temperature as the sole factor.

RESULTS

The value of the relevant model coefficients for a wide range of independently published data, together with the % variance accounted for, are presented in Tables 2–4.

A summary of the model fit to the published data for thermal inactivation of *Cl. botulinum* and that for thiamine (vitamin B_1) denaturation, is given in Table 2. Also shown are previously unpublished model fits to aerobic and anaerobic denaturation of ascorbic acid (vitamin C).

Tables 3 and 4 summarize the model fit to published data for the growth phase and lag phase, respectively.

The predictive equation for the rate coefficient is obtained by substituting for the coefficients from the appropriate

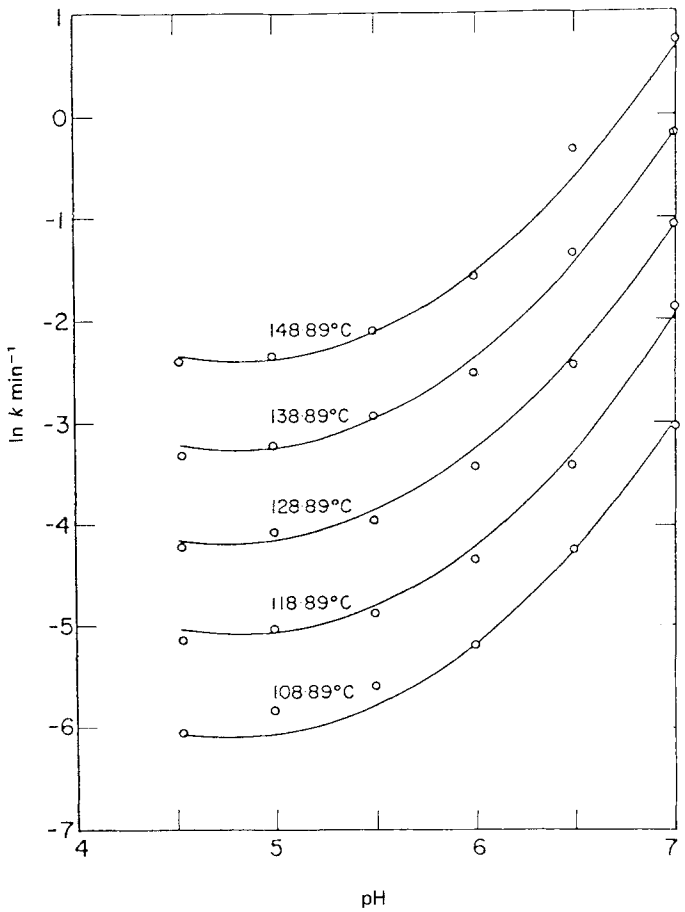

Fig. 5. Plot of the rate coefficient for thiamine denaturation against pH for different temperatures.

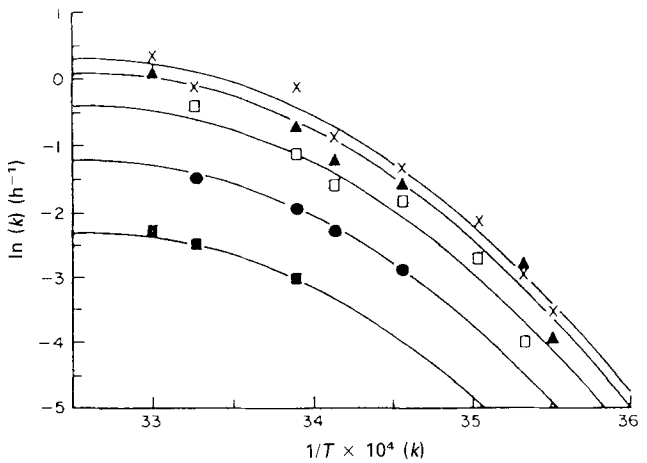

Fig. 6. Fitted lines of model prediction for the rate coefficient to the data of Broughall et al. [6] for various water activities: ×, 0.98; ▲, 0.97; □, 0.96; ●, 0.95; ■, 0.94.

table. For example, from Table 3 the model predictions for the data of Broughall et al. [6] are obtained from:

$$\ln k(\mathrm{h}^{-1}) = -1904.3 + 2.8434 \times 10^5/T - 4.3618 \times 10^7/T^2 + 2935.7\, a_w - 1495\, a_w^2 \quad (9)$$

Integration with other models

The significant and simple relationship revealed by the model permits ready integration with other equations in a range of processing operations, including sterilization in continuous flow, the batch chilling of carcass meats etc. This was demonstrated by Davey [11,16] who quantified the influence of pH on the design characteristics of a continuous flow, tubular sterilizer. The predictive equations for the rate coefficient were included with those for heat and mass transfer and fluid velocity profiles (residence time distribution). The results are summarized in Fig. 7 for sterility requirements (N/N_0) of 10^{-12} and 10^{-14}. Because of the non-linear variation of the rate coefficient with pH, the magnitude of the necessary sterilizer length increases with a rise in pH. A change in pH from 4 to 7 is shown to require a nearly five-fold change in sterilizer length (= holding time).

