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ORIGINAL ARTICLE

Temperature dependence of *F*-, *D*- and *z*-values used in steam sterilization processes

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Abstract

Aims: To develop a model, based on microbiological principles, to safely optimize steam sterilization processes.
Methods and Results: The minimum exposure time *F* for a decontamination process at a certain temperature is usually calculated from an empirical model with the decimal reduction time *D* and the temperature resistance coefficient *z* as parameters. These are implicitly assumed to be independent of temperature. Using a microbiological approach, it is shown that also *D* and *z* depend on temperature, indicating that the usual models provide only reliable results in a limited temperature region. The temperature dependence of *F* resulting from this approach describes the available experimental data very well. Safety margins to assure sterility can be included in a straightforward way.
Conclusions: The results from the present approach can be used to safely optimize decontamination processes. The corresponding mathematical model can be implemented rather directly in process control systems.
Significance and Impact of the Study: Our results show that for steam sterilization and disinfection processes the values of *F* predicted by the usual models largely underestimate the required minimum exposure times at temperatures below 120°C. This has important consequences for the optimization of such processes.

Introduction

To obtain sterile products, items have to be sterilized. To compare different decontamination processes, in practice a description in terms of minimum exposure times *F* is used (Van Asten *et al.* 1982; Russell *et al.* 1992; EN–ISO 15883 2006a). The value of *F* at a certain temperature is the minimum time that the organisms, present in or on an item, have to be exposed to a hostile environment to assure sterility of the item. Worldwide, sterility of medical devices is defined as the chance of finding a viable organism in or on a medical device to be at most 1 in 1 000 000 or a Sterility Assurance Level (SAL) of at most 10^{-6} (Allison 1999; Favero 2001; ANSI/AAMI ST67 2001; EN556 2004; EN–ISO 17665 2006b). Already many decades ago, Perkins reported temperature-time combinations for sterilization of aqueous liquids (Perkins 1956). A few years later, the Working Party on Pressure Steam Sterilizers of the Medical Research Council (MRC) adjusted these values to steam sterilization with 100% saturated steam by including safety margins (MRC 1959). The resulting temperature–time combinations are still in use, e.g. 121°C for 15 min and 134°C for 3 min. For a given temperature and time the value of *F* for a process can be calculated. This value should be equal to or larger than that corresponding to an accepted temperature-time combination, in order to assure sterility.

Apart from comparing steam sterilization processes, *F*-values are used to optimize such processes, in order to safe time, energy, money, or to reduce the exposure time of thermo-labile products to high temperatures. In most cases the exposure time to high temperatures is made as short as possible. This is done by taking into account the contribution of each part of the sterilization process to the inactivation of the organisms.

To calculate the value of F for other temperatures than those reported in the literature, empirical models are used with the decimal reduction time D (min) and the temperature resistance coefficient z (°C) as parameters. Values of D and z can be found in the literature (Van Asten *et al.* 1982; Russell *et al.* 1992). From calculations based on an Imaginary Micro Organism (IMO) concept and using the temperature–time combination of 120°C for 20 min (MRC 1959), Van Asten and Dorpema obtained $D = 3{\cdot}33$ min and $z = 17$°C (Van Asten *et al.* 1982). With this reference point they calculated generic temperature-time combinations for other temperatures. Values for D and z can also be calculated from two accepted temperature–time combinations. Using the values given by the MRC (MRC 1959), 121°C for 15 min and 134°C for 3 min, one obtains $D = 2{\cdot}5$ min and $z = 18{\cdot}6$°C. The values for F reported in the literature do not seem to be very consistent (Perkins 1956; MRC 1959; Van Asten *et al.* 1982; Russell *et al.* 1992). Therefore, we thought it worthwhile to investigate whether a straightforward microbiological approach can be used to obtain a reliable prediction for the temperature dependence of F. The theoretical framework is presented in the next section. In the subsequent section the predictions of the resulting model are compared with experimental temperature-time combinations (Perkins 1956), the combinations given by the MRC (1959), and the behaviour of F resulting from the two models mentioned above. The paper will be concluded with a discussion.

