Lecture 46-Advances in Fermentation Technology

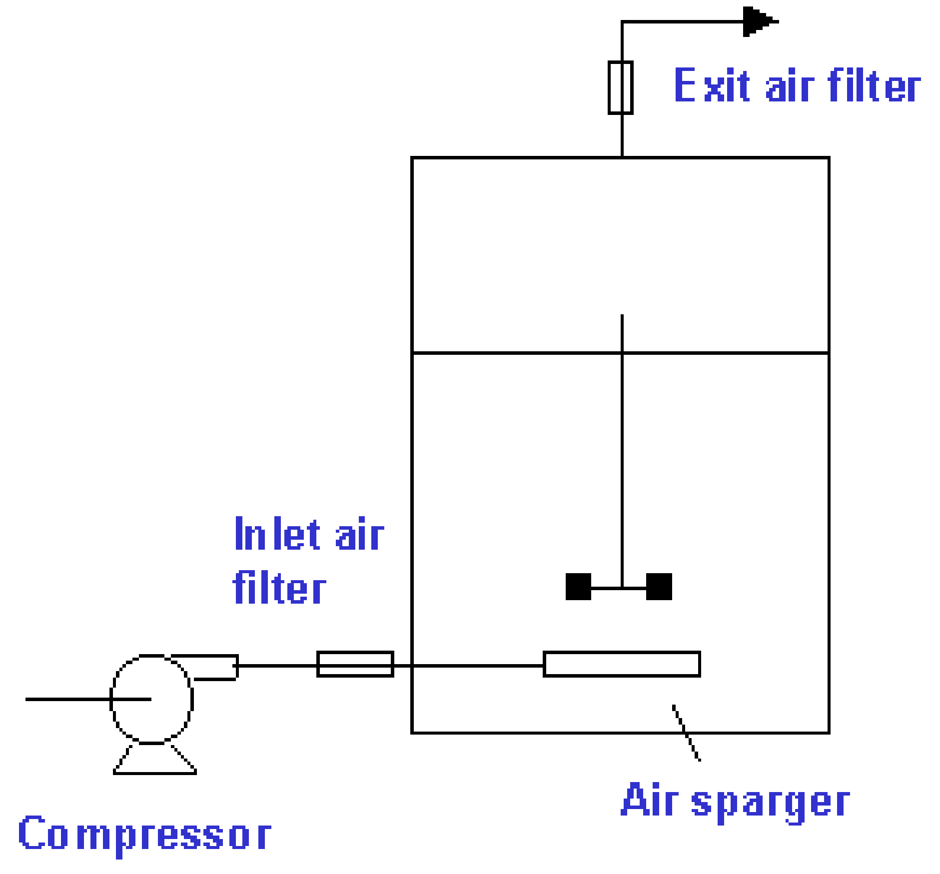
Design:

Oxygen Delivery System

Fermenter Design:

Oxygen Delivery System

The oxygen delivery system consists of: a compressor, an inlet air sterilization system, an air sparger exit air sterilization system.



Fermenter Design

A compressor forces the air into the reactor. The compressor will need to generate sufficient pressure to force the air through the filter, sparger holes and into the liquid.

Air compressors used for large scale bioreactors typically produce air at 250 kPa. The air should be dry and oil free so as to not block the inlet air filter or contaminate the medium.

It is very important that an "instrument air" compressor is not used. Instrument air is typically generated at higher pressures but is aspirated with oil. Instrument air compressors are used for pneumatic control.

Lecture 47-Advanve in Fermentation Technology

Fermenter Design:

Oxygen Delivery System-

Air Sterilization System-1

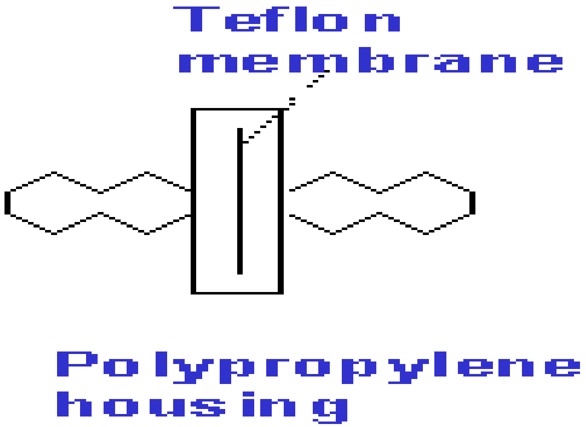
Sterilization of the inlet air is undertaken to prevent contaminating organisms from entering the reactor.

The exit air on the other hand is sterilized not only to keep contaminants from entering but also to prevent organisms in the reactor from contaminating the air.

A common method of sterilizing the inlet and exit air is filtration. For small reactors (with volumes less than 5 litres), disk shaped hydrophobic Teflon membranes housed in a polypropylene housing are used. Teflon is tough, reusable and does not readily block.

Fermenter Design

For larger laboratory scale fermenters (up to 1000 litres), pleated membrane filters housed in polypropylene cartridges are used.



By pleating the membrane, it is possible to create a compact filter with a very large surface area for air filtration. Increasing the filtration area decreases the pressure required to pass a given volume of air through the filter.

Lecture 48-Advances in Fermentation Technology

Fermenter Design:

Oxygen Delivery System-

Air Sterilization System-2

- Sterilization of the inlet and exit air in large bioreactors (>10,000 liters) can present a major design problem. Large scale membrane filtration is a very expensive process. The filters are expensive as they are difficult to make and the energy required to pass air through a filter can be quite considerable.

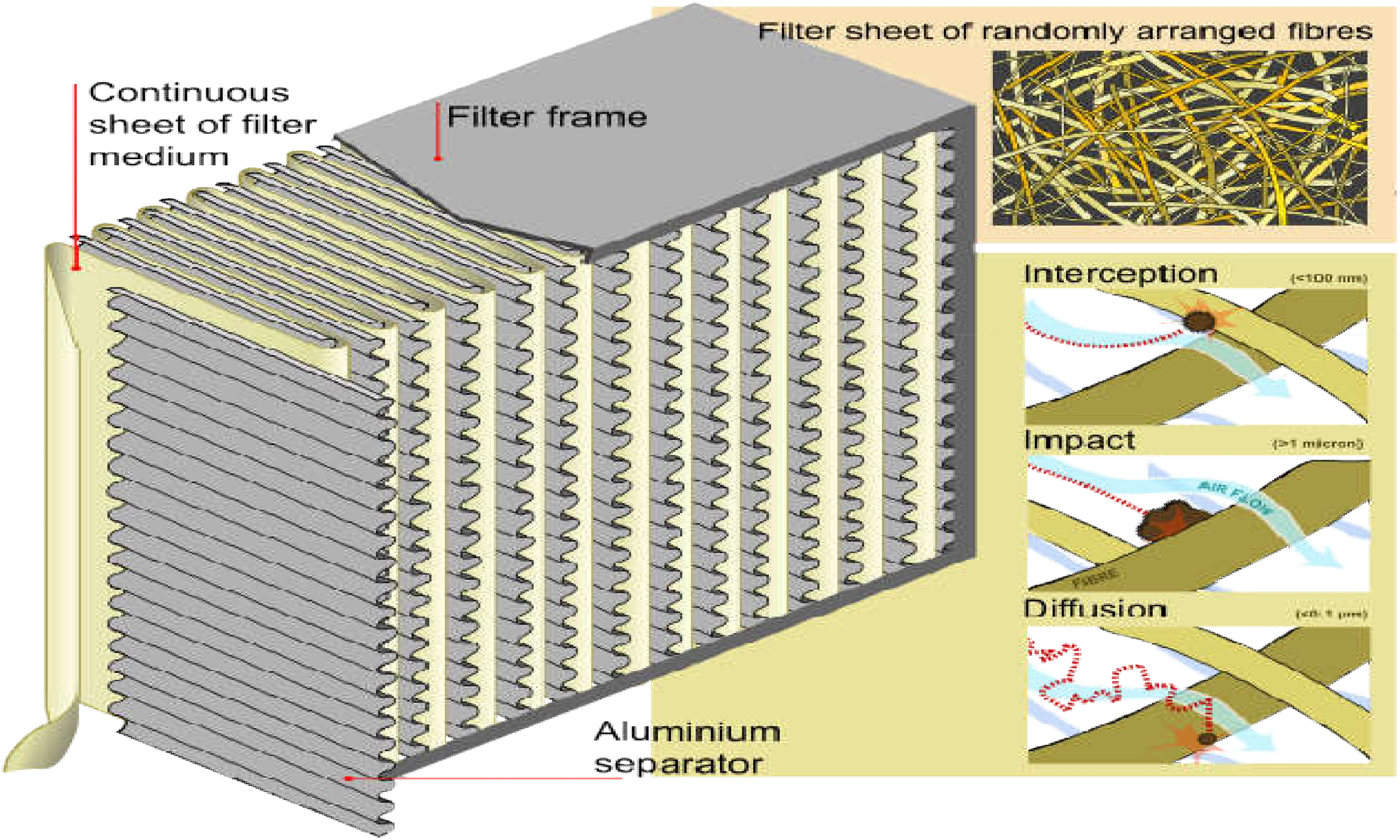
- Heat sterilization is alternative option. Steam can be used to sterilize the air. With older style compressors, it was possible to use the heat generated by the air compression process to sterilize the air. However, compressors are now multi-stage devices which are cooled at each stage and disinfecting temperatures are never reached.