TABLE 1

Comparison of the observed with predicted data of Mackey and Kerridge [24] for *Salmonella* (high inoculum) in minced beef

Temperature (°C)	Lag time (h)	
	Observed	Predicted
10	40.6	41.3
15	10.8	10.4
20	4.2	4.1
25	2.3	2.5
30	2.3	2.2
35	2.6	2.6

ln (1/lag time) = $-623.4 + 3.7668 \times 10^5/T - 5.6970 \times 10^7/T^2$.
T in degree absolute.

DISCUSSION

From the foregoing, it is apparent that the generalized model is of the form:

$$\ln k = C_0 + C_1 V_1 + C_2 V_1^2 + C_3 V_2 + C_4 V_2^2 \quad (10)$$

where V_1 and V_2 are, respectively, environmental factors including the reciprocal of the absolute temperature, a_w or pH.

It is also apparent that predictions obtained using this model are in very good agreement with published experimental data spanning a wide range of independent researchers, media and cell types. These data include both behavior of bacteria (growth and death) and water-soluble vitamin. It is noteworthy that the bacterial data include Gram-negative and Gram-positive bacteria and psychotrophs and mesotrophs, rods and cocci, and spores.

The model fits were obtained using least squares regression [17]. This is something which can be done by unsophisticated users on most desktop calculators and computers. In contrast, the non-linear Arrhenius models of Schoolfield et al. [28], or of Broughall et al. [6] and the non-linear, multiplicative square root model of McMeekin et al. [25] require a much more complex non-linear interative fitting procedure. A comparison with these models has been given elsewhere [13].

TABLE 2

Rate coefficient for *Cl. botulinum* inactivation and water-soluble vitamin denaturation: $\ln k = C_0 + (C_1/T) + C_2\,pH + C_3\,pH^2$

System	C_0	$C_1\times10^{-4}$	C_2	C_3	% Variance accounted for	n	Range		Reference
							T (°C)	pH	
Cl. botulinum in									
spaghetti, tomato sauce and cheese	105.23	−3.7041	−2.3967	0.1695	99.2	32	110–118.3	4.0–7.0	[34]
macaroni creole	104.18	−3.6422	−2.6170	0.1871	99.2	32	110–118.3	4.0–7.0	[34]
Spanish rice	107.56	−3.7511	−2.7940	0.2032	99.3	32	110–118.3	4.0–7.0	[34]
thiamine in									
phosphate buffer	41.478	−1.4808	−4.1027	−0.4678	99.0	35	108.9–1489	3.5–7.00	[20]
ascorbic acid in									
acetate and phosphate buffers	8.2564	−0.50985	1.2434	−0.1222	95.8	23	59.5–85.0	3.52–6.60	[5]
anaerobic system	25.951	−0.9789	−0.7687	0.03269	93.7	12	77.75–95.75	0.38–11.38	[21]

k in min^{-1}. T in degree absolute.

TABLE 3

Fit of the model for rate coefficient during microorganism growth phase: $\ln k = C_0+(C_1/T))+(C_2/T^2)+C_3a_w+C_4a_w{}^{2*}$

Microorganism	C_o	$C_1\times10^{-5}$	$C_2\times10^{-7}$	C_3	C_4	% Variance accounted for	n	Range		Reference
								T (°C)	a_w	
Pseudomonas† on ox muscle	−919.54	1.6033	−2.3784	1317.7	−669	99.0	15	−1–30	0.960–0.993	[29]
Aerobacter aerogenes	−1595.4	3.1597	−4.8378	2149.2	−1069	94.0	18	15–37	0.950–0.975	Wodzinski and Frazier [33] cited by [10]
Pediococcus cerevisiae strain PC4 on GTY media	−1406.0	2.9553	−4.5427	1862.2	−937	92.2	16	24–42	0.944–0.998	[22]
Clostridium botulinum type E strain 103 in NYG media	−2227.8	0.47991	−0.84488	43391.1	−2177	97.9	15	15–30	0.970–0.995	[27]
Microbacterium thermosphactum 22 on APT media	−1347.5	1.3712	−2.0789	2278.7	−1158	98.0	18	0–25	0.930–0.990	[8]
Salmonella typhimurium in UHT milk	−1904.3	2.8434	−4.3618	2935.7	−1495	96.9	28	9–30	0.94–0.98	[6]
Staphylococcus xylosus	−550.95	−2.5162	−3.8381	285.69	−147	97.2	185	7–27	0.848–0.996	[25]

