Degradation of heat labile components

- Certain vitamins, amino acids and proteins may be degraded during a steam sterilization regime
- Thus heat labile compounds can be sterilized by filtration
- However, for the vast majority of fermentations these problems may be resolved by the judicious choice of steam sterilization regime

The activation energy for thermal destruction of *Bacillus steareothermophilus* spores is more than for thermal destruction of nutrient.

Thus it would be advantageous to employ high temperature for shorter period of time to achieve desired probability of sterility, yet causing minimum degradation of nutrients.

Batch sterilization is not possible as high temperature cannot be kept for short period of time by this method thus only solution to this problem is continuous stream sterilization

Advantages of continuous sterilization over batch sterilization

1. Better protection of medium value
2. Ease of scale-up - discussed later
3. Easier automatic control
4. The decrease of flow ability for steam
5. The reduction of sterilization cycle time
6. Under certain conditions, the decrease in corrosion of fermentor

Advantages of batch sterilization over continuous sterilization

1. Lesser assets apparatus expenditure
2. Less chance of contamination - processes require the aseptic inoculums transfer of the sterile broth to the sterile vessel
3. Easier manual control
4. Easier to use with media having a high amount of solid material

The Design of Batch Sterilization Processes

- Main aim of batch sterilization process is still to attain the necessary chance of getting sterility with the least change in nutrient value of the medium
- Continuous sterilization process is better than batch sterilization process in avoiding the damage of nutrients than a continuous sterilization process
- The maximum temperature which is possible in batch sterilization is 121°C therefore a method should be adopted so that medium is exposed to this high temperature for a short period of time
- High temperature and short time sterilization is attained by taking into consideration the heating and cooling time of the batch sterilization

Deindoerfer and Humphrey (1959) offered a method to evaluate the role of heating and cooling periods in sterilization process

The following point should taken into consideration for a batch sterilization process

1. How much temperature of the fermentation medium is increased during heating or decreased during cooling periods of the batch sterilization
2. The initially number of micro-organisms in the medium
3. The thermal death rate of the selected organism

Requisite Del factor can be designed by knowing the initial number of organisms in the medium and the danger of contamination.

Commonly accepted threat of contamination is 1 in 1000, which means number of living organisms after time t is 0.001

For example if any unsterile broth contain 10^{11} number of cells then Del factor for that situation is 32.2

- However, the killing of cells take place during both the heating period and cooling period of the sterilization process in addition to during holding period at 121°C
- So, Del factor can be
- $\blacktriangledown_{overall} = \blacktriangledown_{heating} + \blacktriangledown_{holding} + \blacktriangledown_{cooling}$
- Knowing the temperature and time required to reach that temperature during heating period and cooling period of sterilization process it is possible to determine the overall Del factor by these periods
- Thus, from the Del factors contributed by heating and cooling periods, it is possible to estimate the holding time that may be required for overall Del factor

Batch sterilization Methods

- The batch sterilization of the medium for a fermentation may be achieved either in the fermentation vessel or in a separate mash cooker
- Richards (1966) considered the relative merits of *in situ* medium sterilization and the use of a special vessel

Advantages of a separate medium sterilization vessel

- The medium may be sterilized in a cooker in a more concentrated form than would be used in the fermentation and then diluted in the fermenter with sterile water prior to inoculation. This would allow the construction of smaller cookers
- One cooker may be used to serve several fermenters and the medium may be sterilized as the fermenters are being cleaned and prepared for the next fermentation, thus saving time between fermentations
- In some fermentation, the medium is at its most viscous during sterilization and the power requirement for agitation is not alleviated (lessen) by aeration. Fermenter equipped with a powerful motor would provide sterile medium for several fermenters
- The fermenter would be spared the corrosion which may occur with medium at high temperature.

Disadvantages of a separate medium sterilization vessel

- The cost of constructing a batch medium sterilizer is much the same as that for the fermenter
- If a cooker serves a large number of fermenters complex pipe work would be necessary to transport the sterile medium, with the inherent dangers of contamination
- Mechanical failure in a cooker supplying medium to several fermenters would render all the fermenters temporarily redundant (unneeded). The provision of contingency equipment may be prohibitively costly

The design of continuous sterilization process

- The plan of continuous sterilization system may be advance precisely the same as batch sterilization systems
- In continuous sterilization medium is heated to reach to the sterilization temperature (121°C), holding this temperature to particular period of time and then cooling the medium to reach to the temperature of the fermentation process
- The temperature of the medium is increased in a continuous heat exchanger and is maintained for the holding time in an shielding winding holding coil
- The extent of the holding time is stated by the coil length and the medium stream speed
- The medium after holding time is cooled to the temperature required for fermentation using two chronological heat exchangers - the first using the coming medium as the cooling source and the second using water
- In continuous process high temperature is used which reduce the holding time and nutrient loss
- The necessary Del factor required may be attained by the proper temperature and holding time which decrease the amount of nutrient loss

- Additionally, a continuous process engage heating of small amount of medium and cooling of small amount of medium which is very less in contrast with batch system

- There are two types of continuous sterilizer:

1. The indirect heat exchanger
2. The direct heat exchanger (steam injector)

Continous Sterilizer

A. Indirect Heat Exchanger Double spiral type

The most appropriate indirect heat exchanger is double-spiral type - consist of two sheets of high-grade stainless steel, they are mould around central axis in such a way that they form a double spiral, as shown in Fig. 5.8.

- Steam and Medium is passed through two different plates in opposite direction to attain sterilization temperature

- This sterilizer is also use for cooling of the medium after proper holding time

- Incoming unsterile medium is partially heated which itself is a cooling agent for medium which is there in the sterilizer

- The major advantages of the spiral heat exchanger are

1. There is less chances of contamination between medium and liquid used for cooling or steam, as they are separately moving in a compartment formed by stainless steel plates with gasket seals at the end of the plates.
2. Exchanger will be cleaned by the steam or the liquid used for cooling and continues movement of the media, so that there is less chances of sedimentation, fouling and 'burning on'

- Alternate plates of heat exchanger allow two different liquid or steam to circulate through them in opposite direction
- Two plates are divided by gasket, problem with this gasket can lead to cross contamination of various streams
- This sterilizer is useful for completely soluble media as any suspended solids can block the plates
- Ability of this Sterilizer can be increased by adding extra plates to it
- In continuous stem injector, steam can be directly injected into the unsterile broth

Advantages Indirect heat exchanger

(i) Immediate) heating up times

(ii) Media containing solids can be sterilized by this exchanger

(iii) Less investment

(iv) Easy to maintain and clean

(v) Efficient in using steam

Disadvantages Indirect heat exchanger

(i) Heating may cause foams

(ii) Steam is in direct contact with medium, so medium should be enough concentrated and steam should be free from any agent responsible for anticorrosion

B. Direct Heat Exchanger

- In direct heat exchanger medium is heated with the help of steam and cooled by sudden expansion of the medium in vacuum compartment
- Cooling happens almost instantaneously

Hot water is passed through system for the sterilization of the plant before sterilization of the medium

- Steam is used to sterilize pipe work and fermentor
- Heat is conserved by using incoming media which will cool sterile medium, which in turn get preheated before reaching the sterilizer

Advantages of continuous steam injector

1. very short heating up time
2. it may be used for media containing suspended solids
3. low capital cost
4. easy cleaning and maintenance
5. high seam utilization efficiency

Disadvantages of continuous seam injector

1. foaming may occur during heating
2. direct contact of medium with steam require that allowance be made for condensate dilution and require 'clean' steam, free from anticorrosion additives.