Theory

It is generally accepted that the most essential mechanism for sterilization of aqueous liquids is coagulation; the irreversible change and hardening of the protein chains of the micro-organisms (Sykes 1965). This process can be described in terms of chemical reaction kinetics (McKee and Gould 1988; Reichart 1994), in which the inactivation of organisms is given by

$$\frac{\mathrm{d}N}{\mathrm{d}t} = -kN, \tag{1}$$

with N the number of organisms, t the time, and k the specific inactivation constant for an organism. If the conditions required for steam sterilization are satisfied (Van Doornmalen and Kopinga 2008), the inactivation is identical to that in aqueous liquids, in which case the temperature dependence of k obeys the Arrhenius law:

$$k = A\exp\left(-\frac{E_\mathrm{a}}{RT}\right). \tag{2}$$

In this equation A represents a constant, E_a the activation energy for the inactivation reaction (J mol^{-1}), R the universal gas constant (8·31 J/(mol K)), and T the temperature (K).

If the population of organisms is exposed to a constant temperature, the number of living organisms after a certain time can be obtained from eqn (1):

$$N_e = N_0\exp(-kt) \text{ or } \ln N_e - \ln N_0 = -kt, \tag{3}$$

with N_0 the initial number of organisms and N_e the number of organisms that have survived the sterilization process. This equation shows that the time required to achieve sterility does not only depend on k but also on the initial number of organisms. In papers on sterilization theory (Bigelow 1921) and in the pharmaceutical industry (Sykes 1965; Russell *et al.* 1992), the $^{10}\log$ (referred to as log) is often used instead of the natural logarithm. In that case, eqn (3) reads:

$$N_e = N_0 10^{-k't} \text{ or } \log N_e - \log N_0 = -k't, \tag{4}$$

where $k' = k/\ln(10)$.

Often, a sterility criterion (S) is defined as $S = \log(N_0/N_e)$. Substitution of this criterion in eqn (4) shows that S is equal to the product $k't$. The minimum time (F) to satisfy the sterility criterion can be calculated with:

$$F = \frac{1}{k'}\log(N_0/N_e) = \frac{1}{k'}S. \tag{5}$$

The decimal reduction time D is defined as the time needed to reduce the number of organisms by a factor of 10, e.g., if $N(0) = N_0$ at $t = 0$, then at $t = D$ the value $N(D) = N_0/10$. Equation (4) shows that

$$D = 1/k' = \ln(10)/k \tag{6}$$

and the inactivation of a process can be written as:

$$\log N_e = -\frac{1}{D}t + \log N_0. \tag{7}$$

This equation illustrates that the inactivation can be represented by a straight line in a graph with a logarithmic N-axis (Bigelow 1921; Moats 1971; Briggs 1996).

By substituting eqn (6) in eqn (5) the minimum exposure time (F) for a given sterilization criterion S can be expressed as:

$$F = D\log(N_0/N_e) = DS. \tag{8}$$

The decimal reduction time $D = \ln(10)/k$ depends on the environment conditions. Since in sterilization of aqueous liquids the temperature dependence of k is exponential (see eqn 2), D has a similar temperature

dependence. In the literature (Korczynski 1980; Boom *et al.* 1984; Van Asten *et al.* 1982) the temperature dependence of D is described by a temperature resistance coefficient z (°C), the temperature increase required to reduce D by a factor of 10 with respect to its value D_{ref} at a temperature T_{ref}:

$$\log D = -\frac{1}{z}(T - T_{\mathrm{ref}}) + \log D_{\mathrm{ref}}, \tag{9}$$

or,

$$\log(D/D_{\mathrm{ref}}) = -\frac{1}{z}(T - T_{\mathrm{ref}}). \tag{10}$$

This equation shows that with a known value of z and a known D_{ref} at a certain temperature T_{ref}, values of D at other temperatures can be calculated. If z would be independent of temperature, a plot of $\log(D)$ against T would yield a straight line with a slope equal to $-1/z$. This approximation of D is represented by the dashed line in Fig 1. By substituting eqn (10) into eqn (8) the required exposure time (F) at a given temperature can be calculated:

$$F = F_{\mathrm{ref}} 10^{(T_{\mathrm{ref}} - T)/z}, \tag{11}$$

where F_{ref} is the minimum exposure time at $T = T_{\mathrm{ref}}$.