*k in h^{-1}. T in degree absolute.

†Scott [29] and Empey and Scott [18] reported this organism as *Achrombacter* 7 but it was subsequently identified as *Pseudomonas* by Brown and Weidemann [7].

TABLE 4

Fit of the lag phase model for temperature as the sole factor:
ln (1/lag time) = $C_0 + (C_1/T) + (C_2/T^2)$*

Microorganism	C_0	$C_1 \times 10^{-5}$	$C_2 \times 10^{-7}$	% Variance accounted for	n	Range T (°C)	Reference
Coliforms in blended meat	−312.0	1.9492	−3.0428	98.9	8	8.2–40	[31]
Escherichia coli SF in blended meat	−403.7	2.4792	−3.8083	99.2	8	8.2–40	[31]
Salmonella typhimurium in blended meat	−382.9	2.3808	−3.7001	96.0	7	10–40	[31]
Salmonella in minced beef (high inoculum)	−623.4	3.7668	−5.670	99.7	6	10–35	[24]
Clostridium botulinum on cooked turkey	61.30	−0.19343	†	80.8	5	15–30	[1]
Gram-negative spoilage flora in raw beef mince	−146.9	0.91793	−1.4460	98.3	10	−2–30	[1]
Pseudomonas fluorescens in ice-cream mix	50.27	−0.15271	†	98.0	6	8.0–25	[1]
Staphylococcus aureus in raw shortcrust pastry	−443.3	2.7437	−4.2562	95.0	13	10.6–45.6	[1]

*Lag time in h. T in degree absolute.
†$1/T^2$ term not significant.

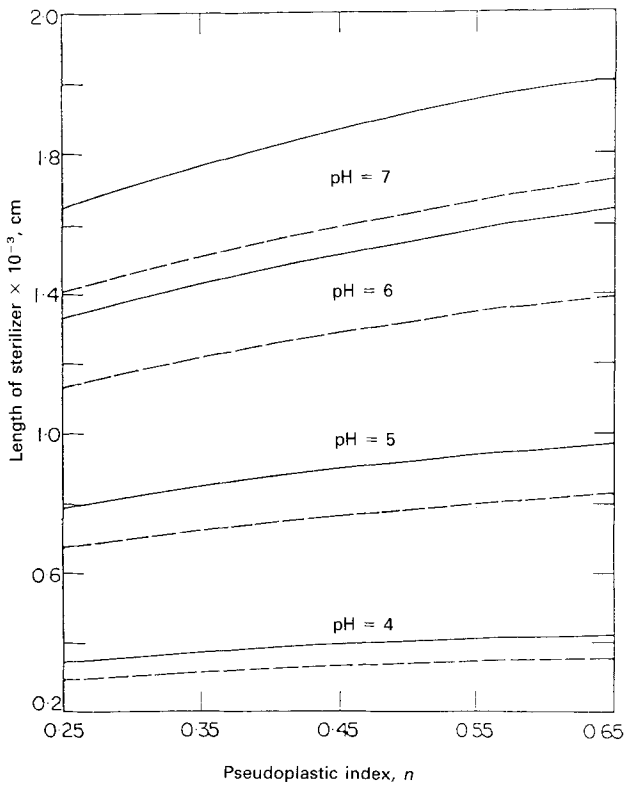

Fig. 7. Effect of pH and sterility requirement on sterilizer length at an operating temperature of 121 °C and a bulk velocity of 10 cm s^{-1}: —— for sterility requirement of 10^{-14}; – – – for sterility requirement of 10^{-12}.

The model is described as linear-Arrhenius, because it is a modified Arrhenius equation, and linear, because in statistical terminology, it is a linear model. A plot of ln k vs $1/T^2$, pH^2, etc. gives a straight line. The need for a uniform approach to terminology for models is addressed elsewhere [15].

