Chapter 9

Media Sterilization

Microorganisms can be removed from fluids by mechanical methods, for example, by filtration, centrifugation, flotation, or electrostatically. They may also be destroyed by heat, chemical agents, or electromagnetic waves. Although cells may be disrupted and killed by mechanical abrasion on a small scale, this method is not satisfactory industrially. Similarly, X-rays, β-rays, ultra-violet light, and sonic irradiations, while useful in the laboratory, are not applicable to the sterilization of large volumes of fluids. Gamma rays on the other hand may prove useful, particularly in the food industry.

Antibacterial agents have an important place in the fermentation industry, particularly for the production of a pure-water supply but have little application for the sterilization of fermentation media. Therefore, a discussion of antibacterial chemicals is beyond the scope of this book. This chapter will be confined to a discussion of the application of moist heat to fermentation media. Despite the fact that heat sterilization of media is the most common method, little attention has been paid until recently to the engineering aspects of heat sterilization.[8]

Interest in continuous methods of sterilizing media is increasing, but for the successful operation of a continuous sterilizer, foaming of the medium must be carefully controlled and the viscosity of the media must be relatively low.[12] The advantages of continuous sterilization of media are as follows:

a. Increase of productivity since the short period of exposure to heat minimizes damage to media constituents,
b. Better control of quality,
c. Leveling of the demand for process steam, and
d. Suitability for automatic control.

At present, most media in the fermentation industry are sterilized by batch methods. Over-exposure of the medium to heat is inherent in batch sterilization processes. Procedures which minimize damage to the medium will be outlined later, but before discussing the design and operation of equipment for sterilizing media, the concept of thermal death of microorganisms will be introduced. This is important since the rational design of sterilizers must be based on knowledge of the kinetics of the death of microorganisms.

In this connection, the deterministic model which has been widely applied in the study of microbial death kinetics will be compared with the probabilistic model. The sterilization of media is necessarily concerned with the progressive reduction of microbes throughout the sterilization cycle, and hence the probabilistic approach is appropriate for the design and operation of equipment.

Although it is known that metallic ions, amino acids, and the pH of the medium all affect the resistance of microorganisms to heat, detailed discussions of these topics are also beyond the scope of this book (see reference 5).

9.1. Thermal Death of Microorganisms

9.1.1. Theory

The destruction of microorganisms by heat implies loss of viability, not destruction in the physical sense. The destruction of organisms by heat at a specific temperature follows a monomolecular rate of reaction as shown in Eq. (9.1).

$$\frac{dN}{dt} = -kN \tag{9.1}$$

where

k = reaction rate constant, min^{-1} (function of temperature; see Section 9.1.2.)
N = number of viable organisms
t = time

Microbiologists sometimes prefer the term decimal reduction time, D, meaning the time of exposure to heat during which the original number of viable microbes is reduced by one-tenth.[6] Integrating Eq. (9.1) under the condition of $N=N_0$ at $t=0$

$$N = N_0 e^{-kt} \tag{9.2}$$

From the above definition of D,

$$\frac{N}{N_0} = \frac{1}{10} = e^{-kD}, \qquad D = 2.303/k \tag{9.3}$$

It is well known that spores are much more resistant to heat than are vegetative cells; in other words, k values for vegetative cells are much greater than those for spores. Although some workers consider that the dipicolinic acid present in spores may be responsible for their increased resistance to heat,[5] further study is necessary before definite conclusions can be drawn as to the mechanism of their increased resistance to heat.

Figures 9.1 and 9.2 show typical death rate data for bacterial spores and vege-

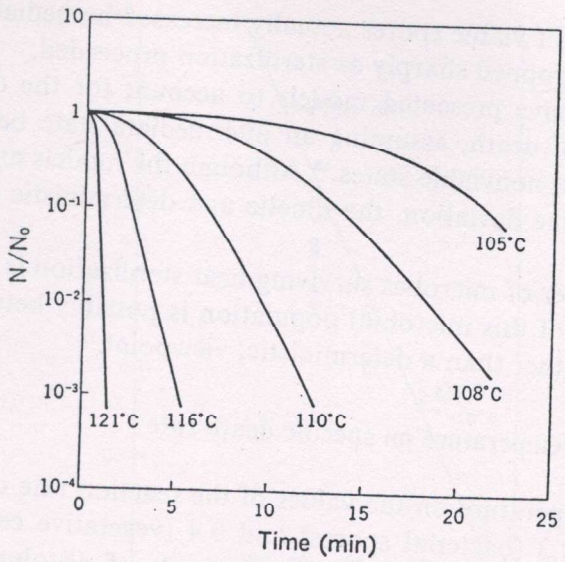

Fig. 9.1. Typical death rate data for spores of *Bacillus stearothermophilus* Fs 7954 in distilled water, where N = number of viable spores at any time, N_0 = original number of viable spores.