Fermenter Design

In small reactors, the exit air system will typically include a condenser.

Fermenter Design

High-efficiency particulate arrestance (HEPA) Filter



Fermenter Design

- The condenser is a simple heat exchanger through which cool water is passed.
- Volatile materials and water vapour condense on the inner condenser surface.
- This minimizes water evaporation and the loss of volatiles.
- Drying the air also prevents blocking of the exit air filter with water.

Lecture 49-Advances in Fermentation Technology

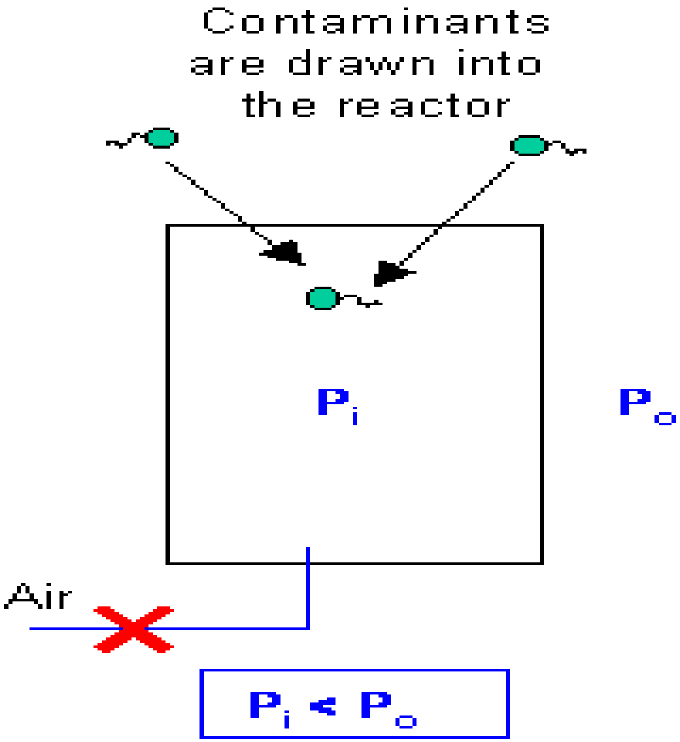
Fermenter Design:

Oxygen Delivery System-

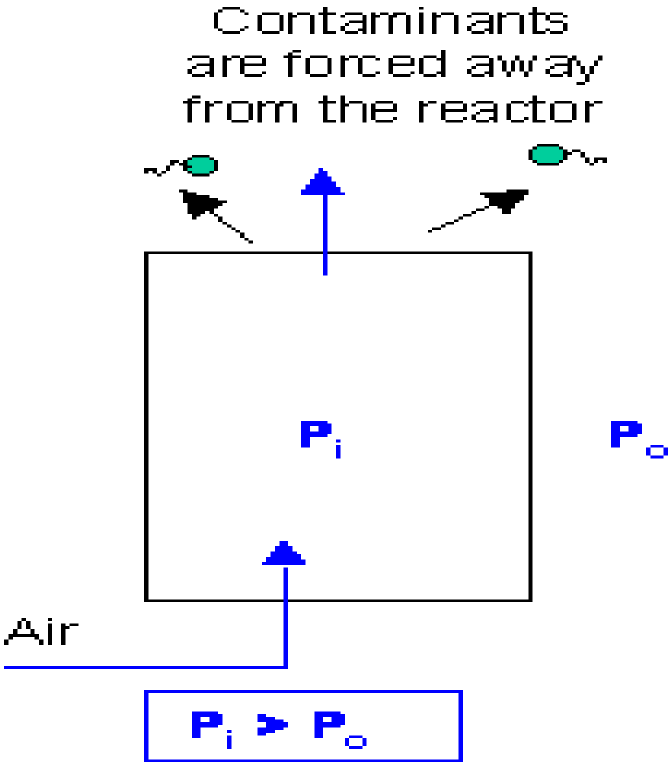
Air Sterilization System-3

- ✓ During sterilization the concept of "maintaining positive pressure" is often used.
- ✓ Maintaining positive pressure means that during sterilization, cooling and filling and if appropriate, the fermentation process, air must be pumped into the reactor.
- ✓ In this way the reactor is always pressurized and thus aerial contaminants will not be "sucked" into the reactor.
- ✓ It is very important that positive pressure is maintained when the bioreactor is cooled following sterilization. Without air being continuously pumped into the reactor, a vacuum will form and contaminants will tend to be drawn into the reactor.

Fermenter Design



Without aeration, a vacuum forms as the reactor cools.



With aeration, positive pressure is always maintained and contaminants are pushed away from the reactor

Fermenter Design

Maintaining positive pressure at all stages of the fermentation setup and operation is an important aspect of reducing the risk of contamination.

Lecture 50-Advances in Fermentation Technology

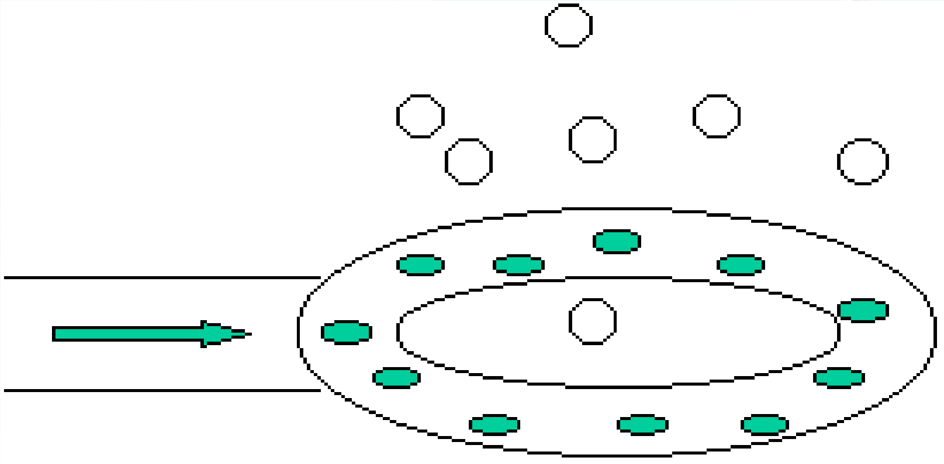
Fermenter Design:

Oxygen Delivery System

- Sparger

The air sparger is used to break the incoming air into small bubbles.

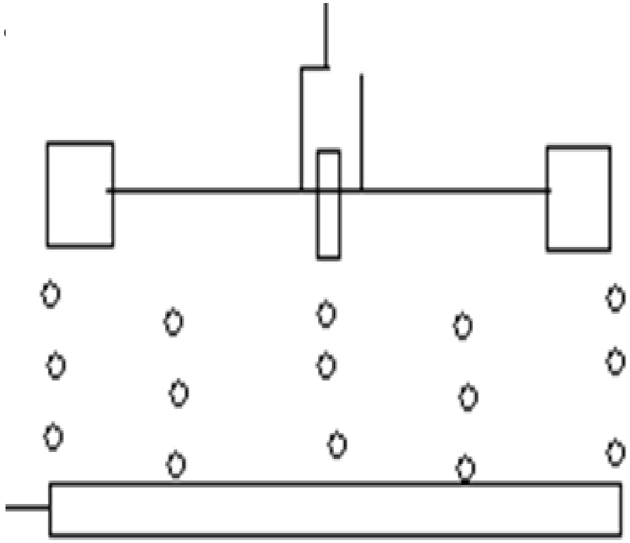
Although various designs can be used such as porous materials made of glass or metal, the most common type of filter used in modern bioreactors is the sparge ring:



Fermenter Design

A sparger ring consists of a hollow tube in which small holes have been drilled. A sparger ring is easier to clean than porous materials and is less likely to block during a fermentation.

During the emptying of a fermenter, it is important that the air feed valve is closed. This will minimize the contamination of the inlet air line.



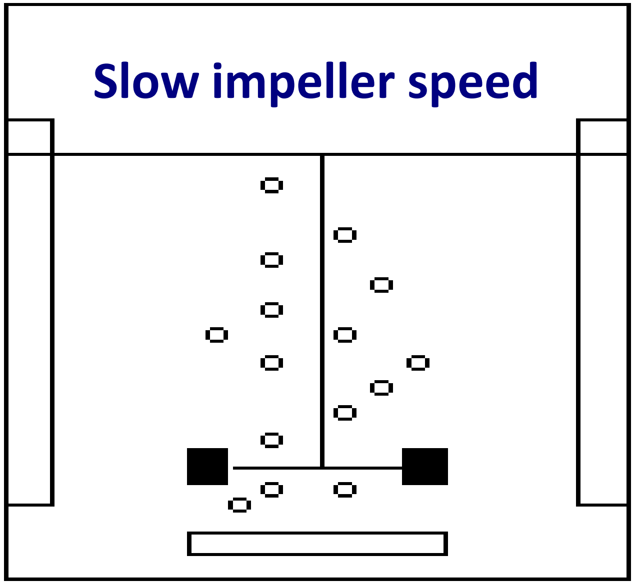
Bubbles rise directly beneath the impeller. Shear forces are highest around the impeller. This maximizes efficiency of bubble break-up.