In principle, D and z may be different for different types of micro-organisms. This complication was taken into account in a straightforward way by Van Asten and Dorpema, who developed the so-called IMO concept (Van Asten *et al.* 1982). The IMO is by definition the most resistant micro-organism for the sterilization method used. By using the values for D and z corresponding to this organism, a safety margin in the sterilization time is implemented.

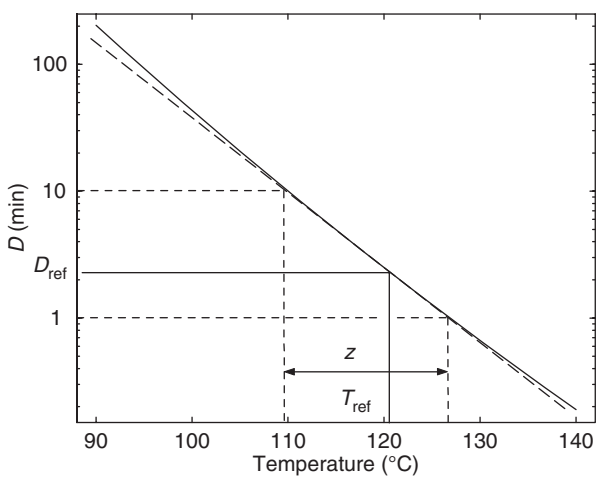

Figure 1 Temperature dependence of the decimal reduction time D. The resistance coefficient z (°C) is defined as the temperature increase needed to reduce D by a factor of 10. The dashed line reflects the approximation in which z is independent of temperature. The solid curve reflects the actual variation of the decimal reduction time D, with T_{ref} = 121°C and z_{ref} = 17°C, yielding a resistance coefficient z that depends on temperature.

A disadvantage of the traditional definition of the temperature resistance coefficient z (eqn 9) is the implicit temperature dependence of this coefficient. This means that z values reported in the literature should only be used in a limited range of sterilization temperatures around the temperature T_{ref} at which z has been determined. This can be demonstrated as follows. If we combine eqns (2) and (6), the decimal reduction time D can be written as:

$$D = \frac{\ln(10)}{A}\exp\left(\frac{E_a}{RT}\right). \tag{12}$$

Because eqn (2) is expressed in the absolute temperature (K), all temperatures have to be expressed in K.

The derivative of eqn (12) with respect to temperature is given by:

$$\frac{\mathrm{d}D}{\mathrm{d}T} = \frac{\ln(10)}{A}\exp\left(\frac{E_{\mathrm{a}}}{RT}\right)\left(-\frac{E_{\mathrm{a}}}{RT^2}\right) = -D\left(\frac{E_{\mathrm{a}}}{RT^2}\right). \tag{13}$$

For an arbitrary temperature T_{ref} this equation can be written as:

$$\frac{\mathrm{d}\log(D)}{\mathrm{d}T} = -\frac{1}{\ln(10)}\left(\frac{E_{\mathrm{a}}}{RT_{\mathrm{ref}}^2}\right). \tag{14}$$

