Essentials of Steam Sterilization Kinetics

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ABSTRACT: Steam sterilization kinetics represent the base for a correct understanding of industrial practice of moist-heat sterilization, but is often a neglected topic. This article by the well-known Italian sterilization practitioner Dario Pistolesi and his former assistant Vittorio Mascherpa provides a simple but rigorous introduction to it and is preliminary and propaedeutic to any useful discussion of the concept of equivalent time Fo. Basic mathematical relationships and concepts as D and z parameters are the object of this article.

KEYWORDS: moist-heat sterilization, steam sterilization kinetics, equivalent time Fo, temperature.

GENERALS

Let us suppose to immerse in pressurized saturated steam, at constant temperature, a system contaminated by a micro biological species (which we assume, for the sake of simplicity, to be pure and homogeneous): e.g. a vial containing an aqueous suspension of a certain sporogenous microorganism.

It has been experimentally shown that, under the above conditions, the reaction of thermal degradation of the microorganism at issue obeys the laws of chemical reactions.

Using N to indicate the number of microorganism present in the system at a given moment, the variation of this number as the function of a chosen time t of exposure to the selected sterilization temperature can be written as:

$$\frac{dN}{dt} = -KN$$

where K is a constant which is typical of the species and conditions of the chosen microorganism.

The degradation reaction, i.e. the sterilization reaction, therefore develops like a first order chemical reaction (i.e. like a chemical decomposition reaction) in which the reaction rate is proportional, in each moment, only to the amount of product still to be degraded (or decomposed).

This seems to be obvious for dry sterilization, but less rigorous for steam sterilization, in which the water vapour molecules also seem to take part in the reaction. Actually, this bimolecular reaction is of the first order, since the steam is present in high excess all the reaction long and its concentration may be regarded as constant.

The above expression can be developed as follows:

$$\frac{dN}{N} = -Kdt \quad (1)$$

$$\int \frac{dN}{N} = -K \int dt$$

and, by converting to base 10 logarithms (from base e or Naperian logarithms, which are less practical in this specific case), the following is obtained:

$$\log N = -kt + \text{constant}$$

where $k = \frac{K}{2.303}$ due to the shift from base e logarithms to base 10 ones.

At time zero, the following is true:

$$t = 0$$

$$N = N_0$$

therefore

$$\log N_0 = \text{constant}$$

from which

$$\log N = -kt + \log N_0 \quad (2)$$

which leads to

$$\log \frac{N}{N_0} = -kt$$

and therefore

$$\frac{N}{N_0} = 10^{-kt} \quad (3)$$

where:

N_0 = initial number of microorganism

t = elapsed exposure (= sterilization) time

N = number of microorganism after the exposure time t

k = reaction rate constant which depends on the species and conditions of the microorganism

Expression (3) shows that the number of microorganism decreases exponentially depending on the sterilization time. If this expression is converted into a chart, with log N as the function of t, Diagram 1 is obtained:

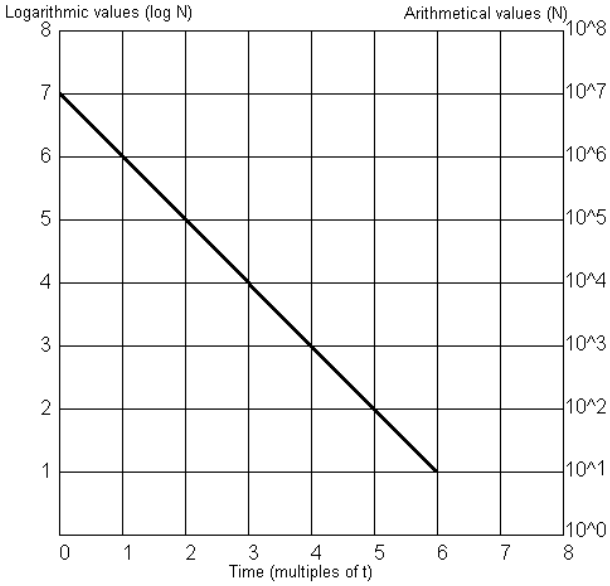

Diagram 1

Here we see that a constant percentage reduction of the concentration of viable microorganism occurs for each arbitrary time interval t. We can therefore draw a first conclusion:

The time required to reduce the microorganism concentration to any pre-set value is the function of its initial concentration.