Fermenter Design

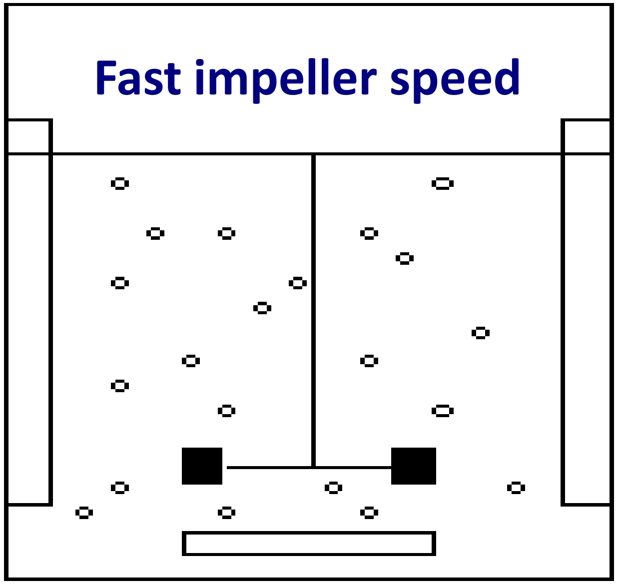
- The sparger ring must be located below the agitator and will have approximately the same diameter as the impeller.
- Thus, the bubbles rise directly into the impeller blades, facilitating bubble break up.

The shear forces that an impeller generates play a major role in determining bubble size. If the impeller speed is to slow then the bubbles will not be broken down. In addition, if the impeller speed is too slow, then the bubbles will tend to rise directly to the surface due to their buoyancy

Fermenter Design



The bubbles will not be sheared into smaller bubbles and will tend to rise directly towards the surface



Smaller bubbles will be generated and these bubbles will move with throughout the reactor increasing the gas hold up and bubble residence time

Fermenter Design

- Another consequence of too slow impeller speed is a flooded impeller.
- Under these conditions, the bubbles will accumulate and coalesce under the impeller, leading to the formation of large bubbles and poor oxygen transfer rates.
- A similar phenomenon will happen when aeration rate is too high. In this case, the oxygen transfer efficiency will be low.

Lecture 51-Advances in Fermentation Technology

Fermenter Design:

Oxygen Delivery System-

Air Flow Rate

Air flow rates are typically reported in terms of volume per volume per minute or *vvm*, which is defined as:

$$vvm = \frac{\text{Volumetric air flow rate}}{\text{Liquid volume}}$$

Note the unit convention, The air flow rate and liquid volume must have the same basal unit. The air flow rate must be expressed in terms of volume per minute (L/L/m).

Lecture 52 Advances in Fermentation Technology

Fermenter Design:

Foam Control System-1

Foam control is an essential element of the operation of a sparged bioreactor. The following photograph shows the accumulation of foam in a 2 liter laboratory reactor.

Fermenter Design

- Excessive foam formation can lead to blocked air exit filters and to pressure build up in the reactor.

- The latter can lead to a loss of medium, damage to the reactor and even injury to operating personnel.

- Foam is typically controlled with aid of antifoaming agents based on silicone or on vegetable oils.

- Excessive antifoam addition can however result in poor oxygen transfer rates.

Lecture 53-Advances in Fermentation Technology

Fermenter Design:

Foam Control System-2

- Antifoam requirement will depend on:

i. The nature of the medium: Media rich in proteins will tend to foam more readily than simple media.

ii. The products produced by the fermentation: Secreted proteins or nucleic acids released as a result of cell death and hydrolysis have detergent like properties.

iii. The aeration rate and stirrer speed: Increasing the aeration rate and stirrer speed increases foaming problems.

iv. The use of mechanical foam control devices: Foam control devices such as mechanical and ultrasonic foam breakers help to reduce the antifoam requirement.

Fermenter Design

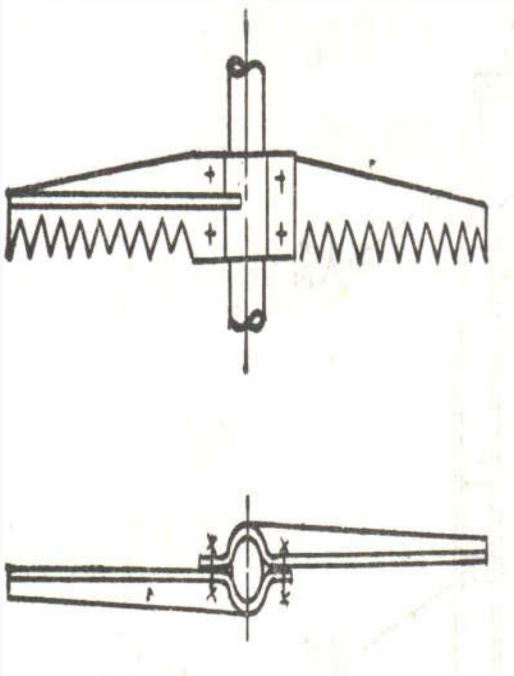
- Antifoam requirement will depend on:

v. The head space volume: The larger headspace volume, then the greater the tendency for the foam to collapse under its own weight. For example, for fermentations in which high levels of foam is produced, a 50% headspace volume may be required.

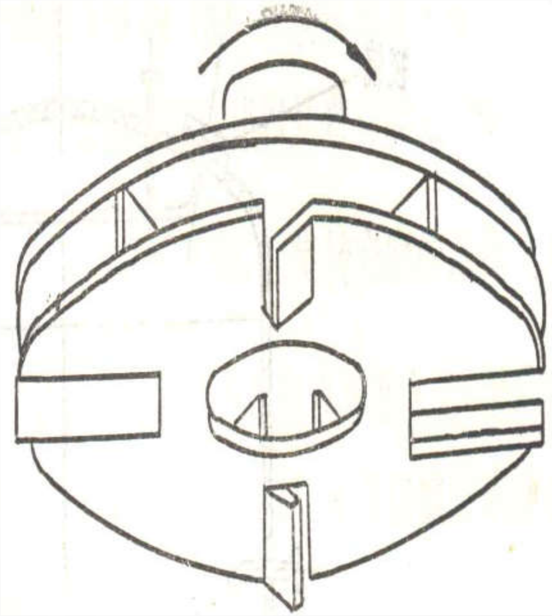
vi. Condenser temperature: In laboratory scale reactors, a cold condenser temperature can help to control the foam. The density of the foam increases when it moves from the warm headspace volume to the cold condenser region. This causes the foam to collapse.

Fermenter Design

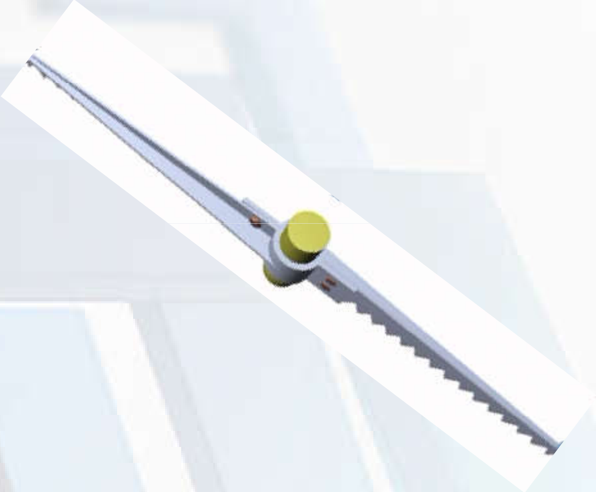
- **Foam Cutters**



- **Foam Cutters**



- **Foam Cutters**



Lecture 54-Advances in Fermentation Technology

Fermenter Design:

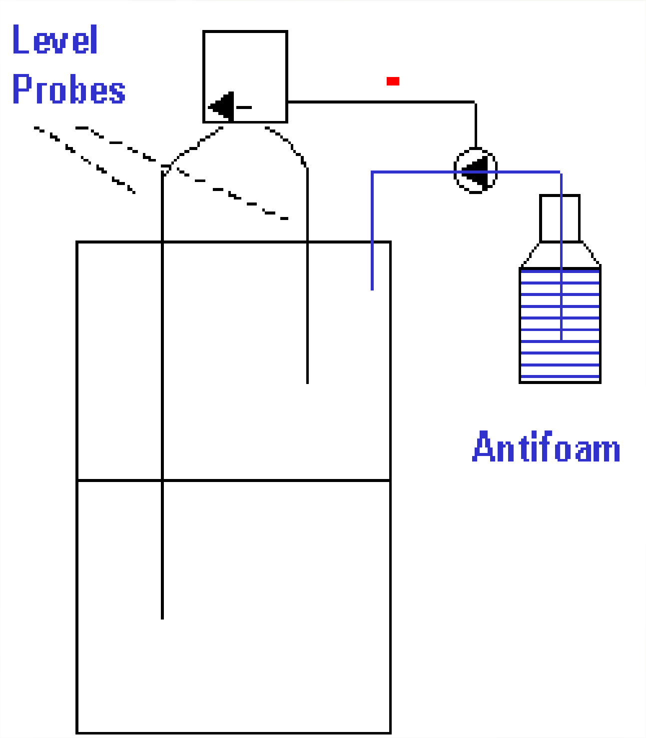
Foam Control System-3

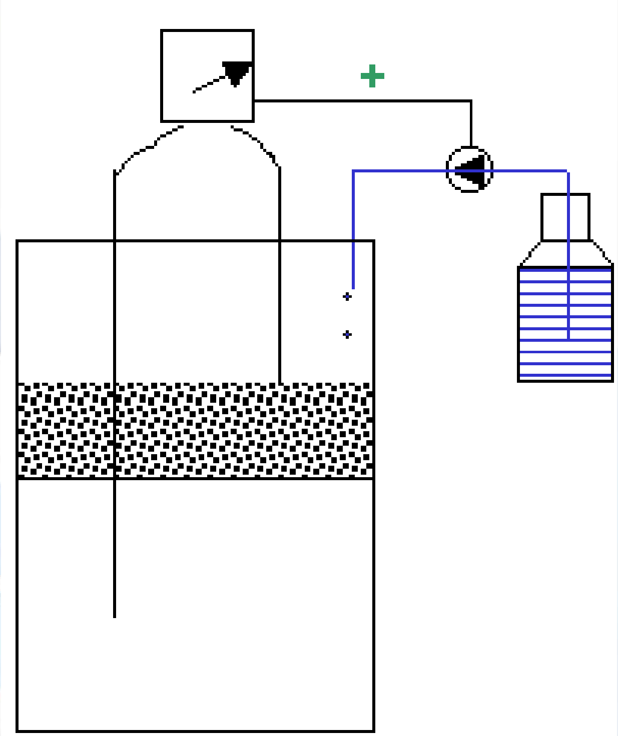
Foam is typically detected using two conductivity or "level" probes.

One probe is immersed in the fermentation liquid while the other placed above the liquid level.

When the foam reaches the upper probe, a current is carried through the foam. The detection of a current by the foam controller results in the activation of a pump and the antifoam is then added until the foam subsides.

Fermenter Design





Lecture 55-Advances in Fermentation Technology

Fermenter Design:

pH Control System-1

❑Neutralizing Agents:

The neutralizing agents used to control pH should be non-corrosive. They should also be non-toxic to cells when diluted in the medium.

- **Potassium hydroxide is preferred to NaOH, as potassium ions tend to be less toxic to cells than sodium ions. However KOH is more expensive than NaOH. Sodium carbonate is also commonly used in small scale bioreactor systems.**
- **Hydrochloric acid should never be used as it is corrosive even to stainless steel.**
- **Likewise, sulphuric acid concentrations should not be between 10% and 80% as between this range, sulphuric acid is most corrosive.**

Fermenter Design

❑Neutralizing Agents:

- For laboratory fermenters, a peristaltic pump is used to add the pH adjusting agents. Silicone tubing is often used. However, note that silicone tubing will decay in the presence of high alkali concentrations. Thick walled slicone tubing should be used.
- Alternatively Tygon or Neoprene tubing can be used. Tygon is not autoclavable but can be sterilized by passing the NaOH through the tubing for about 1 hour. Neoprene is autoclavable but is not transparent or translucent as is Tygon or silicone.

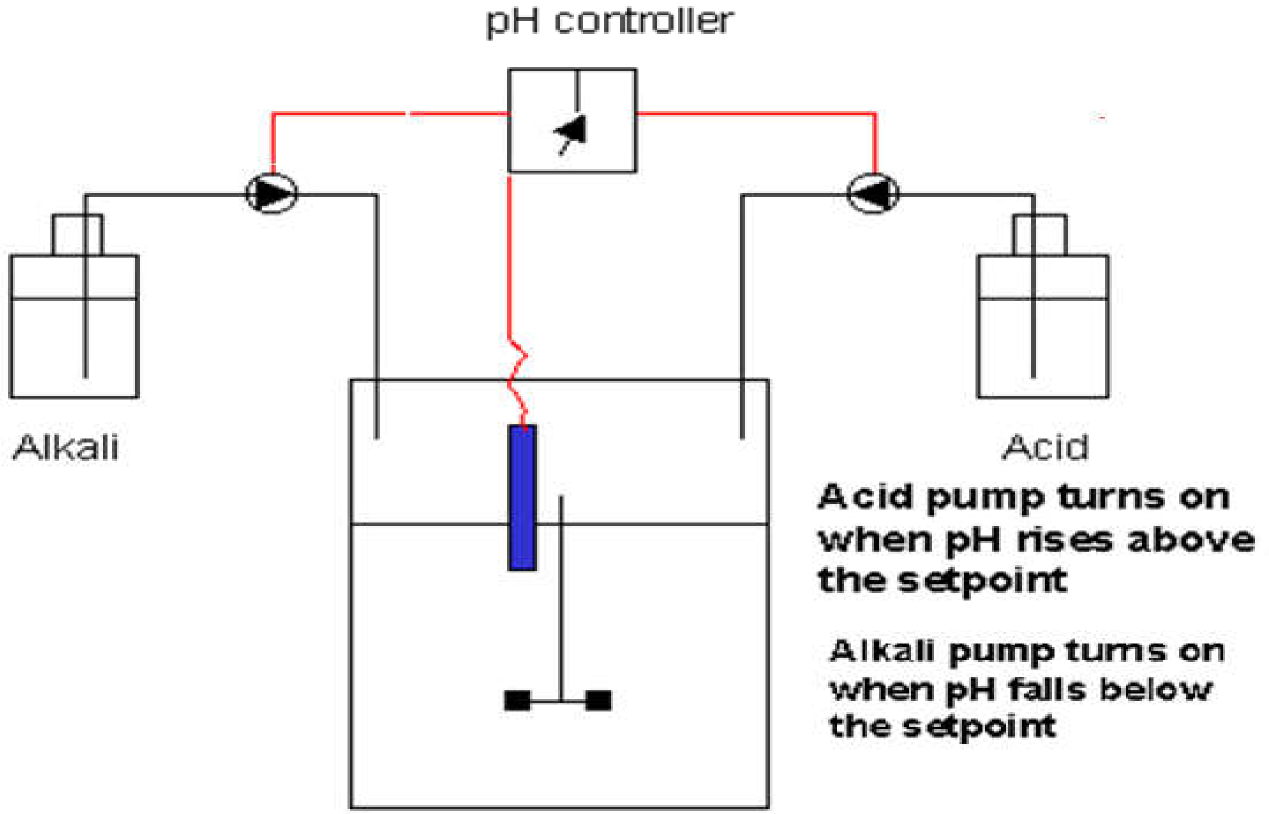
For fermentations that produce large amounts of acids, for example lactic acids fermentation using media containing high sugar concentrations, high concentrations of alkali (4 M and above) are preferred. This will prevent dilution of the medium due to the addition of excessive addition of the alkali solution.

Lecture 56-Advances in Fermentation Technology

Fermenter Design:

pH Control System-2

The pH control system consists of: a pH probe, alkali delivery system & acid delivery system. The pH probe is typically steam sterilizable.

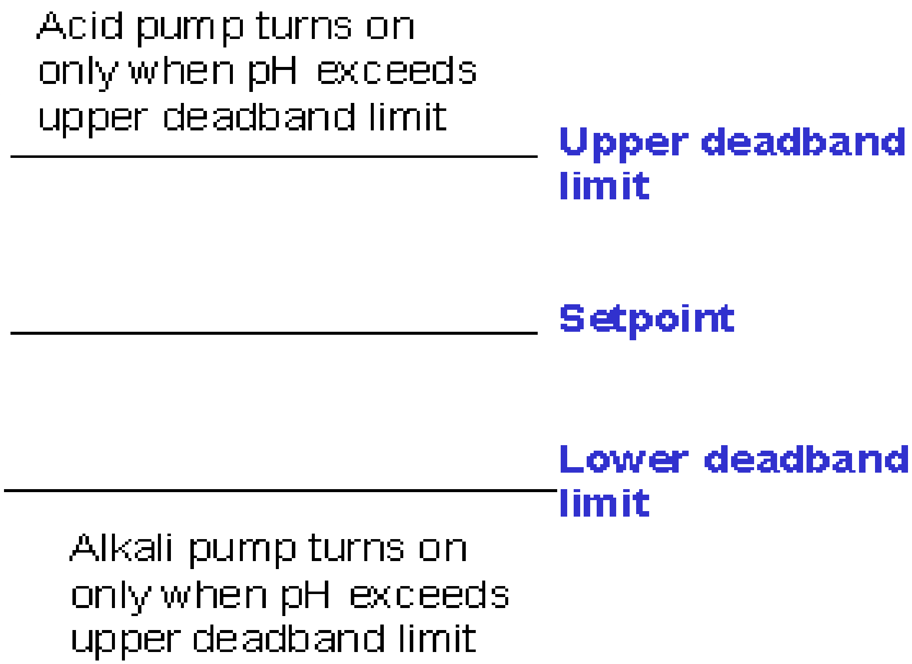


Acid pump turns on when pH rises above the setpoint

Alkali pump turns on when pH falls below the setpoint

Fermenter Design

The pH control system (and indeed all other fermenter control systems) are designed to have a dead-band. A dead-band is used to prevent excessive alkali and acid addition. The pH control dead-band is shown in following diagram:



Acid pump turns on only when pH exceeds upper deadband limit

Upper deadband limit

Setpoint

Lower deadband limit

Alkali pump turns on only when pH exceeds upper deadband limit

Fermenter Design

The set-point is the pH at which the fermenter is being attempted to be controlled at. For example, if the fermentation is to be run at a constant pH of 6.5, then the set-point is set to 6.50.