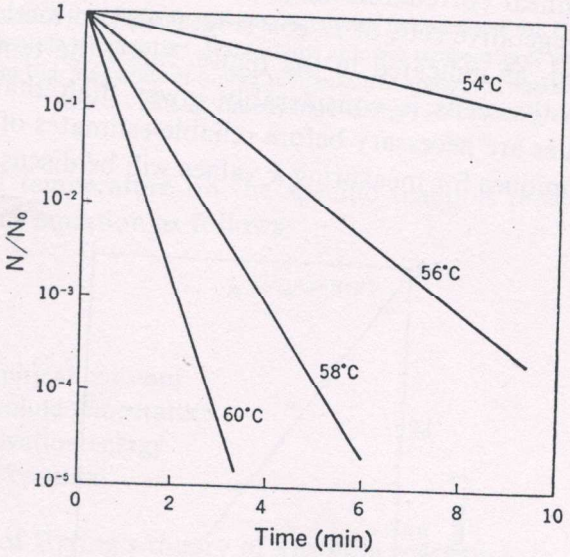

Fig. 9.2. Typical death rate data for *E. coli* in buffer, where N=number of viable cells at any time and N_0= original number of viable cells.

tative cells respectively; the parameters are different temperatures. For simplicity, data points are excluded from these figures. It is apparent from Figs. 9.1 and 9.2 that the resistance of bacterial spores to heat is much more marked than that of vegetative cells. It is clear from Fig. 9.1 that the logarithmic rate of death, as stated in Eq. (9.1), does not always hold for bacterial spores, particularly during the short period immediately following exposure to heat. There are cases reported

where the number of viable spores actually increased immediately after exposure to heat and then dropped sharply as sterilization proceeded.

Some workers have presented models to account for the deviation from the logarithmic rate of death, assuming an intermediate state between the original viable and the final nonviable states.[13] Although the models are partially successful in explaining the deviation, the kinetic and deterministic models will not be elaborated here.

Since the number of microbes surviving heat sterilization is usually less than 1, the rate of death of this microbial population is possibly better considered from a probabilistic, rather than a deterministic, viewpoint.

9.1.2. Effect of temperature on specific death rate

The effect of temperature on the values of the reaction rate constant, k, is exemplified in Figs. 9.3 (bacterial spores) and 9.4 (vegetative cells). In both figures the values of k are plotted against reciprocals of absolute temperature, $1/T$. Although the data points are scattered considerably in the case of vegetative cells (Fig. 9.4), a linear correlation between k and $1/T$ is shown in these figures.

Regarding the vegetative cells of *E. coli* (Fig. 9.4), two methods of determining k values were used, as indicated in the figure. Since the temperature range for inactivating vegetative cells is considerably lower than that for spores, some elaborate techniques are necessary before reliable estimates of k can be obtained. Experimental techniques for measuring k values will be discussed in the following section.

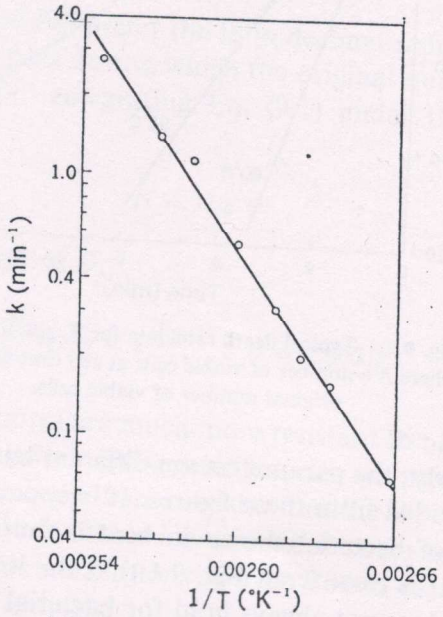

Fig. 9.3. Correlation of isothermal death rate data for *Bacillus stearothermophilus* Fs 7954, where k= reaction rate constant and T=absolute temperature. Value of E (activation energy)= 68.7 kcal/g mole.

suming the temperature of operation and the species of microbes are identical (k = constant). In other words, the latter way of evaluating N_0 (total number rather than concentration) is a more severe criterion for assessing the degree of sterilization.

