Highlights on "equivalent time" F_0

VITTORIO MASCHERPA

R&D, Fedegari Autoclavi SpA, SS 235 km 8, Albuzzano, PV 27010, Italy
VIM@fedegari.com

ABSTRACT: This paper summarizes the concept of F_0 and its related parameters (D, z). Essential notions on sterilization kinetics are explained. The idea of physical and biological "equivalent time" is presented and its application in moist-heat sterilization processes is discussed.

KEYWORDS: moist-heat sterilization, equivalent time, sterilization kinetics, F_0 value, D-value, z-value, autoclaving, autoclave, single or double autoclaving

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INTRODUCTION

It has been experimentally shown that the reaction of *thermal* degradation of microorganisms obeys the laws of 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).

If N_0 is the initial microbial population, after an exposure time t to defined and steady conditions, the population N will be expressed by each one of the following formulas:

$$\mathrm{Log}\ (N_0 / N) = t / D;$$

$$\mathrm{Log}\ N_0 - \mathrm{Log}\ N = t / D;$$

$$N = N_0 * 10^{(-t/D)}$$

Parameter *D* in these formulas is physically a time and is defined decimal (or decadal) decay (or reduction). This parameter expresses in a quantitative way the resistance of a microbial species to a sterilizing treatment; for this reason, the Parenteral Drug Association of USA (PDA) calls it *resistance value*. *In a thermal sterilization process, D-value is the time required to reduce to one tenth of the initial value the population of*:

- a specified microbial species
- prepared in a defined way
- treated on a specific substrate
- underspecified and ideally steady process conditions.

Resistance value D is:

- expressed in minutes and tenths of minutes
- referred always and explicitly to a temperature, that is usually indicated as foot index: D_T, for example D_{121}, D_{134}
- independent of the residual amount of the microbial population.

If liquid H_2O (superheated water or condensing steam) is steadily in contact with the microorganisms to inactivate, the process is called moist heat sterilization. As well as on all the above conditions, in this type of process the resistance value D depends very strongly on the temperature T. If there is no contact between microorganisms and liquid H_2O, the process is called dry heat sterilization. In this type of process, D-values useful for industry can be obtained only at notably higher temperature than in the case of moist heat sterilization, and their dependency on temperature is less sensible.

For the moist heat sterilization of most microbial species, the reference temperature is 121°C (formerly 250°F, exactly 121.11°C). The following are experimental facts:

- provided that all the other conditions, including the steady contact between microorganisms and liquid H_2O remain unchanged, the resistance value D_T becomes smaller if the temperature increases;
- inversely, D-values grow bigger if the temperature decreases.

For each microorganism at a given temperature, the knowledge of its Resistance value D_T derives from *experimental* evaluations of initial microbial population N_0 and of the surviving population N after the exposure time t_T at the ideally constant temperature T. The value of D_T is calculated as:

$$\mathbf{D_T = t_T / Log\ N_0 - Log\ N}$$

The real difficulty when using this formula lies both in the reliable knowledge of N0 and N and in the correct evaluation of the dwelling time t_T at the foreseen conditions of constant temperature T under steady contact with liquid H_2O. Regardless to the mathematical calculation finally involved, D-values are intrinsically experimental data and cannot be predicted on a theoretical basis.

THE EFFECT OF TEMPERATURE

A *temperature coefficient* and is the common way to express the dependence of D-value on temperature for a specified microorganism. Temperature coefficient is defined as *the number z of degrees of temperature variation required to change ten times a D-value*. As already said, the D-value increases if the temperature decreases and decreases if the temperature increases.

Parameter z is:

- expressed in temperature degrees
- relevant to a specified microbial species
- referred to a small temperature range.

The definition of z-coefficient involves that:

$$\mathbf{D_{(T-z)} = D_T * 10}$$

Thanks to the definition of Parameter z, D-value undergoes a ten-fold variation if the temperature varies by z°C. For 1°C, the variation of D is given by the formula:

$$\mathbf{D_T / D_{(T-1)} = {}^{z}\sqrt{10}}$$

For instance:

- if z = 10°C, the ratio of D-values at two temperatures which differs by 1°C is given by 10√ 10 = 1.2589, i.e. D changes by about 26 % every degree centigrade
- if z = 5°C, the ratio of D-values at two temperatures which differs by 1°C is given by 5√ 10 = 1. 5849, i.e. D changes by more than 58 % every degree centigrade
- if z = 20°C, the ratio of D-values at two temperatures which differs by 1°C is given by 20√ 10 = 1.1220, i.e. D changes by about 12 % every degree centigrade

