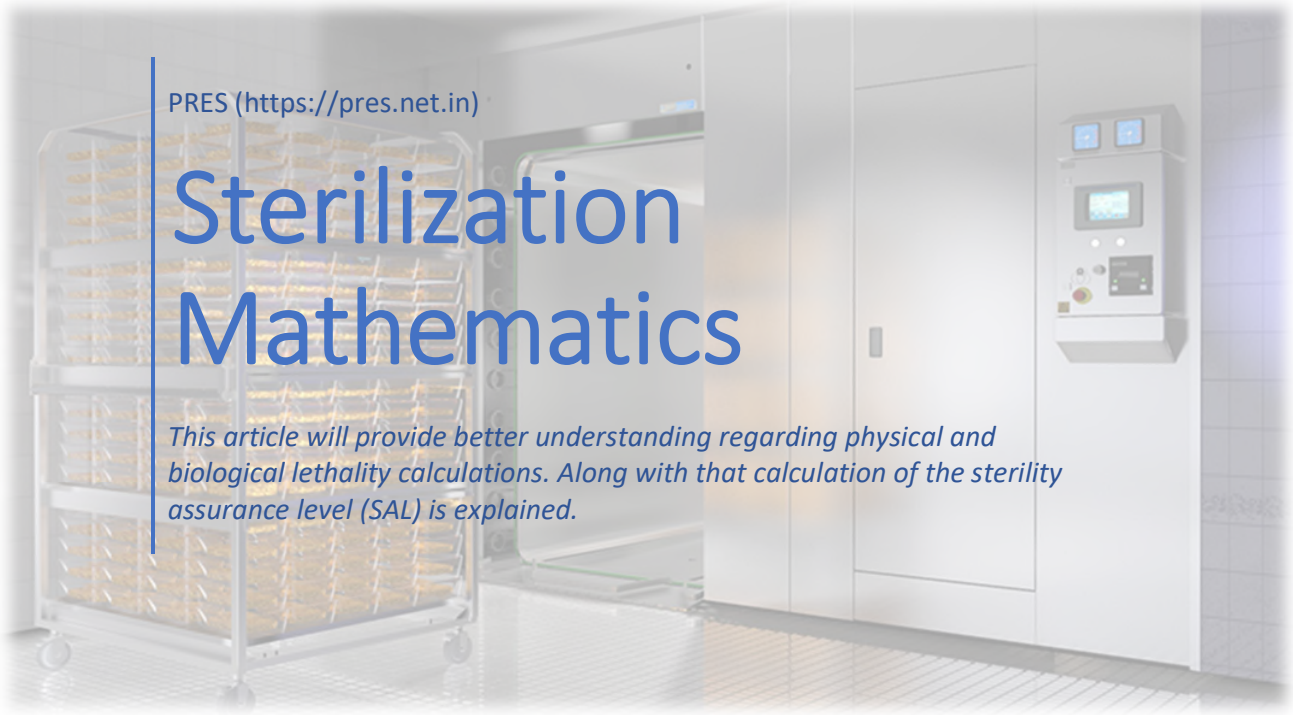

PRES (https://pres.net.in)

Sterilization Mathematics

This article will provide better understanding regarding physical and biological lethality calculations. Along with that calculation of the sterility assurance level (SAL) is explained.

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11-22-2020

Sterile means free from viable microorganisms and sterilization is any physical or chemical process which destroys all life forms, with special regard to microorganisms (including bacteria and sporogenous forms) and inactivates viruses.

Therefore, the terms “sterile” and “sterilization”, in a strictly biological sense, describe the absence and, respectively, the destruction of all viable microorganisms. In other words, they are absolute terms: an object or system is either “sterile” or “non-sterile”.

The destruction of a microbial population subjected to a sterilization process follows a logarithmic progression: only a treatment of infinite duration can provide the absolute certainty that the entire microbial population has been destroyed, and that the system is sterile.

