Bioreactor Design and Analysis
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Lecture – 30
Heat Transfer Operations in Bioreactors – Part 3

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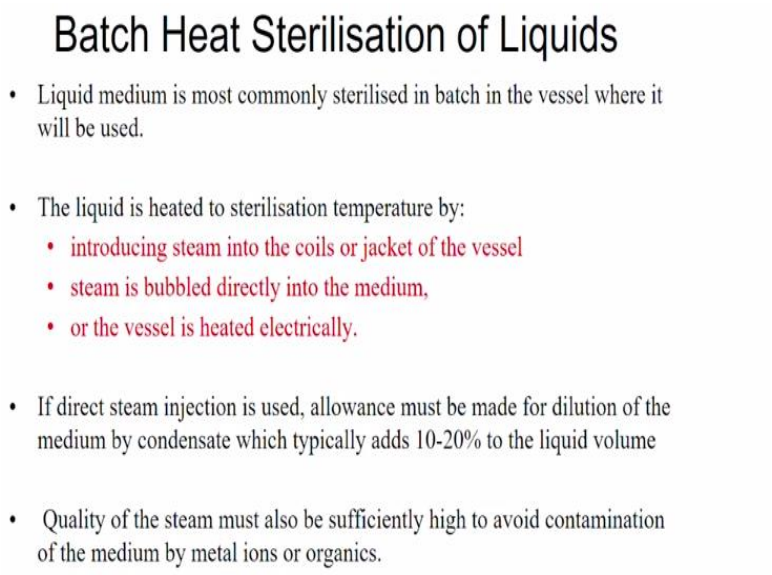

Welcome back students. So, in the last class we were discussing about heat transfer operations in the reactors where the major heat transfer operations included the metabolic heat generation due to the growth of the culture inside the reactor. Then the heat which is dissipated due to mixing by the moving parts inside the reactor and the major part of the heat transfer operation during fermentations involves heat sterilisation operations in order to make the medium sterile for monoseptic fermentations.

So, we characterized depending on the heat yield based on the stoichiometry, the metabolic heat rate generated during growth added up with the heat of mixing to calculate the total amount of heat which is required to be removed during fermentation by the cooling jacket or by any cooling arrangement or the coolant which is flown around the reactor. So, in terms of designing we generally design knowing the coolant which is to be used.

We work around two parameters. One is the temperature of the coolant and the other is the surface area available for the heat transfer. Then we discussed about the sterilisation process wherein in the bath sterilisation process we discussed about the time for sterilisation taking

into account the death of the contaminants during the heating period, the cooling period and the holding period.

So just to summarize liquid medium this is most commonly sterilized in batch in the vessels where it will be used. The liquid which is heated to sterilisation temperature we do it by introducing the steam into the jacket of the vessel. The steam is directly either bubbled inside the medium or the vessel can even be heated electrically to reach to the sterilisation temperature.

If the direct steam injection is being used, then allowance for dilution of the medium due to the condensate which generally adds up to 10 to 20% of the liquid volume. The quality of the steam must also be sufficiently high to avoid any contamination of the medium by the metal ions or any organics which can get mixed with the medium due to the steam condensate.

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- A typical temperature-time profile for batch sterilisation is shown in Figure 13.35.
- Once the holding or sterilisation temperature is reached, the temperature is held constant for a period of time t_{hd}.
- Cooling water in the coils or jacket of the fermenter is then used to reduce the medium temperature to the required value.

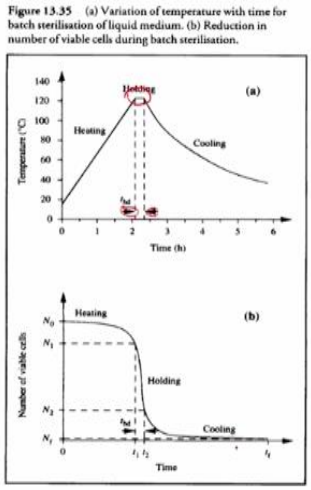

Figure 13.35 (a) Variation of temperature with time for batch sterilisation of liquid medium. (b) Reduction in number of viable cells during batch sterilisation.

What you see on the slide is a typical temperature time profile for batch sterilisation operation. Once the holding or the sterilisation temperature is reached, the temperature is held constant for a period of time which is shown here as t hd with these black arrows. The cooling water in the coils or the jacket of the fermenter is then used to bring the temperature of the medium back to the required temperature.