If for example, a 5% dead-band is used, then the upper dead-band limit will be

$$1.05 \times 6.5 = 6.83$$

and the lower dead-band limit will be

$$0.95 \times 6.5 = 6.18$$

If the dead-band is too small, then it is possible that pH will often overshoot and undershoot the dead-bands leading to excessive alkali and acid addition. The trade off is that a wide dead-band will lead to less precise pH control.

As many fermentations tend to produce acids rather than substances that increase the pH, acid addition is often not required. Indeed not all fermentations need continuous pH control.

Lecture 57-Advances in Fermentation Technology

Fermenter Design:

Cleaning, Sterilization &Sampling Facilities

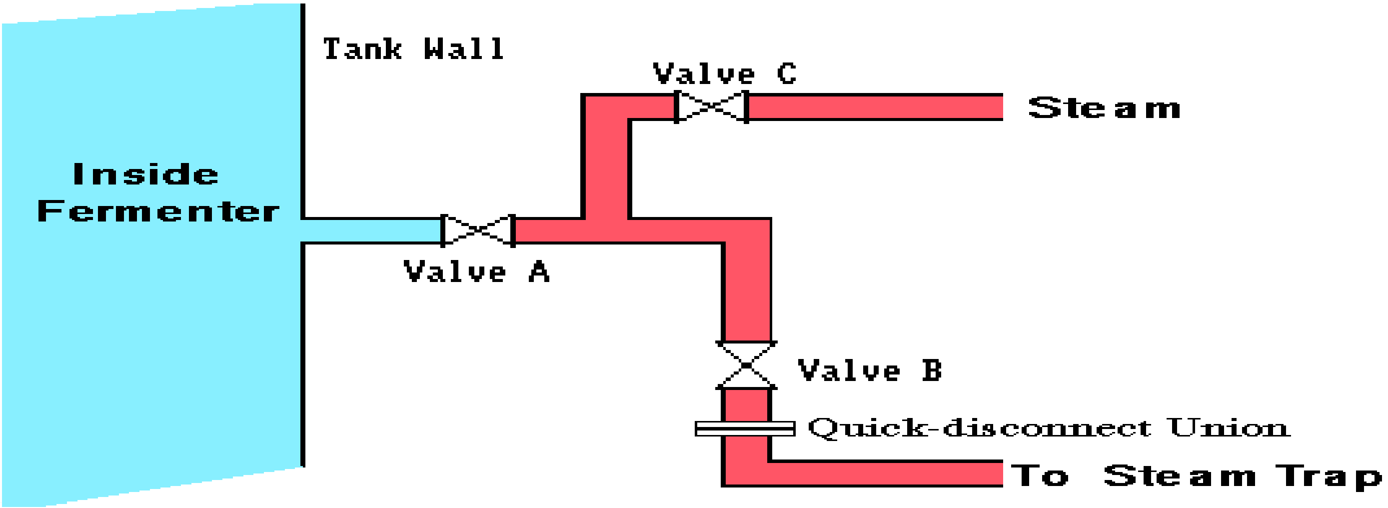
❑Cleaning & Sterilization Facilities:

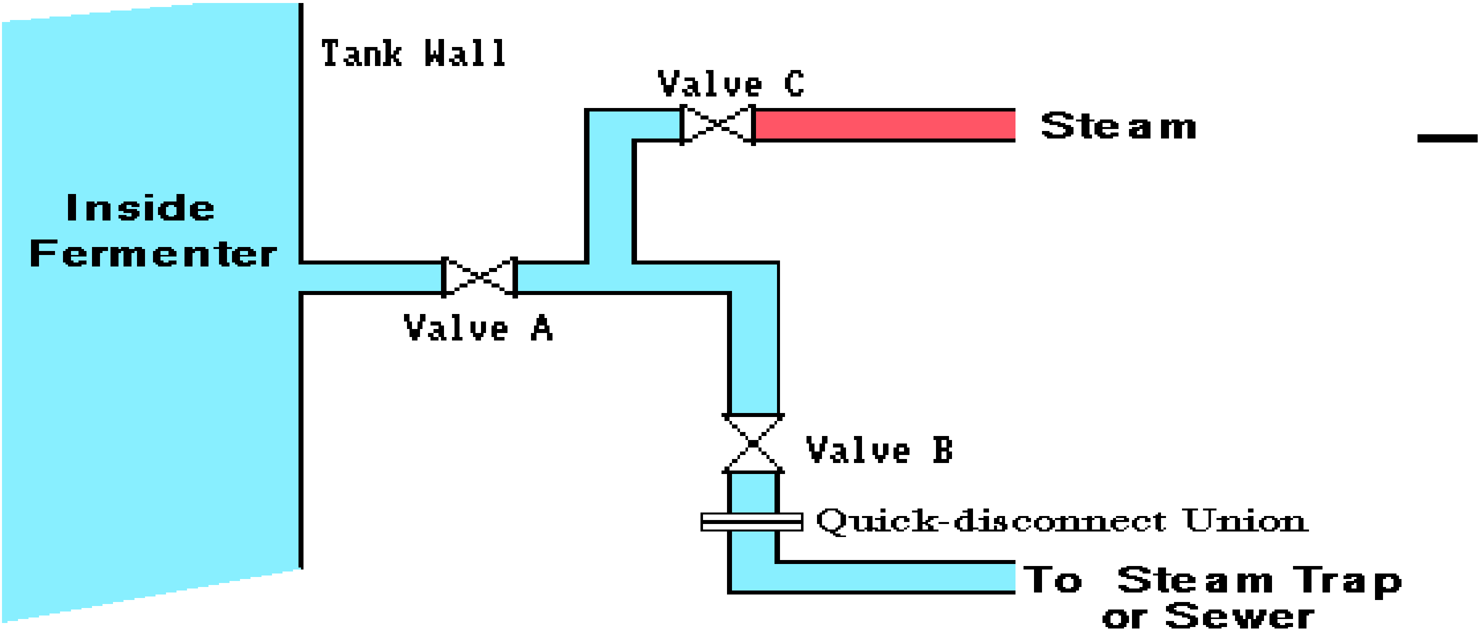
Small scale reactors are taken apart and then cleaned before being re-assembled, filled and then sterilized in an autoclave.

However, reactors with volumes greater than 5 liters cannot be placed in an autoclave and sterilized. These reactors must be cleaned and sterilized "in place". This process is referred to "Clean in Place".

CIP involves the complete cleaning of not only the fermenter but also all lines linked to the internal components of the reactor. Steam, cleaning and sterilizing chemicals, spray balls and high pressure pumps are used in these processes. The process is usually automated to minimize the possibility of human error.

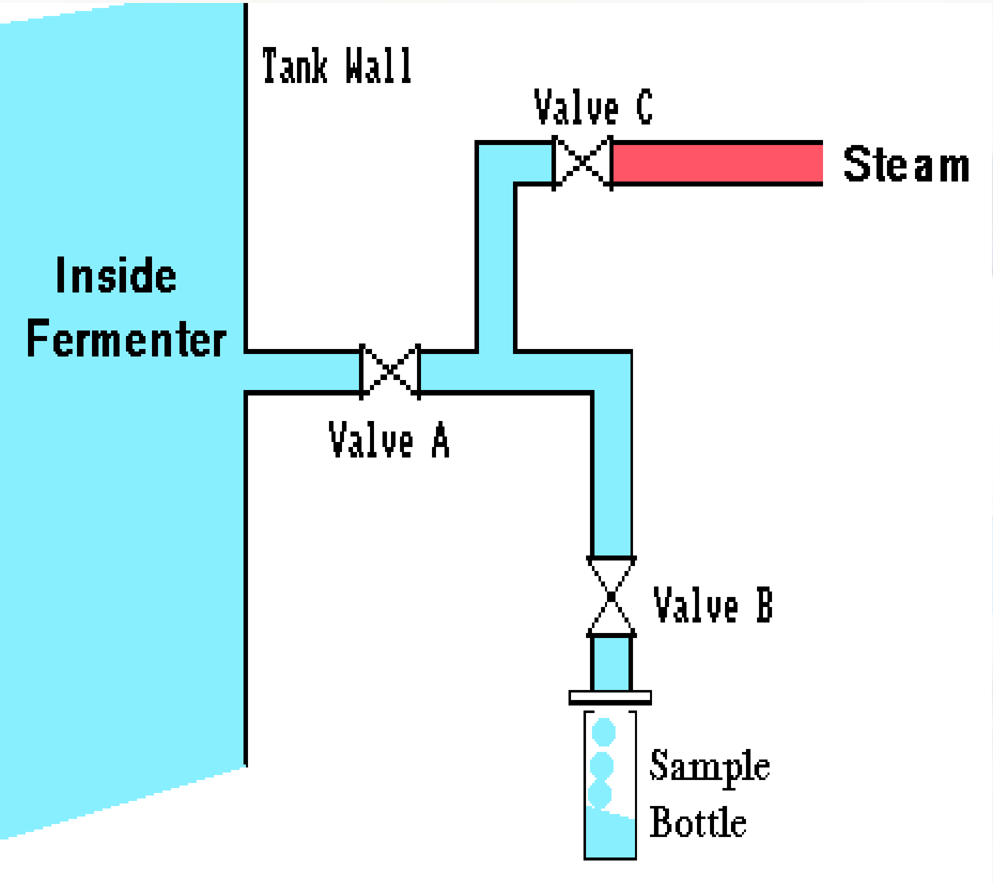
Fermenter Design





Fermenter Design

❑Sampling:



END

Lecture 58-Advances in Fermentation Technology

Sterilization:

Introduction–1

A FERMENTATION product is produced by the culture of a certain organism, or organisms, in a nutrient medium. If the fermentation is invaded by a foreign microorganism then the following consequences may occur:

Summary
Sterilization is the removal or destruction of all living microorganisms.
Heating is the most common method used for killing microbes, including the most resistant forms, such as endospores. A sterilizing agent is called a sterilant. Liquids or gases can be sterilized by filtration.