This equation describes the slope of a plot of $\log(D)$ against T at a temperature T_{ref}. Since, by definition, this slope is equal to $-1/z$ (see eqn 9), it follows that

$$z = \frac{T_{\mathrm{ref}}^2 R \ln(10)}{E_{\mathrm{a}}}. \tag{15}$$

This equation shows that z depends on the square of the (absolute) temperature. Since z is not constant, the temperature increase to reduce D by a factor of 10 is also not constant. This is illustrated in Fig. 1, where the solid curve reflects a typical variation of D with temperature calculated from eqn (12). The dashed line represents a linearization of this curve, corresponding to the traditional definition of z, which is clearly only valid in a temperature region close to T_{ref}. If the actual temperature T differs significantly from a temperature T_{ref}, at which a z value has been documented, eqn (15) shows that the actual z value may be obtained using:

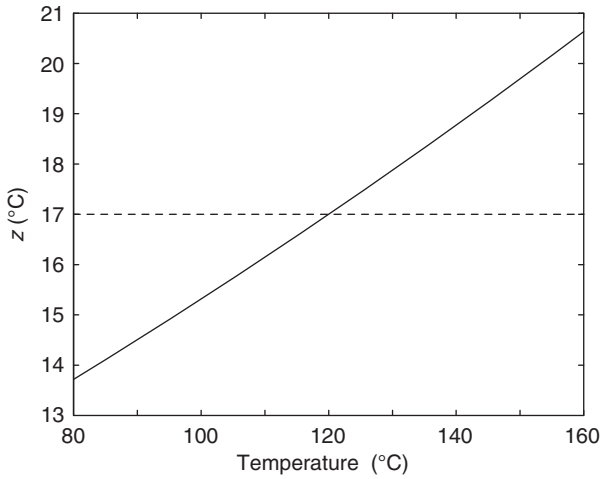

Figure 2 Variation of the resistance coefficient z with temperature. The horizontal dashed line represents the constant value z = 17°C used in various theoretical models [Van Asten *et al.* 1982; Russell *et al.* 1992]. The solid curve represents $z(T)$ calculated from eqn (16), using T_{ref} = 120°C and z_{ref} = 17°C.

$$z(T) = z(T_{ref})\left(\frac{T^2}{T_{ref}^2}\right). \tag{16}$$

This is illustrated in Fig. 2, where we used a standard model reported in the literature (Van Asten *et al.* 1982): T_{ref} = 120°C (293 K) and z_{ref} = 17°C. Alternatively, the temperature dependence of D can be expressed in T_{ref} and D_{ref} using eqn (12). This yields

$$\ln\left(\frac{D}{D_{ref}}\right) = \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right). \tag{17}$$

Substitution of eqn (15) gives:

$$\log\left(\frac{D}{D_{ref}}\right) = \frac{T_{ref}^2}{z(T_{ref})}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right). \tag{18}$$

In contrast to the usual description of sterilization processes, eqns (16)–(18) are valid in the entire temperature region in which eqn (2) can be applied.

Results

As already mentioned in the introduction, the temperature–time combinations required to assure sterility in aqueous liquids reported by Perkins (Perkins 1956) are still the basis of the present sterility criteria for steam sterilization processes. To test to what extent the theory described above can be a useful extension of the traditional description in terms of D and z, we fitted the data of Perkins by the following equation:

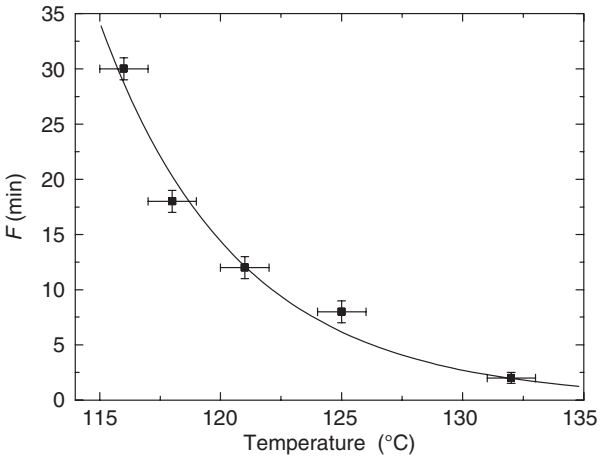

Figure 3 Time-temperature combinations to achieve sterility for aqueous liquids given by Perkins (1956) (black squares) and a fit of eqn (19) to these data.