So, this is the profile of the temperature during sterilisation process. So, if you see the plot given below in which we have number of viable sets versus time there is a reduction in the

number of cells happening in all the three phases during the heating, then during the holding and also during cooling.

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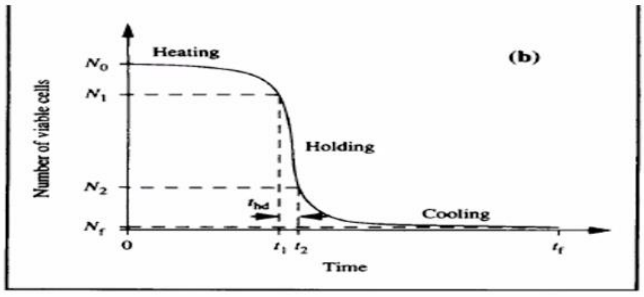

- Normally, cell death below about 100°C is minimal;
- However, when heating and cooling are relatively slow, temperatures remain close to the maximum for considerable periods of time and, as indicated in Figure 13.35, cell numbers can be reduced significantly outside of the holding period.
- Usually, holding periods are of the order of minutes whereas heating and cooling of large liquid volumes take hours.

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So normally cell death below 100 degrees C assuming these are all aqueous solutions, they are minimal. However, when the heating and cooling are relatively slow temperatures remain close to the maximum for considerable period of time as indicated in the figure. You can see that. The cell numbers therefore can still be reduced significantly outside the holding period. So usually holding periods are of the order of minutes whereas heating and cooling of large liquid volumes can even take up to hours.

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- For operation of batch sterilisation systems, we must be able to estimate the holding time required to achieve the desired level of cell destruction.
- As well as destroying contaminant organisms, heat sterilization also destroys nutrients in the medium. Minimizing this loss, relates with holding time.
- Cell death occurs at all times during batch sterilization, including the heating-up and cooling-down periods.
- The holding time t_{hd} can be minimized by taking into account cell destruction during these periods.

So, for operations of bad sterilisation systems, we must be able to estimate the holding time which will be required to achieve the desired level of sterility or cell destruction. As well as

destroying contaminant organisms heat sterilisation also in parallel is destroying the nutrients in the medium. So, in order to minimize this loss, the holding time becomes crucial. The cell death occurs because at all times during batch sterilisation process which includes the heating up and the cooling down period.

So, if we take into account the cell death during the heating and the cooling period, we may be able to reduce the holding period which may prevent the loss of the medium quality.

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- The final number of contaminant after cooling is N_f. Ideally, N_f is zero; at the end of the sterilisation cycle we want to have no contaminants present.
- It is theoretically impossible to achieve (require an infinitely-long sterilisation time.)
- Normally, the target level of contamination is expressed as a fraction of a cell, which is related to the *probability of contamination.*
- For example, one can aim for an N_f value of 10^{-3}; this means we accept the risk that one batch in 1000 will not be sterile at the end of the process.
- If N_o and N_f are known, the holding time required to reduce the number of cells from N 1 to N 2 can be determined by considering the kinetics of cell death.

So, the final number of contaminants after cooling let us suppose it is N subscript f, so ideally the final number of contaminants would be 0 in a sterilisation process by the end of one sterilisation cycle. But it is theoretically impossible to achieve at large scales which may require infinitely long sterilisation time. So normally the target level of contamination it is expressed as a fraction of a cell which we had in the last class described as the probability of contamination.

For example one can aim for an N f value of 10 to the power of –3 which implicates that it is equivalent to the risk of one batch in thousand batches not being successfully sterilized. So, if N 0 is the initial number of cells, N f is the final number of cells which we desire, the holding time period required to reduce the number of cells between them can be determined by considering the kinetics of cell death.