The model expresses that the influence of environmental factors (temperature and/or a_w and pH) are 'additive'. The very good agreement of the model with these wide ranging and diverse data strongly suggests environmental factors act independently on cell growth and thermal inactivation, and also on vitamin denaturation. That environmental factors act independently on cell growth is also revealed by the model approaches of McMeekin et al. [25] and Broughall et al. [6]. It is interesting to observe that this finding (together with yet unpublished findings on the appropriateness of the model) suggests a parallel with the 'hurdle effect' developed by Leistner [23]. The so-called 'interchangeable(ness)' of the environmental factors in hurdle theory suggests that these factors act independently on microbial activities. The linear-Arrhenius model presented here could, therefore, be a useful means to quantify these 'hurdles'.

The values of the coefficients tabulated for the model were all significant. Testing indicated that there were no significant interaction terms, such as: $1/T^*a_w$; $1/T^{2*}pH^2$; etc. There appeared no structure left in the residuals from the model, suggesting that improvement in the model (for these data) was unlikely.

No physiological interpretation has been offered for the significance of the value of the regression coefficients. Clearly one or more of the coefficients can have a zero value. This contrasts with the approaches of Broughall et

al. [6] and McMeekin et al. [25]. Broughall et al. [6] uses regression coefficients to define 'an enthalpy of activation' for cell growth at higher and lower temperatures. McMeekin et al. [25] forced a common coefficient for individual water activity data which they described as a 'theoretical minimum temperature (T_{min})' for cell growth.

Because the linear-Arrhenius model is 'quadratic' in terms of the environmental factors, it should be realized that the minimum data that can be fitted must involve at least three values of each factor. Much published data are not suitable for model fits of combined environmental factors, for example, the data of Scott [30], only one temperature was reported for a wide range of a_w, and the data of Baird-Parker et al. [4] because only one temperature was reported for a range of a_w or only one a_w was reported for a range of temperatures.

Extrapolation of data through predictions from the model must be done with caution. The model, for example, does not predict limiting values of a_w or temperature or pH. However, the very good agreement between model predictions and the published data suggests limited extrapolation may be done without introducing large errors.

As noted by McMeekin et al. [26], some attempts have been made to use 'broken' curves to improve the fit of simple Arrhenius models. This was done by defining an 'activation term' as a function of T_{min}. Davey [13] found no justification in the necessary truncating of data at low or high temperatures to include such a term. The present author believes that because of the *Unstructured* and *Unsegregated* nature of these models such approaches can only be qualitative. After all, the models implicitly assume an idealized cell phase which is represented by an 'average cell'.

Because Arrhenius based his model on an analysis of single-reaction-rate theory, some researchers felt that it was not appropriate to model the complexities of a living cell. Arrhenius initially considered E (Eqn. 3) to represent the energy difference between the reactants and the activated species of the reacting molecules [19]. Later he modified the form and replaced E with μ, the *temperature characteristic* of the process. Despite that E and μ were considered constants, biological processes characteristically gave rise to non-linear or 'curved' Arrhenius plots. The excellent agreement between predictions of the modified model and these extensive data, nevertheless, suggests a rate-controlling step, analogous to reaction rate theory.

It might be expected that because of the *Unstructured* and *Unsegregated* nature of the model it could reasonably be expected to apply to a homogeneous mix of identical molecules of water-soluble vitamin (Table 2). That the model explains such diverse cell data (Tables 3–4) suggests that the concept of an average cell, although an idealization, is an adequate perspective for mathematical formulation.

The values of the 'average cell' coefficients for this, and other models will reflect such factors [12] as substrate, thermal history and laboratory technique. A comparison of model coefficients could be made all the more meaningful were laboratory techniques standardized.

The illustrated applications of the model demonstrate it is suitable for modeling the bacterial growth phase, and bacterial lag phase and denaturation of water-soluble vitamins. The model appears to be a 'simple, universal and significant relationship' [12].

CONCLUSIONS

The linear-Arrhenius model of Davey for the rate coefficient is a mathematical formulation of published experimental data. When applied to bacterial cells in defined media it is a model form described as *Unstructured* and *Unsegregated*.

It appears to be universally applicable for predicting the combined influence of environmental factors (especially T, a_w and pH) on both bacterial and water-soluble vitamin behavior in defined media.

The concept of 'additive' terms for each of the environmental factors is an appropriate form. This, together with its apparent simplicity and relative ease of use of the model, should result in it being of wide practical use.

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