UNDERSTANDING F_{phy} OR EQUIVALENT EXPOSURE TIME AT 121°C:

The F0 value of a saturated steam sterilisation process is the lethality expressed in terms of the equivalent time in minutes at a temperature of 121 °C delivered by the process to the load in its container with reference to micro-organisms possessing a theoretical Z-value of 10.

$$F_0 = \Delta t \sum 10^{\frac{T-121}{z}} \qquad \text{-------} \quad \textit{Equation 1.0}$$

where:

Δt = time interval between two next measurements of T
T = temperature of the sterilized product at time t
z = temperature coefficient, assumed to be equal to 10°C

A	B	C	D	E	F	G	H
Lethality calculated after 100 ° C	Data capture in hh:mm:ss	Chamber Setpoint=120.5	z=10° C Tref=121.0 ° C	Accumulated lethality F=Σ L × Δt	Chamber Setpoint=120.5	z=10° C Tref=121.0 ° C	Accumulated lethality F=Σ L × Δt
Phase	**Cycle time**	**Temperature (T1)**	**Lethality (L1)**	**Fphy(1)**	**Temperature (T2)**	**Lethality (L2)**	**Fphy(2)**
Heating Phase	12:10:35	101.10	0.01	0.01	101.50	0.01	0.01
	12:11:35	103.80	0.02	0.03	104.20	0.02	0.03
	12:12:35	106.40	0.03	0.06	106.70	0.04	0.07
	12:13:35	108.90	0.06	0.13	109.10	0.06	0.13
	12:14:35	111.30	0.11	0.23	111.40	0.11	0.24
	12:15:35	113.40	0.17	0.41	113.50	0.18	0.42
	12:16:35	115.50	0.28	0.69	115.50	0.28	0.70
	12:17:35	117.40	0.44	1.12	117.40	0.44	1.14
	12:18:35	119.20	0.66	1.79	119.20	0.66	1.80
	12:19:35	120.30	0.85	2.64	120.40	0.87	2.67
Exposure Phase	12:20:35	120.50	0.89	3.53	120.50	0.89	3.56
	12:21:35	121.00	1.00	4.53	121.10	1.02	4.59
	12:22:35	121.20	1.05	5.58	121.30	1.07	5.66
	12:23:35	121.30	1.07	6.65	121.50	1.12	6.78
	12:24:35	121.40	1.10	7.74	121.60	1.15	7.93
	12:25:35	121.50	1.12	8.87	121.60	1.15	9.08
	12:26:35	121.50	1.12	9.99	121.70	1.17	10.25
	12:27:35	121.50	1.12	11.11	121.70	1.17	11.43
	12:28:35	121.50	1.12	12.23	121.70	1.17	12.60
	12:29:35	121.60	1.15	13.38	121.70	1.17	13.78
	12:30:35	121.60	1.15	14.53	121.70	1.17	14.95
	12:31:35	121.60	1.15	15.68	121.70	1.17	16.13
	12:32:35	121.60	1.15	16.82	121.80	1.20	17.33
	12:33:35	121.60	1.15	17.97	121.80	1.20	18.53
	12:34:35	121.60	1.15	19.12	121.80	1.20	19.73
Time interval (Δt)	1 Minute						
Max Cycle Fphy	19.73						
Min Cycle Fphy	19.12						

Lethality rate (L) = $10^{(T-Tref)/z}$ ------- *Equation 2.0*

Where,

T= Temperature of load article

T_{ref} = Set sterilization temperature 121° C

Z Value = 10 (if not known for challenge organism)

Fphy or/ $F_{physical}$ = Time interval (Δt) × Accumulated Lethality (ΣL) ------- *Equation 3.0*

To demonstrate over kill approach Fphy must be > 12. Lower F0 can be acceptable for product specific approach based on the detailed knowledge of bioburden.

UNDERSTANDING BIOLOGICAL LETHALITY OR F_{Bio} (PRODUCT SPECIFIC APPROACH VS OVER KILL APPROACH):

F_{Bio} represent actual kill of microorganism.

Biological Lethality/F_{BIO}

$F_{BIO} = D_T[\log(N_0) - \log(N_F)]$ ------- *Equation 4.0*

Where,

D_T = $D_{121^{o}C}$value of BI

N_0 = Starting Population of BI *(Considering worst case 10^6)*

N_F = Surviving Population of BI

For BI Drop Test: N_F = ln (Number of BI Units Tested/Number of Sterile BI Units)

Table: Lists "average" D-values and z-values for some "typical" microorganisms; 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.