The tdp (thermal death point) and tdt (thermal death time) tests have been used in the food industry as alternative techniques to the tdr (thermal death rate) used for testing sterility in the fermentation industry (*cf.* Section 9.1.3.). The tdp test determines the thermal death temperature (point) of a spore suspension which is exposed to heat for a definite period of time; the commonly used tdt test measures the length of time required for inactivating a spore suspension at a given temperature. Experimental data on either the tdp or the tdt tests may become more useful with reference to the sterilization curves as summarized in Fig. 9.9.[17]

Readers who are interested in either the experimental check of Fig. 9.9 or an application of the figure in assessing the sterilization "aftereffect," are recommended to consult the original papers.[1,4,16]

9.3. Batch Sterilization of Media

9.3.1. Temperature-time profile and design calculation

Figure 9.10 shows different types of equipment for the batch sterilization of media, while Table 9.2 summarizes the design equations with which the temperature-time profile can be calculated for each type of equipment. Heat losses are neglected in these equations.

From Eqs. (9.1) and (9.4),

$$\frac{dN}{dt} = -kN = -\alpha' e^{-E/RT} N$$

Integrating,

$$\Gamma_{\text{total}} = \ln \frac{N_0}{N} = \int_0^t k\,dt = \alpha' \int_0^t e^{-E/RT} dt \tag{9.34}$$

where

Γ_{total} = design criterion

It is apparent from Table 9.2 that the values of absolute temperatures, T, are functions of the time of exposure, t. In the design of batch sterilizers, the time, t, applying Eq. (9.34) must be determined. It is clear that some of the contaminating microbes will be inactivated during the time required to heat (t_1) and cool (t_3) the bulk of the medium.

TEMPERATURE TIME PROFILE

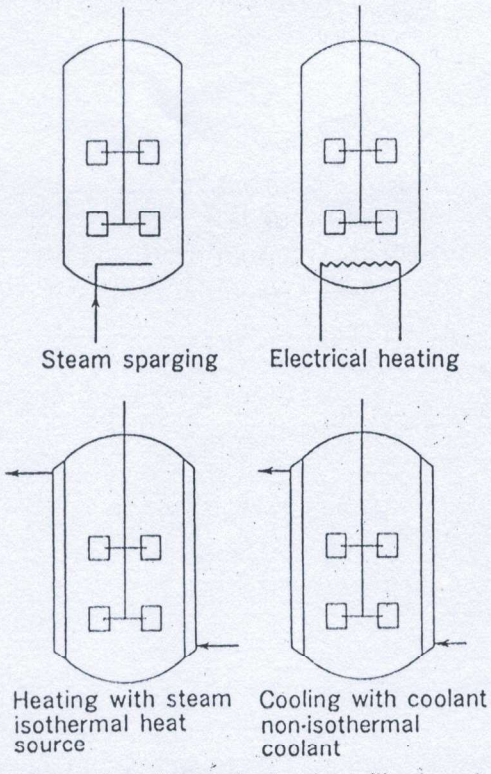

Fig. 9.10. Types of equipment for batch sterilization of media.[9]

TABLE 9.2
TEMPERATURE-TIME PROFILE IN BATCH STERILIZATION.[9]

Type of heat transfer	Temperature-time profile	α, β, or γ
Steam sparging	$T = T_0\left(1 + \frac{\alpha t}{1+\gamma t}\right)$ (Hyperbolic) (9.30)	$\alpha = \frac{hs}{Mc_pT_0}$ $\gamma = \frac{s}{M}$
Electrical heating	$T = T_0(1+\alpha t)$ (Linear) (9.31)	$\alpha = \frac{q}{Mc_pT_0}$
Heating with steam	$T = T_H(1+\beta e^{-\alpha t})$ (Exponential) (9.32)	$\alpha = \frac{UA}{Mc_p}$ $\beta = \frac{T_0 - T_H}{T_H}$
Cooling with coolant	$T = T_{co}(1+\beta e^{-\alpha t})$ (Exponential) (9.33)	$\alpha = \left(\frac{wc_p'}{Mc_p}\right)\left\{1 - \exp\left(-\frac{UA}{wc_p'}\right)\right\}$ $\beta = \frac{T_0 - T_{co}}{T_{co}}$