It may be easily verified that by iteration of these variations over all the temperature range equal to z-coefficient, the D-value changes by ten times, according to the definition of z. z-coefficient for a specified microorganism within a given temperature range is calculated by the following formula from two experimentally known values D_2 e D_1 at two temperatures T_2 e T_1:

$$\mathbf{z = (T_2 - T_1) / (Log\ D_1 - Log\ D_2)}$$

It is worthy to repeat that a value of z-coefficient is reliable only within the bounds of the temperature range to which are referred the experimentally known D-values used for the calculation of it. The use of a z-coefficient outside this temperature range is not sound (extrapolations are always very risky). Inside the meaningful range, z-coefficient provides the instrument to calculate D-values at intermediate temperatures as mathematical issues of the two experimental ones at the bounds of the temperature range.

THE EQUIVALENT TIME

The trend of Resistance value D *vs* Temperature T may thus be known by the calculation of z-coefficient from at least two experimental D-values. To provide this mathematical relationship with a biological meaning, a continuous presence of liquid H_2O (superheated water or condensing steam) in contact with the microorganisms is necessary.

The following question may now be discussed:

How many minutes of sterilization at an ideally constant reference temperature (i.e. 121°C) are tantamount to* t *minutes of sterilization at a different and / or variable temperature?

To answer this question, one has to start from the different resistance of a reference microorganism to the moist heat at different temperatures; this fact is expressed through the parameter D by the above formula:

$$\mathbf{D_T = D_0 * 10^{(T0 - T) / z}}$$

The equivalent time at the reference temperature will be the time that yields the same reduction of the microbial population as under the actual conditions. In finite terms, this time is given by the formula:

$$F_{(T_0,z)} = \Delta t * \sum 10^{(T - T_0) / z}$$

where Δt is a constant and suitably short interval of time between next measurements of actual exposure temperature T. When using this finite formula, T is regarded as constant during a time interval Δt. This equivalent time F is also called by PDA Lethality (factor). Lethality is thus the integration over the exposure time to moist heat of the Lethal rates L expressed by the formula:

$$L_{(T0,z)} = 10^{(T - T0) / z}$$

Lethal rates are dimensionless and thus have no unit of measurement. They are a function only of the difference between actual temperature and reference temperature and of the value of z-coefficient.

If the references temperature T_0 is set equal to 121°C (formerly 250°F, i.e. 121.11°C) and z-coefficient is set equal to 10°C (formerly 18°F), the equivalent time is conventionally called F_0. In finite terms, it is given by the formula:

$$F_0 = \Delta t * \sum 10^{(T - 121[.11]) / 10}$$

where Δt is the constant and suitably short interval of time between next measurements of actual exposure temperature T. When using this finite formula, T is regarded as constant during a time interval Δt.

If the actual temperature T is lower than the reference temperature, the equivalent time is always shorter than the actual one, as the exponent of 10 in the formula is negative and the resulting power of 10 is lower than 1. On the other side, if the actual temperature T is higher than the reference temperature, the equivalent time is always bigger than the actual one, as the exponent of 10 in the formula is in this case positive and the resulting power of 10 bigger is than 1. Exactly at the reference temperature, equivalent time and actual time are identical, and the formula confirms this obvious fact because the exponent of 10 is exactly ought and the power of 10 is exactly 1. In other words, these formulas are valid throughout the entire temperature range within which the z-coefficient is deemed to be constant.

"PHYSICAL" AND "BIOLOGICAL" EQUIVALENT TIME

The "physical" equivalent time, F_{PHY}, is that one calculated from the actual data of time and temperature measured during a sterilization process. If an autoclave maintained exactly the reference temperature for a time t, the consequence would be F_{PHY} = t, regardless to the value of z-coefficient. On the contrary, assuming for instance z = 10°C:

· while the temperature is 0.5°C lower than the reference one, the equivalent time is equal to t * 10(-0.5/10) = 0.89 t
· while the temperature is 2.0°C higher than the reference one, the equivalent time is equal to t * 10(2/10) = 1.585 t.

As said above, the number that multiplies the actual time to produce equivalent time is called L, lethal rate.