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- Rate of heat sterilisation is governed by the equations for thermal death. For first-order death kinetics, in a batch vessel where cell death is the only process affecting the number of viable cells:

$$\frac{dN}{dt} = -k_d N \qquad (13.95)$$

- where N is number of viable cells, t is time and k_d is the specific death constant.
- Eq. (13.95) applies to each stage of the batch sterilisation cycle: heating, holding and cooling.
- However, because k_d is a strong function of temperature, direct integration of Eq. (13.95) is valid only when the temperature is constant, i.e. during the holding period. The result is:

$$\ln \frac{N_1}{N_2} = k_d t_{hd} \qquad t_{hd} = \frac{\ln \frac{N_1}{N_2}}{k_d}$$

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Then in the last class we characterize the thermal death kinetics of the organisms. So, rate of heat sterilisation it is governed by the equations for thermal death which is defined as a first-order death kinetics in a batch vessel so where cell death is the only process affecting the number of viable cells. So, if you can see on the slide the rate at which the cell death is happening being a first-order equation is proportional to the number of cells.

And that proportionality constant is called the thermal death rate constant and it has a negative sign because it is death, the reduction in the cells which is happening. Now this equation applies to each stage of the path sterilisation cycle be it heating, holding or cooling. Now because k d is a very strong function of temperature which is being governed by Arrhenius equation.

The direct integration of this equation is valid only when the temperature is constant, now which is only during the holding period. However, during the heating and the cooling period the temperature may not be constant but it will be following a profile. So, your holding time period can easily be obtained because the k d is constant there. So as shown here if N 1 is the number of viable cells remaining at the start of the holding period and N 2 are the number of cells remaining at the end of the holding period.

Then your time for holding period which is written here as t hd can be given as ln N 1 by N 2 divided by k d from the equation given here. So, once you integrate put the limits N 1 to N 2 and here the time th d, then you can obtain the holding time in terms of k d.

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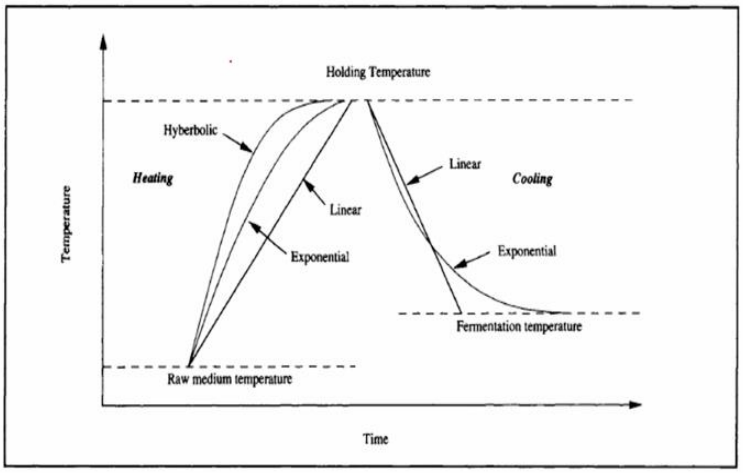
Figure 13.36 Generalised temperature–time profiles for the heating and cooling stages of a batch sterilisation cycle. (From F.H. Deindoerfer and A.E. Humphrey, 1959, Analytical method for calculating heat sterilization times. *Appl. Microbiol.* 7, 256–264.)



So here for example you can see the different temperature time profiles for various heating and cooling stages in batch sterilisation cycles where depending on the heat source it can follow different profile. It can be hyperbolic function, can be an exponential function, can be a linear function with respect to time.

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Table 13.3 General equations for temperature as a function of time during the heating and cooling periods of batch sterilisation

(From F.H. Deindoerfer and A.E. Humphrey, 1959, Analytical method for calculating heat sterilization times. Appl. Microbiol. *7, 256–264)*

Heat transfer method	*Temperature–time profile*
Heating	
Direct sparging with steam	$T = T_0\left(1 + \frac{\frac{h\hat{M}_s t}{M_m C_p T_0}}{1 + \frac{\hat{M}_s}{M_m} t}\right)$ (hyperbolic)
Electrical heating	$T = T_0\left(1 + \frac{\hat{Q} t}{M_m C_p T_0}\right)$ (linear)
Heat transfer from isothermal steam	$T = T_S\left[1 + \frac{T_0 - T_S}{T_S} e^{\left(\frac{-UA}{M_m C_p}\right)}\right]$ (exponential)

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So, bringing it into a mathematical form here there are some examples. If the heat transfer method let us say is a direct sparging with steam then this will be the hyperbolic function with respect to time and you can see the temperature time profile function. Similarly, electrical heating it is a linear function and for heat transfer from an isothermal steam the function is an exponential function which is given here.