$$\ln\left(\frac{F}{F_{ref}}\right) = \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right), \tag{19}$$

which can be obtained directly from eqn (17) for any arbitrary value of the sterility criterion S (see eqn 8). Since the experimental errors in the data of Perkins are not given, they are estimated from the precision of the reported values, i.e., an uncertainty of 1°C in the temperature and 0·5–1 min in the time. In Fig. 3 the results of this procedure are shown. The data of Perkins are denoted by the black squares. The solid curve is a fit of eqn (19) to these data. It is evident that this equation describes the data very well. For a reference temperature T_{ref} = 120°C (393 K) the fit yields an inactivation temperature $E_a/R = 2{\cdot}643 \times 10^4$ K and F_{ref} = 14·38 min. One should note that the choice of T_{ref} in eqn (19) is arbitrary. For instance, by taking T_{ref} = 134°C (407 K) an equivalent description of the data is obtained for the same inactivation temperature and F_{ref} = 1·423 min. Values for F_{ref} at different values of T_{ref} are directly related by eqn (19).

The Working Party on Pressure Steam Sterilizers of the MRC has added safety margins to the data of Perkins to assure sterility for steam sterilization processes (MRC 1959). At 121°C the safety margin equals 25%, increasing via about 90% at 126°C to about 110% at 134°C. The corresponding temperature-time combinations are denoted by the open circles in Fig. 4. The MRC states that these safety margins have been included to allow for deviations in steam quality (MRC 1959), but no rationale is given for their magnitude, in particular, the large relative increase at higher temperatures. These temperature-time

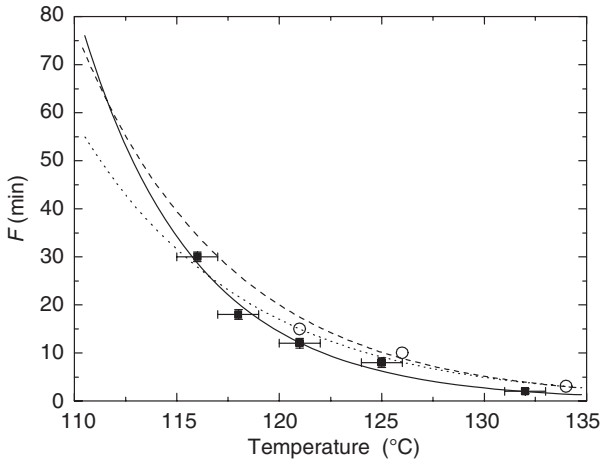

Figure 4 Various time-temperature combinations used in steam sterilization processes. The black squares represent the data reported by Perkins (1956), the open circles represent those given by the MRC MRC (1959). The solid curve is a fit of eqn (19) to the data of Perkins. The dashed curve represents a theoretical model with F_{ref} = 20 min, T_{ref} = 120°C, and z_{ref} = 17°C, the dotted curve represents a theoretical model with F_{ref} = 15 min, T_{ref} = 121°C, and z_{ref} = 18.6°C.