The sterilization reaction is therefore neither an "all-or-nothing" process nor a "potential barrier" process as was once thought

D-VALUE OR DECIMAL DECAY TIME

The D-value is defined as the decimal (or decadal) decay (or reduction) time: i.e. it is the time required, at a specified temperature T, to reduce the microbial population being considered by one logarithmic value, i.e. from 100% to 10% of the initial value.

On the base of the above expression (3) it is obvious that the D-value: *it is the reciprocal of the reaction rate k*, since if $t = k^{-1}$, it is $N = 0.1N_0$.

At the temperature of 121 °C, the D-values generally oscillate between 0.2 and 2 minutes: very often $D_{121} = 1$ is assumed in the absence of more specific experimental data.

It is immediately evident that the result of sterilization at constant temperature can be very different depending on the D-value of the contaminating microbial species (or on the largest D-value, in case of mixed contamination). The following graph shows that a residual contamination of 10^{-6} is achieved in eight minutes, starting from an initial unit contamination of 10^2, at 121 °C if D = 1. Sixteen minutes are required for the same result if D = 2 and 4 are sufficient if D = 0.5 (see Diagram 2).

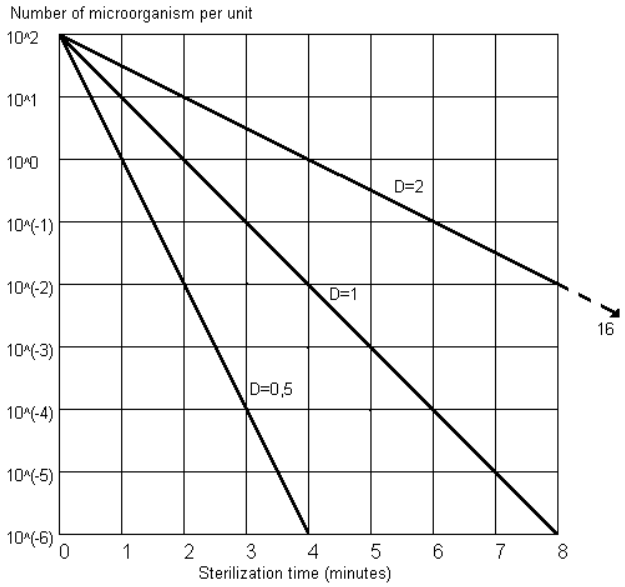

Diagram 2

STERILITY AS "PROBABLE EFFECT" OF EXPOSURE TIME

Let us now consider what happens within a batch of units (vials, bottles or others) with an initial constant unit contamination of 100 microorganisms = 10^2. If the D-value at 121 °C is assumed = 1, after one minute at 121 °C, the reduction = to 10^1 = 10 microorganisms is achieved; after another minute, only 10^0 = 1 microorganism is still surviving. After another minute the surviving microbial population would be 10^{-1} = 1/10 microorganism.

A contamination of 1/10 must not be understood to mean that each unit contains 1/10 of a microorganism: this would be biologically meaningless, even if in this case the unit would probably be sterile, but *in the sense that there is a*

probability of having 1/10 of the units still contaminated within the batch of sterilized units.

In fact, three minutes would be the necessary time to reduce the microbial population to a single surviving microorganism if the initial population were ten times larger than the one at issue. This higher initial contamination could be regarded either as a ten times larger number of microorganism in the same unit, or as the initial contamination of a ten times larger unit.