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

The bioburden count in the controlled manufacturing facility is generally far lower than 10^6.To achieve a 1-log reduction means to decrease the microbial population by a factor of 10. A sterilization cycle that provides a SAL of 10^-6 effectively means that the microorganisms that “could” be present (i.e., bioburden) are killed, and an additional 6-log reduction safety factor has been provided. The following provides an example of a cycle achieving a SAL of 10^-6.

Bioburden (worst case) = 170 CFU (colony forming unit).

To reduce the microbial population from 170 to 1 = log (170) = 2.23 (i.e., a 2.23 log reduction is required to reduce the population from 170 to 1).

Applying an additional 6-log reduction will theoretically reduce the microbial population from 1 to 0.000001. This provides a SAL of 10^-6 or a one in one million opportunity of a survival of single microorganism.

Total log reduction required = 2.23 + 6 = 8.23.

Therefore, to achieve a SAL of 10^-6 with a bioburden of 170 CFU requires a sterilization cycle that should provide 8.23 log reduction.

$F_{bio} = D_{121°C} \times (\text{Log } 172 - \text{Log } 10^{-6}) = D_{121°C} \times (2.23+6) = D_{121°C} \times 8.23 = 0.5 \times 8.23 = 4.115$

Means $F_0 = 4.1$ is enough to achieved PNSU 10^{-6}. The over kill and product specific approaches differ in the values that are used for D and N_0.

But in case of over kill considering BI $D_{121°C}$ value in 2.0 and initial worst-case spore population is 10^6

$F_{bio} = D_{121°C} \times (\text{Log } 10^6 - \text{Log } 10^{-6}) = 2.0 \times (6+6) = 24$

Means $F_0 = 24$ is enough to achieved PNSU 10^{-6}.

For Over kill approach $D_{121°C} > 1.5$ need to be considered but in the product specific approach based on the BI and Bio-burden concept or absolute bio-burden concept $D_{121°C}$ can be optimized. Therefore, high level of understanding required for bioburden /or high resistance microorganism/ or spore former.

Sterility Assurance Level (SAL)

The SAL for a given sterilisation process is expressed as the probability of micro-organisms surviving in a product item after exposure to the process. SAL of 10^{-6}, for example, denotes a probability of not more than 1 non-sterile item in 1×10^6 sterilised items of the final product. The levels of bacterial endotoxins in the finished product can be impacted by the bioburden and bacterial endotoxins in the components (i.e. active substance, excipients and containers), and by microbiological contaminants introduced during manufacture. To ensure an acceptable level of bacterial endotoxins in the finished product, the level of microbiological contaminants of the components should be minimal. Acceptance criteria for bioburden and, where relevant, bacterial endotoxins in components and bulk solutions should be specified.

Calculation of Sterility Assurance Level (SAL)

Requirement: SAL ≤ 10^{-6}

Log $N_F = -F_{PHY}/D_T + \text{Log } N_0$ [by rearranging equation 4.0] ------ Equation 5.0

Alternate way of representing SAL is,

SAL = $10^{(1\text{og}N_0\text{-SLR})}$ Where SLR= F_{PHY}/D_T ------ Equation 6.0

For Example,

The process equivalent time is 58.5 min, therefore $F_{PHY} = 58.5$

The process D-Value is 4.5 minutes

Process usages spore challenge of 1.5×10^6 therefore $N_0 = 1.5 \times 10^6$

Log $N_F = -58.5/4.5 + \text{Log } 1.5 \times 10^6 = -13 + 6.17 = -6.82$

$N_F = 10^{-6.82}$

Calculated SAL is observed $10^{-6.82}$ which is better than $10^{-6.0}$.

Definition:

F0 value
Microbiological lethality of a sterilization process expressed in terms of the equivalent time, in minutes, at a temperature of 121.1 °C with reference to microorganisms with a z value of 10 °C

bioburden
population of viable microorganisms on and/or in a product and/or sterile barrier system

biological indicator
test system containing viable microorganisms providing a defined resistance to a specified sterilization process

***D*10 value**
time or dose required to achieve inactivation of 90 % of a population of the test microorganism under stated conditions

sterility assurance level (SAL)
probability of a single viable microorganism occurring on an item after sterilization

sterilization
validated process used to render product free from viable microorganisms

NOTE In a sterilization process, the nature of microbial inactivation is exponential and thus, the survival of a microorganism on an individual item can be expressed in terms of probability. While this probability can be reduced to a very low number, it can never be reduced to zero