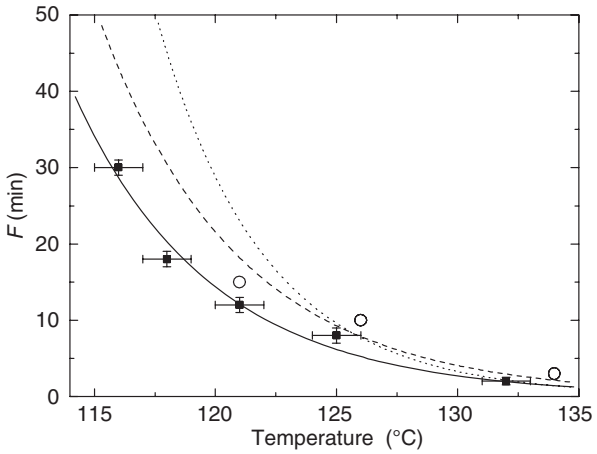

Figure 5 Various time-temperature combinations that might be used for steam sterilization processes. The black squares represent the data reported by Perkins (1956), the open circles represent those given by the MRC (1959). The solid curve is a fit of eqn (19) to the data of Perkins. The dashed curve reflects the effect of increasing the sterility by 50%, the dotted curve represents the effect of increasing the inactivation energy by 30%.

combinations have no direct relation with a microbiological inactivation process. Nevertheless, several theoretical models based on the MRC temperature-time combinations have been developed and are used in practice, as already mentioned in the introduction. Two of these are included in Fig. 4. The dashed curve represents a model with T_{ref} = 120°C, F_{ref} = 20 min and z = 17°C (see eqn 11), whereas the dotted curve represents a model with T_{ref} = 121°C, F_{ref} = 15 min and z = 18·6°C. Inspection of the figure shows that both models describe the MRC temperature-time combinations reasonably well and respect adequate safety margins between 120 and 135°C. However, at temperatures below about 115°C, the values of F following from these models drop below those resulting from the fit to the data of Perkins, where no safety margins have been included. We will return to this point below.

From a microbiological point of view, adding safety margins to the data of Perkins can be done in two ways. First, one might increase the sterility criterion S, which results in a proportional increase of the minimum exposure time, as can be seen from eqn (8). This is reflected by the dashed curve in Fig. 5, where we used the fit of eqn (19) to the data of Perkins and increased F_{ref} by 50%. This value was chosen because it yields the best description of all three temperature-time combinations given by the MRC. If we would have chosen an increase of F_{ref} by 100%, an excellent description of the MRC temperature-time combinations at 126 and 134°C would have been obtained, but the value of F at 121°C would have been overestimated by 60%. This illustrates the somewhat arbitrary magnitude of the safety margins introduced by the MRC. Second, one might increase the inactivation energy of the most resistant micro-organism. This approach, however, results in exposure times that increase much faster with decreasing temperatures than the temperature-time combinations given by the MRC. As an example, we included in Fig. 5 the fit of eqn (19) for T_{ref} = 134°C to the data of Perkins, where we increased the inactivation energy by 30% (dotted curve). Also this value was chosen because it yields the best description of all three temperature-time combinations given by the MRC. In view of these results we conclude that the most adequate way to add safety margins is by increasing the sterility criterion S and, consequently, F_{ref} by about 50%. We will refer to this description (dashed curve) as F_{theor}.

In general, steam sterilization processes are optimized as much as possible, in order to safe time, energy, money, or to reduce the exposure time of thermo-labile products to high temperatures. In most cases the exposure time to high temperatures is made as short as possible. This is done by taking into account the contribution of each part of the sterilization process to the inactivation of the organisms. For a part of the process at temperature T_i during a time interval Δt_i it follows from eqn (7) that

$$\log\left(\frac{N_0^i}{N_e^i}\right) = \frac{\Delta t_i}{D(T_i)}, \qquad (20)$$

where N_0^i and N_e^i denote the number of organisms at the start and the end of the time interval Δt_i, respectively. For the entire process (time intervals i to N) this gives

$$\log\left(\frac{N_0}{N_e}\right) = \sum_{i=1}^{N} \frac{\Delta t_i}{D(T_i)}. \quad (21)$$

For any chosen value of the sterility criterion S the decimal reduction time $D(T_i)$ at a certain temperature can be expressed in the minimum exposure time $F(T_i)$ at that temperature (see eqn 8), which yields

$$S_{\text{process}} = \log\left(\frac{N_0}{N_e}\right) = S\sum_{i=1}^{N} \frac{\Delta t_i}{F(T_i)}. \quad (22)$$

From this equation it can be seen that a process meets the sterility criterion if $S_{\text{process}} \geq S$ *or*

$$\sum_{i=1}^{N} \frac{\Delta t_i}{F(T_i)} \geq 1. \quad (23)$$

The sum in this equation can be evaluated as follows. The process is divided in sufficiently small time intervals Δt_i. From the temperatures T_i during these time intervals the values $F(T_i)$ are calculated using eqn (19) with appropriate values of the parameters F_{ref} and T_{ref}.