If the unit is not considered any longer as the single vial or bottle, but as the whole of all the items produced over a period of time, the initial number of microorganism present in each item has to be multiplied times the number of items produced and the exposure time to achieve the reduction to the same number of viable microorganism left in the whole of the items produced, has to be correspondingly increased.

The following example will be helpful to focus the matter.

A new sterile product in ampoules has to be launched; the number of ampoules to be produced over all the life period of the product is expected to be 10^{10}. The maximum number of contaminated ampoule deemed to be acceptable is $10^0 = 1$: this obviously means

that the probability of having non sterile ampoules after the sterilization must not exceed 10^{-10}. Let us also suppose that the microbial population within each ampoule after the filling and the sealing does not exceed 10^3 microorganisms: these must be destroyed by mean of moist heat terminal sterilization at 121 °C. The applicable D-value is 1 minute.

The total number of microorganism to be destroyed during the life of the product will be:

$$10^{10+3} = 10^{13}$$

If this whole microbial population were exposed to moist heat at 121 °C over a period of thirteen minutes, it would be reduced to 10^{-13} times it initial number, i.e. to $10^{13-13} = 10^0 = 1$.

The exposure time of thirteen minutes would thus be sufficient (under all the other above hypotheses) to prevent the total number of contaminated ampoules from exceeding the value of one.

From the point of view of each single ampoules, thirteen minutes of exposure would reduce the microbial population to the theoretical value of:

$$10^{3-13} = 10^{-10}$$

To interpret this numeric value as the probability of still having one contaminated ampoule in ten thousand million sterilized ampoules means that a single ampoule will still be contaminated out of a whole of 10^{10} (or ten ampoules out of a whole of 10^{11}).

This probability value is defined as PNSU (Probability of Non Sterile Unit) or, mainly in European documents, SAL (Sterility Assurance Level).

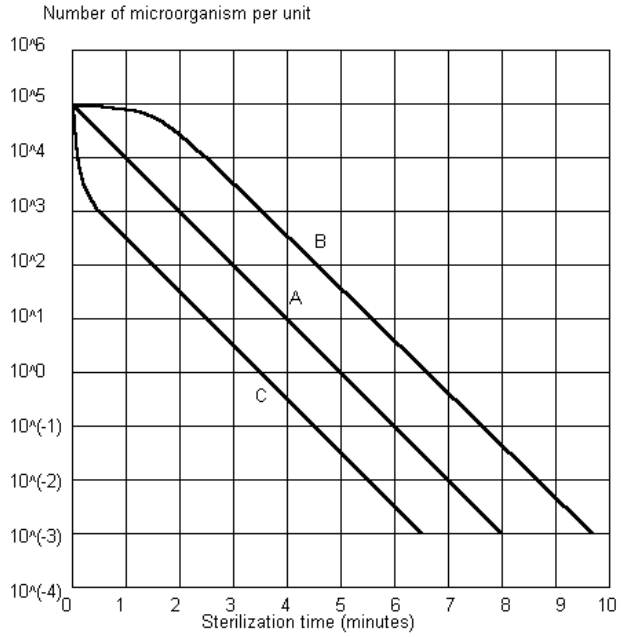

Diagram 3

The above discussion and example lead to the conclusion that the optimum exposure time of a sterilization process must take in due account not only the initial microbial population within the single item to be sterilized and the species and conditions of the contaminating microorganism, but also the total number of items expected to be sterilized over the life period of the product.

Straight lines so far examined are strictly theoretical. Actually, they are concave or convex, especially for high concentrations: i.e. they resemble the path of curves B and C with respect to the theoretical straight-line path A (see Diagram 3).

Z-VALUE OR TEMPERATURE COEFFICIENT

All the above considerations have been developed under the basic assumption that the temperature is kept constant all the exposure time long. It seems rather obvious and is experimentally confirmed that the D-value changes as the temperature changes. If the D-values experimentally obtained for a given microbial species are plotted on a semi-logarithmic chart as the function of the temperature T, a path similar to Diagram 4 is obtained.