Sterilization

i. The medium would have to support the growth of both the production organism and the contaminant, resulting in a loss of productivity.

ii. If the fermentation is a continuous one then the contaminant may 'outgrow' the production organism and displace it from the fermentation.

iii. The foreign organism may contaminate the final product, e.g. single-cell protein where the cells, separated from the broth, constitute the product.

iv. The contaminant may produce compounds which make subsequent extraction of the final product difficult.

Sterilization

v. **The contaminant may degrade the desired product; this is common in bacterial contamination of antibiotic fermentations where the contaminant would have to be resistant to the normal inhibitory effects of the antibiotic and degradation of the antibiotic is a common resistance mechanism, e.g. the degradation of *β*-lactam antibiotics by *β*-lactamase producing bacteria.**

vi. **Contamination of a bacterial fermentation with phage could result in the lysis of the culture.**

Lecture 59-Advances in Fermentation Technology

Sterilization:

Introduction–2

Avoidance of contamination may be achieved by:

1. Using a pure inoculum to start the fermentation.
2. Sterilizing the medium to be employed.
3. Sterilizing the fermenter vessel.
4. Sterilizing all materials to be added to the fermentation during the process.
5. Maintaining aseptic conditions during the fermentation.

Sterilization

The extent to which these procedures are adopted is determined by the likely probability of contamination and the nature of its consequences. Some fermentations are described as 'protected' - that is, the medium may be utilized by only a very limited range of microorganisms, or the growth of the process organism may result in the development of selective growth conditions, such as a reduction in pH. The brewing of beer falls into this category; hop resins tend to inhibit the growth of many micro-organisms and the growth of brewing yeasts tends to decrease the pH of the medium.

Sterilization

Thus, brewing worts are boiled, but not necessarily sterilized, and the fermenters are thoroughly cleaned with disinfectant solution but are not necessarily sterile. Also, the precautions used in the development of inoculum for brewing are far less stringent than, for example, in an antibiotic fermentation.

However, the vast majority of fermentations are not 'protected' and, if contaminated, would suffer some of the consequences previously listed.

Lecture 60-Advances in Fermentation Technology

MEDIUM

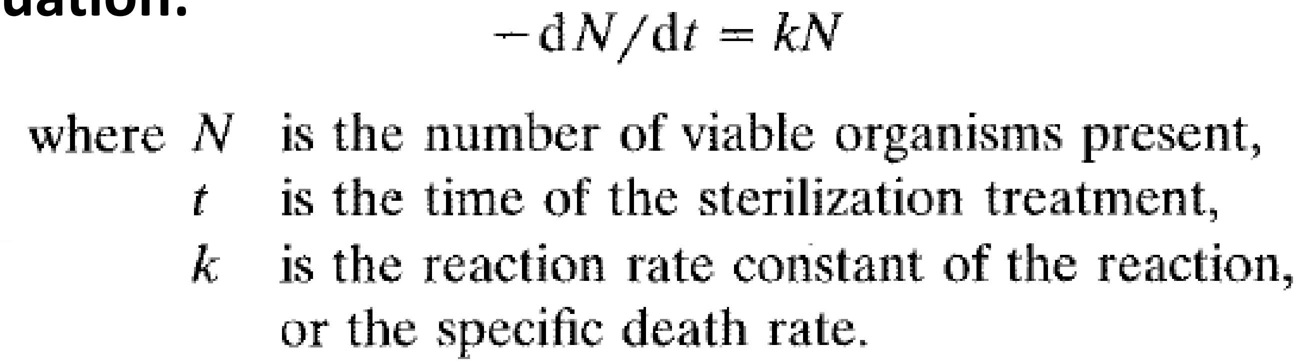
Sterilization–1

Media may be sterilized by filtration, radiation, ultrasonic treatment, chemical treatment or heat. However, for practical reasons, steam is used almost universally for the sterilization of fermentation media. The major exception is the use of filtration for the sterilization of media for animal-cell culture - such media are completely soluble and contain heat labile components making filtration the method of choice. Filtration techniques will be considered in later modules.

Before the techniques which are used for the steam sterilization of culture media are discussed it is necessary to discuss the kinetics of sterilization.

Sterilization

The destruction of microorganisms by steam (moist heat) may be described as a first-order chemical reaction and, thus, may be represented by the following equation:



$$-\mathrm{d}N/\mathrm{d}t = kN$$

where N is the number of viable organisms present,
t is the time of the sterilization treatment,
k is the reaction rate constant of the reaction, or the specific death rate.

It is important at this stage to appreciate that we are considering the total number of organisms present in the volume of medium to be sterilized, not the concentration - the minimum number of organisms to contaminate a batch is one, regardless of the volume of the batch.

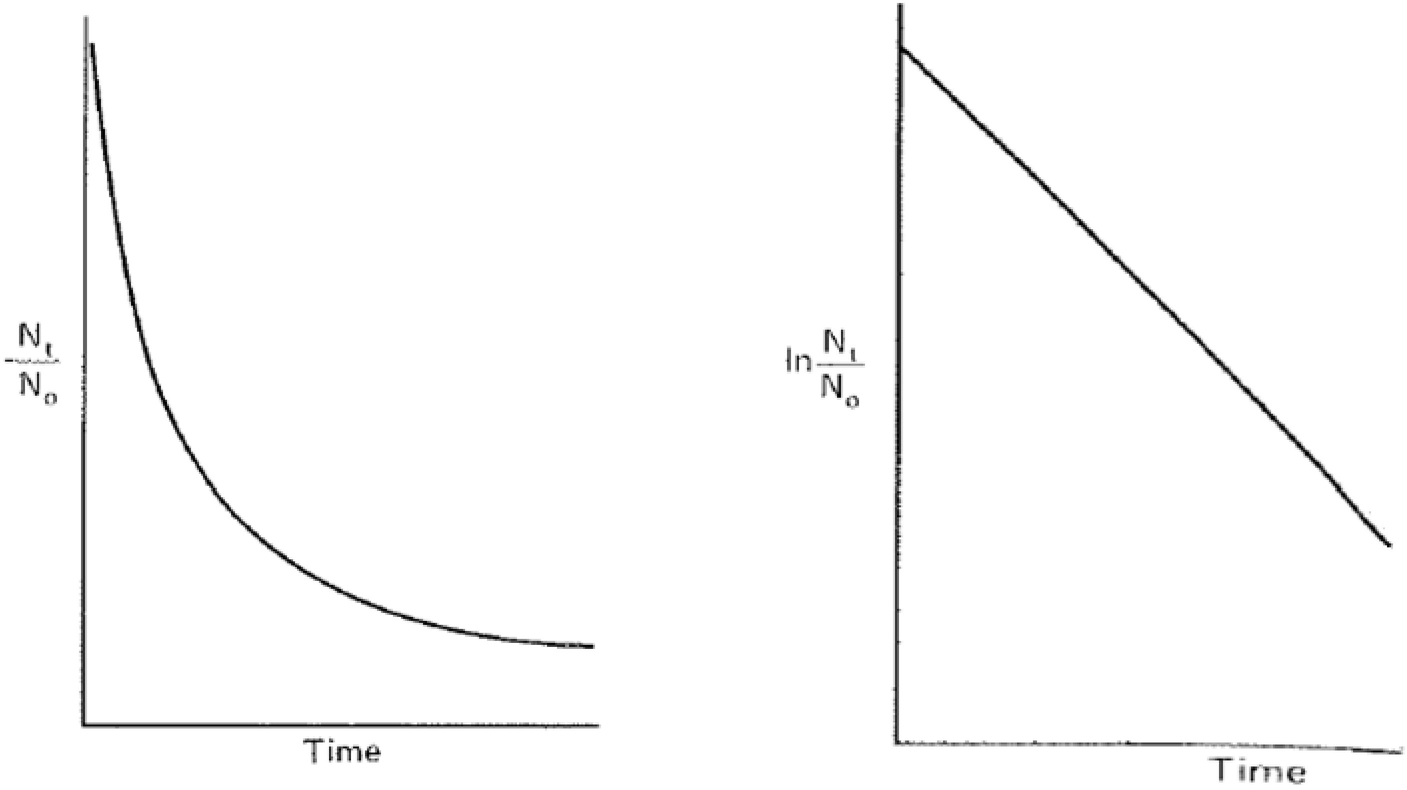
Sterilization

On integration of equation (in previous slide) the following expression is obtained:

$$\ln(N_t/N_0) = -kt$$

The graphical representations of above two equations are illustrated in Figure (in next slide), from which it may be seen that viable organism number declines exponentially over the treatment period. A plot of the natural logarithm of N_t/N_o against time yields a straight line, the slope of which equals $-k$.