If we compare Figs 4 and 5 it is clear that in the temperature range between 121 and 134°C both F_{theor} introduced above and the two models mentioned above represent the MRC temperature-time combinations with an acceptable degree of accuracy. At lower temperatures, however, the latter two models predict minimum exposure times that are much lower than those resulting from a microbiological approach. If the $F(T)$ relations for these models would be used to evaluate the sum in eqn (22), the contribution of the low-temperature parts of the process to the inactivation would be overestimated. In such cases it cannot be guaranteed that the chosen sterility criterion is actually satisfied. The errors in the calculations will be most pronounced for processes that involve temperatures below 115°C during significant time intervals.

Discussion

Although the minimum exposure times F calculated according to various empirical models (Van Asten *et al.* 1982; Russell *et al.* 1992) are in line with the reported experimental data of Perkins (Perkins 1956) and the MRC (1959), the results presented in this paper show that these methods should only be applied in the temperature region between 121 and 134°C. Moreover, it is shown that these calculations are only valid within a limited temperature range close to some reference temperature (T_{ref}), because the parameters D and z used in these calculations actually vary with temperature. Consequently, the parameters D_{ref} and z_{ref} used to calculate the value of F must be chosen close to used T_{ref}, and should be available in literature or determined from experiments close to T_{ref}.

This limited temperature range is a major disadvantage of the current methods to calculate values of F and may lead to incorrect conclusions and even to a false sense of safety. For instance, when sterilization temperatures are located significantly below T_{ref}, the calculated exposure times to sterilization conditions appear to be too short. This error may be very pronounced for sterilization processes in which the items are warming up and cooling down slowly.

The straightforward microbiological approach presented in this paper to calculate $F(T)$ by eqn (19) perfectly describes the available experimental data (Perkins 1956). The resulting mathematical model contains only two independent parameters and can be implemented in a similar way as the generally used empirical models (Van Asten *et al.* 1982; Russell *et al.* 1992). There is no need to improve the description by using a double Arrhenius function, such as the log R–fa_t function (Lambert 2003), which involves up to five adjustable parameters. By increasing the sterility criterion S by 50% the model gives a fair overall description of the time–temperature combinations of the MRC (MRC 1959). It has the major advantage that it is applicable in the entire temperature range where the Arrhenius equation can be applied. For sterilization of aqueous liquids or surface steam sterilization this temperature range extends at least from 115 to 134°C, as is shown by the perfect fit of our model to the data of Perkins. As long as the dominant killing process is coagulation, which is basically a mechanistic process, the Arrhenius equation remains valid. To investigate this point in detail, it may be worthwhile to supplement the data of Perkins with data at temperatures between, e.g. 105 and 115°C.

The model can be implemented rather directly in the computer program used to collect and analyse the data during validation and monitoring of a sterilization process. Another advantage of the use of this model is that no false sense of safety is introduced and products are not exposed to high temperatures longer than necessary. This will result in sterilized products, saving energy and costs.

In its present simple form, our model cannot directly be used to optimize the thermal preservation cycles used in the food industry (see, for instance, Peleg 2006). Various experiments on the death kinetics of some microorganisms that are relevant for food product quality show significant deviations from log-linear behaviour, in the form of 'shoulders', lags and tails (Lambert 2003). If data

on the death kinetics of all relevant micro-organisms would be available from controlled experiments in the entire temperature range of interest, a mathematical model might be developed following the approach presented in this paper, but it would be far more complex.

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