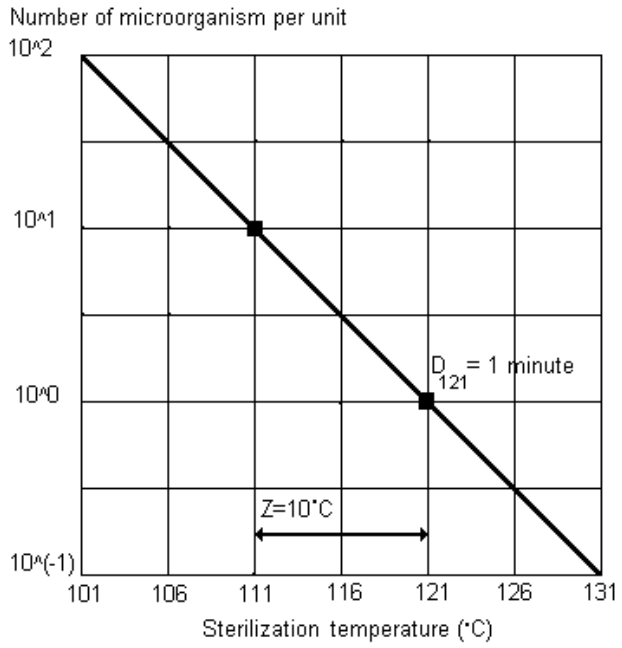

Diagram 4

In this case, it can be seen that D-value is 1 minute at 121 °C (i.e. the average value which is very often assumed to be acceptable in the absence of more exact experimental data). It can also be seen that D-value varies by a factor of 10 if the temperature varies by 10°C.

The z-value is defined as the temperature coefficient of microbial destruction, i.e. as the number of degrees of temperature which causes a 10-fold variation of D (or, more generally, of the sterilization rate).

The z-values generally oscillate between 6 and 13 for steam sterilization in the range 100 to 130 °C; z-value is often assumed to be equal to 10 in the absence of more precise experimental data.

The fact that D-value varies by 10 times for a variation of 10 °C when z = 10 must not lead to the false assumption that D varies by one time (i.e. doubles) for an increase of 1 °C; obviously this is not true.

It is actually the matter of finding the number which yields 10 when multiplied ten times by itself, i.e. raised to the tenth power. This number is 1.2589…

Therefore a variation of 1 °C entails a variation of D-value of 26 %.

As can be seen, this is quite a large value which illustrate the dramatic effects generated when the sterilization temperature is also only a few degrees lower than the expected value, perhaps only in some point of the load.

This means that the sterilization rate is approx. reduced by 50 % any time the sterilization temperature is reduced by 3 °C

It is also useful to remember that the effect of temperature variation decreases considerably as the temperature raises and drops to approximately one half (and even less) for dry sterilization at approximately 200 °C. Under these condition z-value is about 20 instead of about 10. Therefore, the small temperature differences which can be so dramatic in steam sterilization are much less effective in dry sterilization.

Table 1 lists "average" D-values and z-values for some "typical" microorganism; in fact the actual D-values and z-values depend to a large extent on the medium which contains the microorganisms and on their history.

Table 1

AVERAGE VALUE OF D AND z FOR SOME TYPICAL MICROORGANISMS		
Microorganism	D_{121} (min.)	z (°C)
Clostridium botulinum	0.2	10
Bacillus stearothermophilus	2.0	6
Bacillus subtilis	0.5	10
Bacillus megaterium	0.04	7
Bacillus cereus	0.007	10
Clostridium sporogenes	0.8 - 1.4	13
Clostridium histolyticum	0.01	10

Actually, at 121 °C no microorganism has exactly D = 1 minute and z = 10 °C. However, the combined use of these two parameters in sterilization calculations provides ample margins of safety as regards the microorganisms which are commonly dealt with.