Sterilization



Plots of the proportion of survivors and the natural logarithm of the proportion of survivors in a population of microorganisms subjected to a lethal temperature over a time period.

Sterilization

This kinetic description makes two predictions which appear anomalous:

i. An infinite time is required to achieve sterile conditions (i.e. $N_t = 0$).

i. After a certain time there will be less than one viable cell present.

Lecture 61 Advances in Fermentation Technology

MEDIUM

Sterilization–2

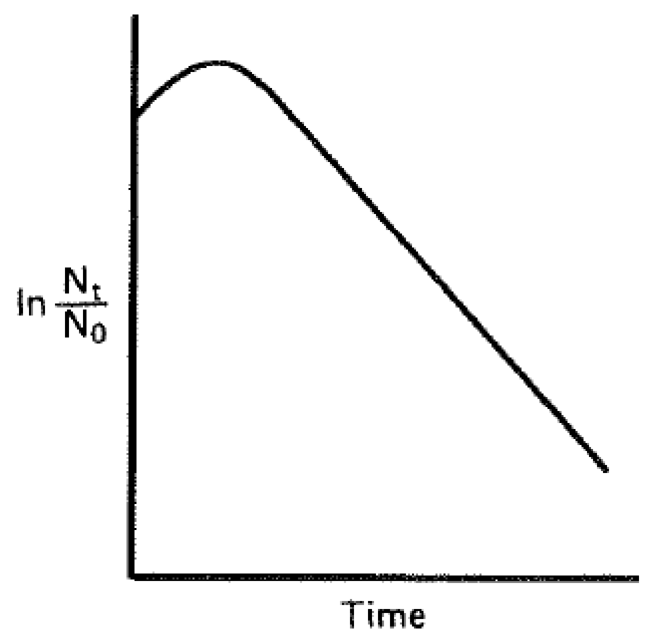
As per context of the discussion in previous module, a value of N_t of less than one is considered in terms of the probability of an organism surviving the treatment. For example, if it were predicted that a particular treatment period reduced the population to 0.1 of a viable organism, this implies that the probability of one organism surviving the treatment is one in ten. This may be better expressed in practical terms as a risk of one batch in ten becoming contaminated.

The value of k is not only species dependent, but dependent on the physiological form of the cell; for example, the endospores of the genus *Bacillus* are far more heat resistant than the vegetative cells.

Sterilization

Figures in next slides illustrate the effect of the time of heat treatment on the survival of a population of bacterial endospores.

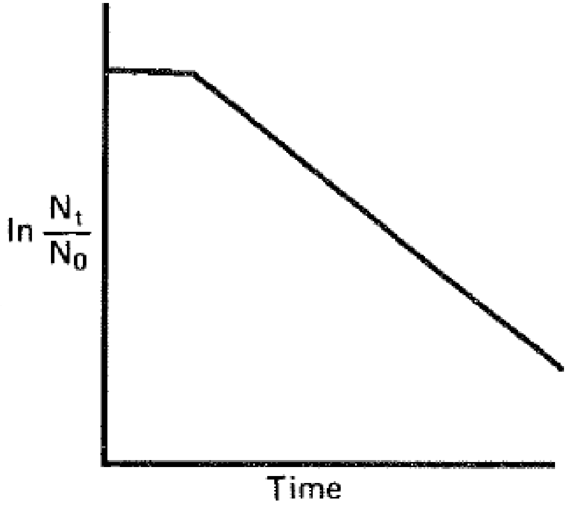
The activation of spores is significantly more than their destruction during the early stages of the process and, therefore, viable numbers increase before the observation of exponential decline.



Initial population increase resulting from the heat activation of spores in the early stages of a sterilization process

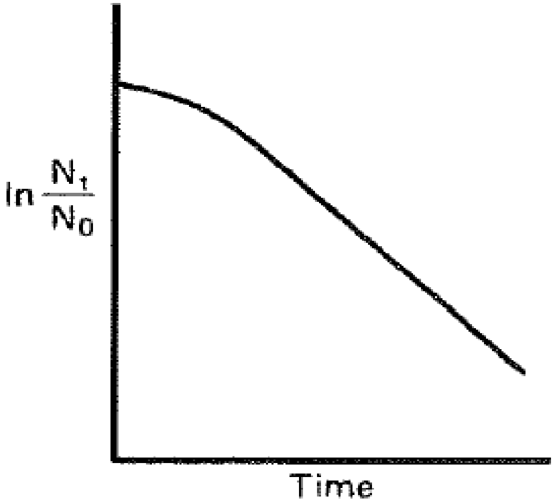
Sterilization

The activation of spores is balanced by spore death.



An initial stationary period observed during a sterilization treatment due to the death of spores being completely compensated by the heat activation of spores

The activation of spores is less than spore death.



Initial population decline at a sub-maximum rate during a sterilization treatment due to the death of spores being compensated by the heat activation of spores

Sterilization

The deviation from an immediate exponential decline in viable spore number is due to the heat activation of the spores, that is the induction of spore germination by the heat and moisture of the initial period of the sterilization process.

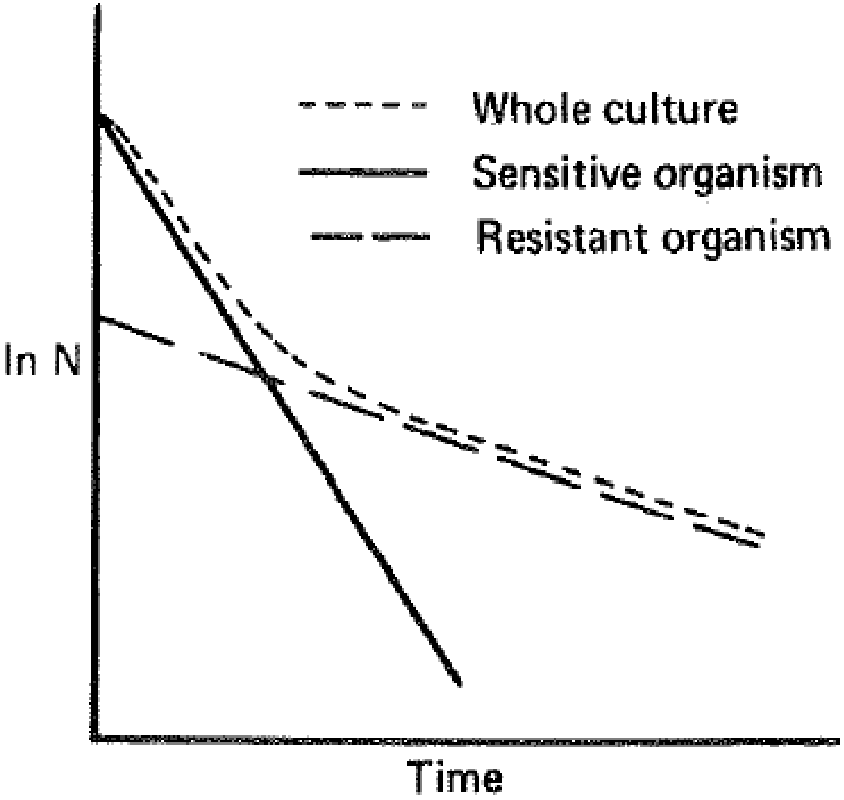
Lecture 62-Advances in Fermentation Technology

MEDIUM

Sterilization–3

Figures in next slides illustrate typical results of the sterilization of mixed cultures containing two species with different heat sensitivities.

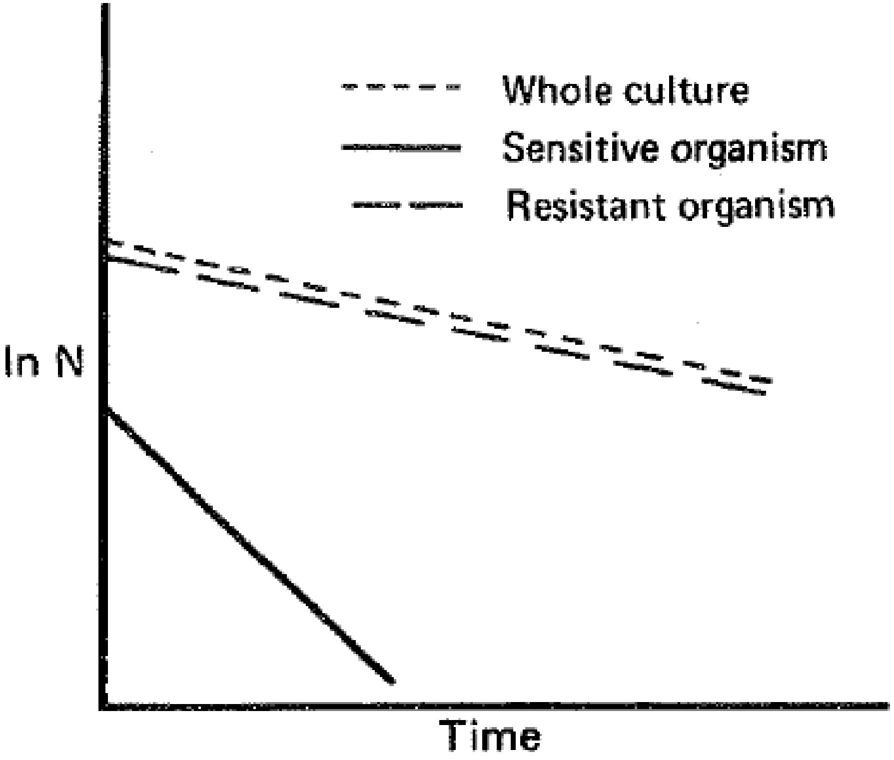
Sterilization



The effect of a sterilization treatment on a mixed culture consisting of a high proportion of a very sensitive organism

In this case, the population consists mainly of the less-resistant type where the initial decline is due principally to the destruction of the less-resistant cell population and the later, less rapid decline, is due principally to the destruction of the more resistant cell population.