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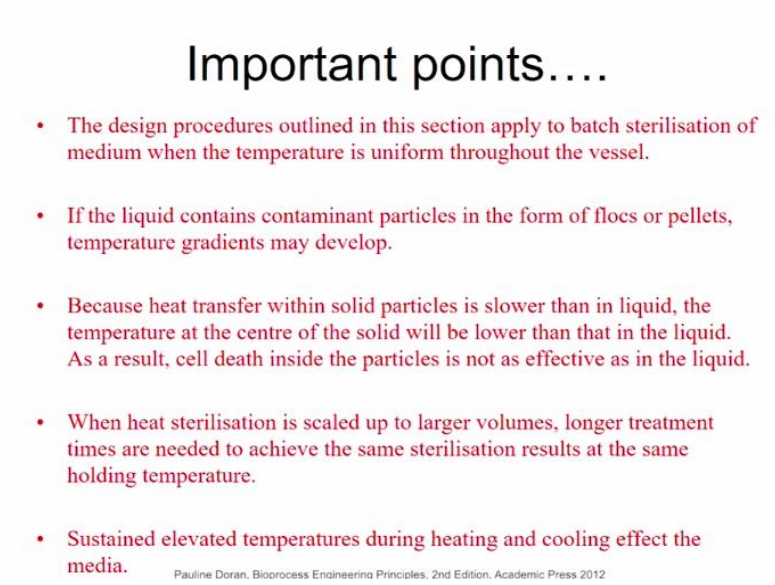

So what are the key points? The design procedures outlined here apply to batch sterilisation of the medium when the temperature is assumed to be uniform throughout the vessel. If the liquid contains contaminant particles in the form of flocs or pellets, there can be temperature gradients which may develop. So, heat transfer within solid particles we know would be slower than in a uniformly mixed liquid.

So, the temperature at the centre of the solid will be lower than in the bulk liquid. As a result, the cell death inside the particle is not as effective as in the bulk liquid of a free cells. When heat sterilisation is scaled up to large volumes, longer treatment times are needed to achieve the same sterilisation results at the same holding temperature. Sustained elevated temperature during heating and cooling will certainly affect the quality of the medium.

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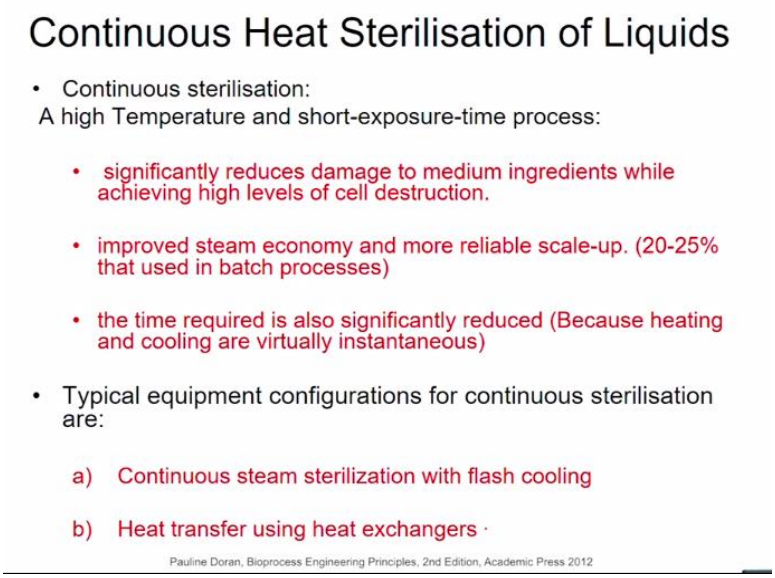

Now we have been discussing about the batch sterilisation cycles. Let us see what is a continuous sterilisation process used generally in industry. So continuous sterilisation process is generally based on a high temperature and short exposure time process. This can significantly reduce the damage to medium ingredients while achieving high levels of cell destruction. Further this can improve steam economy and with scale-up.

The efficiencies are more reliable. The time required is also significantly reduced the reason being the heating and cooling are virtually instantaneous. So, the typical equipment configurations for container sterilisation they involve generally two ways. One is continuous steam sterilisation with flash cooling and the other is heat transfer using heat exchangers.