The formulas to calculate the lethal rates and the equivalent time are obtained by comparing D-values at the bounds of a temperature range within which the D-values undergoes a ten-fold change. This means that the concept of L (and F) depends on the concept of D, but this origin does not entail that any D-value of microorganisms is to be used in the calculation of the lethal rates L and of the physical equivalent time F_{PHY} produced by an autoclave. Lethal rates and physical equivalent time depend only on the actual temperature, on the reference temperature and on z-value, i.e. the temperature range within which the Resistance of a microorganism assumed as reference changes by ten times, regardless to the actual values that express it.

Independently of the above, the exposure time t_T necessary to obtain the aimed reduction of a given microbial population at sterilization temperature T is often called "biological" equivalent time, F_{BIO}. Thanks to the definition of D_T, F_{BIO} is given by the formula:

$$F_{BIO} = t_T = D_T * (\text{Log } N_0 - \text{Log } N)$$

F_{BIO} may be regarded as the equivalent target time of a sterilization process. If:

$$F_{PHY} > F_{BIO}$$

the sterilization process reduces the microbial species deemed to be initially present with a population N_0 to a final population of N. Thanks to the formulas of F_{PHY} and of F_{BIO}, the condition may also be written as:

$$\Delta t * \sum 10^{(T - T_0)/z} \geq D_T * (Log\ N_0 - Log\ N)$$

Another parameter has been introduced to "rectify" the actual F_{PHY}-value, in order to comply directly with the actual D-value of a reference microorganism: the ratio F_0 / D_0. has been called F_D. By dividing the above equations through D and using this parameter, the above equations may also be written:

$$F_D \geq Log\ N_0 - Log\ N$$

This simplified way of writing must not hide the essential requirement that **the reference temperature has always to be the same for F_{PHY} and $_D$**.

HOW TO USE EQUIVALENT TIME F_0

When discussing equivalent sterilization time, it is always necessary to assure the compliance with essential conditions for the effectiveness of sterilization process. In the case of moist-heat sterilization, the exposure to temperature is effective as purported to be only as long as the microorganisms are in contact with liquid H_2O (superheated water o condensing steam). If this condition is not compliant, equivalent time becomes only a mathematical formula without any biological meaning and its use has no sense.
If *water containing products* are sterilized (including simple saline solutions and most of the food specialties in sealed containers), we can be sure that liquid H_2O (in this case superheated water) is present in contact with microorganisms, because it is already contained in the bulk of the items to be sterilized. This case is also called *bulk sterilization* and the calculation of equivalent time may usefully include heating and cooling phases, even if temperatures lower that 110°C supply no appreciable lethality to the load. In this first case, the steam injected into the autoclave or the water circulated is not the sterilizer agent, but only the heating one.

On the contrary, the sterilization of the so-called *porous/hard goods* (P/HG) is a *surface sterilization* process. The effective contact of the microorganisms with liquid H_2O (in this case almost always condensing steam) may be obtained only after a complete *removal of the air* initially surrounding the load and by the presence of *saturated steam* in contact with the external (and internal if any) load surfaces. In this second case, the steam fed into the autoclave or the water circulating is both the heating agent and the sterilizing one.

As the effectiveness of the sterilization of P/HG does not depend only on temperature, it would be irrational to start the calculation of the equivalent time for such goods before attaining all the sterilization conditions. For example after the so-called plateau period, when no residual air is supposed to remain in the chamber or around the load. The equivalent time itself may be regarded in this case only as an additional instrument of control. If a programmable temperature threshold is provided to start the calculation of the equivalent sterilization time, this threshold has thus to be set equal or very close to the minimum sterilization temperature. This is very important not only before the exposure phase, but also after it, because both the drying vacuum and the cooling by air circulation immediately destroy the condition of contact of condensing steam with the load.

Also in the case of some special products, as dialysis filters or multi-bag systems for blood, the calculation of equivalent sterilization time is not useful and may be very misleading, regardless to the fact that a counterpressure autoclave is often used to sterilize them.

For these products, the effective sterilization conditions depend on equilibria of partial steam and air pressure inside the products and the validation exercise must include an accurate biological study. Due to the possible presence of residual air or other non-condensable gases inside these products, the sterilization effective temperature, i.e. the steam condensation temperature, is often different in a non-predictable way from the measurable temperature inside. Therefore, for these products it makes no sense to consider equivalent sterilization time based on thermometric data: the calculation of the temperature-time function F would become meaningless and its results would be misleading.