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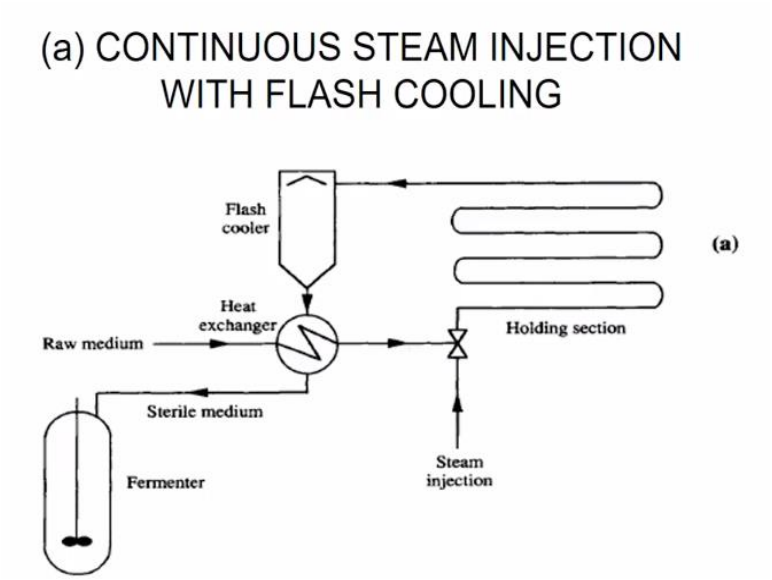

So, if you see the figure shown here it is a schematic for container steam injection with flash cooling. Here the raw medium before the direct sparging of steam inside the medium it passes through a heat exchanger where its temperature is increased close to sterilisation temperatures by the heat exchange from a sterile medium which is being cooled through this heat exchanger.

So, there is a heat transfer between already sterile medium with the raw medium which is going for sterilisation. So, in this process the sterile medium gets cooled which directly then at the required temperature can be fed in to the fermenter for fermentation and the raw medium in this process gets heated up so that to economize the use of steam. So, then there is direct steam injection once it reaches high temperature or the sterilisation temperature.

It is passing through the holding section where the holding time is being governed by the length of this section and then it is very quickly brought down to low temperatures using a flash cooler. So, in flash cooler it expands to bring down the temperature. So, in this process the time for heating and the cooling is minimized.

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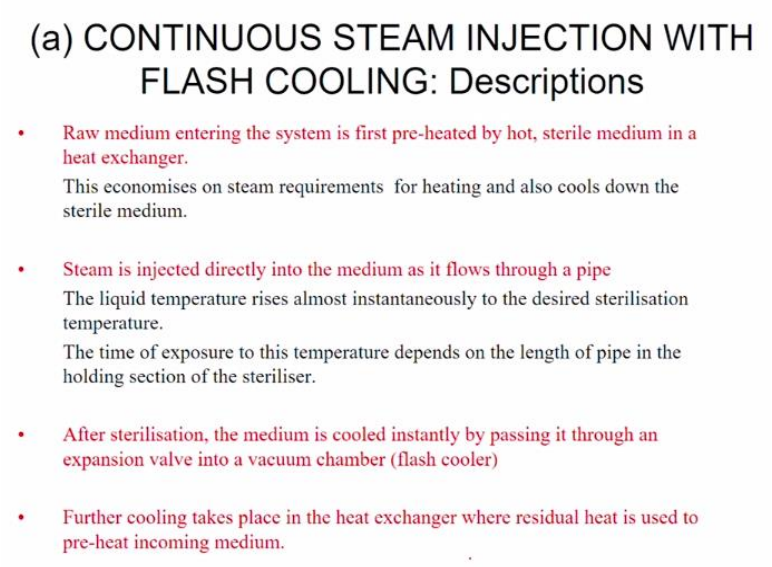

So, as I described earlier the raw medium entering the system is first preheated by the hot and the sterile medium in the heat exchanger. This would economize the steam requirements for heating and also will cool down the sterile medium. Then the steam is injected directly into the medium as it flows through a pipe. The liquid temperature rises almost instantaneously to the desired sterilisation temperature.

The time of exposure to this temperature would depend on the length of the pipe in the holding section of the sterilizer. After sterilisation, the medium is cooled instantaneously by passing it through an expansion valve into a vacuum chamber which we have shown here as a flash cooler. Further the cooling takes place in the heat exchanger where the residual heat is used to preheat the incoming medium.

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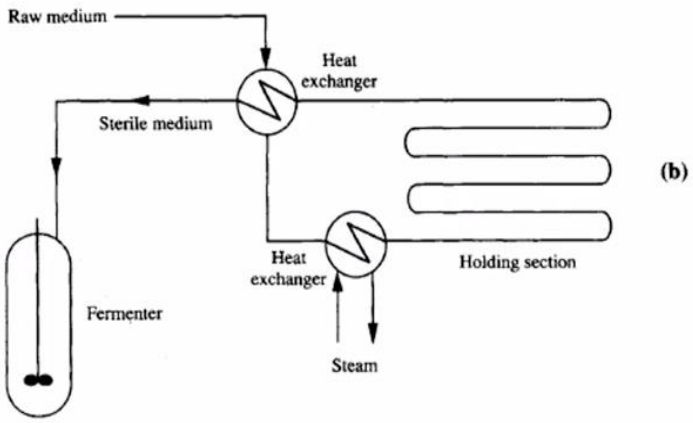
(b) HEAT TRANSFER USING HEAT EXCHANGERS.



Now the second type where heat exchangers are used for the sterilisation process. So if you see the schematic here rather than having direct steam sparging inside the medium, the steam exchanges heat with the medium in a heat exchanger.

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(b) HEAT TRANSFER USING HEAT EXCHANGERS: Descriptions

- Raw medium is pre-heated with hot, sterile medium in a heat exchanger
- then brought to the sterilisation temperature by further heat exchange with steam.
- The sterilisation temperature is maintained in the holding section
- Temperature is reduced to the fermentation temperature by heat exchange with incoming medium.

So, in this process also the raw medium is preheated with the hot and sterile medium in a heat exchanger. Then it is brought to the sterilisation temperature by further heat exchange with the steam. The sterilisation temperature is again maintained in the holding section. Temperature is reduced to the fermentation temperature by heat exchange with the incoming medium.

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Disadvantages

- Heat-exchange systems :
  - are more expensive to construct than injection devices;
  - fouling of the internal surfaces also reduces the efficiency of heat transfer between cleanings.

- Steam injection:
  - dilution of the medium by condensate
  - foaming from direct steam injection can also cause problems with operation of the flash cooler.

Now to compare the two types of container sterilisation equipments, let us see the merits and the demerits associated with them. In a heat exchange system, it is going to be more expensive to construct rather than direct steam injection. There fouling of the internal surfaces also reduces the efficiency of heat transfer between cleanings.

In case of steam injection dilution of the medium by the condensate can happen which may account to 10 to 20% of the volume. Foaming because of the direct steam injection can also cause problems with the operation of the flash cooler.

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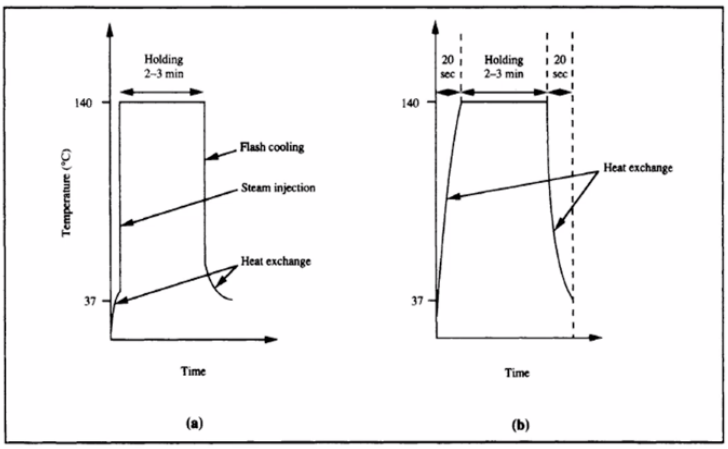
Figure 13.38 Variation of temperature with time in the continuous sterilisers of Figure 13.37.



From Figure 13.38 above, rates of heating and cooling in continuous sterilisation are much more rapid than in batch.

While the temperature time profile is shown here in the container sterilisers you can see if you compare it with the batch, the heating and the cooling is almost instantaneous, the holding time in comparison to the heating and the cooling time is higher.