Sterilization



The effect of a sterilization treatment on a mixed culture consisting of a high proportion of a relatively resistant organism

This case represents the reverse situation where the more resistant type predominates and its presence disguises the decrease in the number of the less resistant type.

Sterilization

As with any first-order reaction, the reaction rate increases with increase in temperature due to an increase in the reaction rate constant, which, in the case of the destruction of micro-organisms, is the specific death rate *(k).* Thus, *k* is a true constant only under constant temperature conditions.

Sterilization

The relationship between temperature and the reaction rate constant was demonstrated by Arrhenius and may be represented by the equation:

$$\mathrm{d}\ln k/\mathrm{d}T = E/RT^2$$

where E is the activation energy,
R is the gas constant,
T is the absolute temperature.

On integration, above equation gives:

$$k = A\mathrm{e}^{-E/RT}$$

where A is the Arrhenius constant.

On taking natural logarithms, this equation becomes:

$$\ln k = \ln A - E/RT$$

Sterilization

From the last equation it may be seen that a plot of ln k against the reciprocal of the absolute temperature will give a straight line. Such a plot is termed an Arrhenius plot and enables the calculation of the activation energy and the prediction of the reaction rate for any temperature.

Lecture 63-Sterilization

MEDIUM Sterilization–4

By combining together equations $\ln(N_t/N_0) = -kt$ and $k = Ae^{-E/RT}$, the following expression may be derived for the heat sterilization of a pure culture at a constant temperature:

$$\ln N_0/N_t = A \cdot t \cdot e^{-E/RT}$$

Deindoerfer and Humphrey (1959) used the term ln N_o/N_t as a design criterion for sterilization, which has been variously called the Del factor, Nabla factor and sterilization criterion represented by the term ∇ . Thus, the Del factor is a measure of the fractional reduction in viable organism count produced by a certain heat and time regime. Therefore:

$$\nabla = \ln(N_0/N_t)$$

Sterilization

but $\ln(N_0/N_t) = kt$

and $kt = A \cdot t \cdot e^{-(E/RT)}$

thus $\nabla = A \cdot t \cdot e^{-(E/RT)}$.

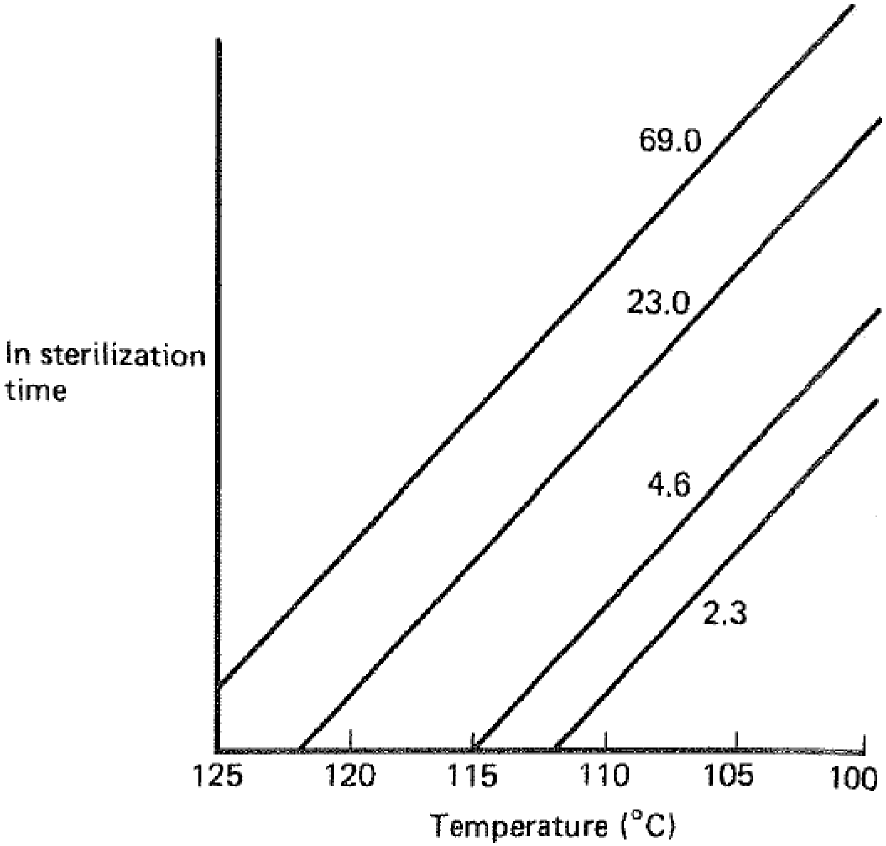
On rearranging, above equation becomes:

$$\ln t = E/RT + \ln(\nabla/A)$$

Thus, a plot of the natural logarithm of the time required to achieve a certain ∇ value against the reciprocal of the absolute temperature will yield a straight line, the slope of which is dependent on the activation energy, as shown in Fig. on next slide.

Sterilization

From Fig. it is clear that the same degree of sterilization (∇) may be obtained over a wide range of time and temperature regimes; that is, the same degree of sterilization may result from treatment at a high temperature for a short time as from a low temperature for a long time.



The effect of sterilization and temperature on the Del factor achieved in the process. The figures on the graph indicate the Del factors for each straight line.

Sterilization

This kinetic description of bacterial death enables The design of procedures (giving certain ∇ factors) for the sterilization of fermentation broths. By choosing a value for N_t, procedures may be designed having a certain probability of achieving sterility, based upon the degree of risk that is considered acceptable.

Lecture 64 Advances in Fermentation Technology

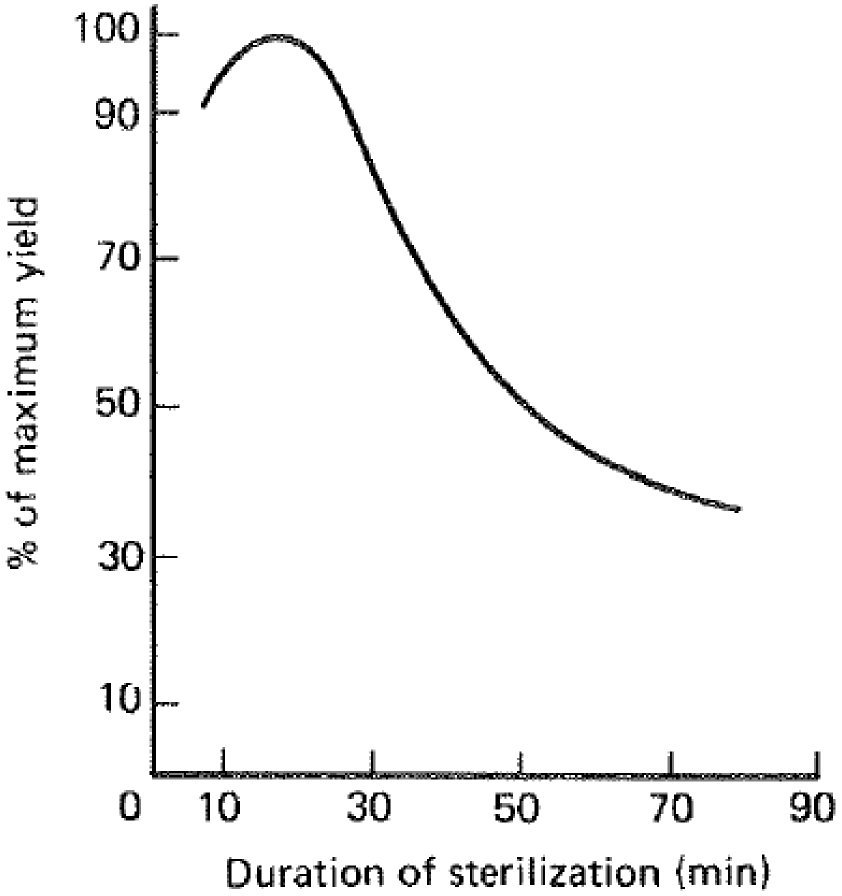
MEDIUM

Sterilization–5

A fermentation medium is not an inert mixture of components and deleterious reactions may occur in the medium during the sterilization process, resulting in a loss of nutritive quality. Thus, the choice of regime is dictated by the requirement to achieve the desired reduction in microbial content with the least detrimental effect on the medium.

Figure in net slide, illustrates the deleterious effect of increasing medium sterilization time on the yield of product of subsequent fermentations. The initial rise in yield is due to some components of the medium being made more available to the process micro-organism by the 'cooking effect' of a brief sterilization period.

Sterilization



The effect of the time of sterilization on the yield of a subsequent fermentation

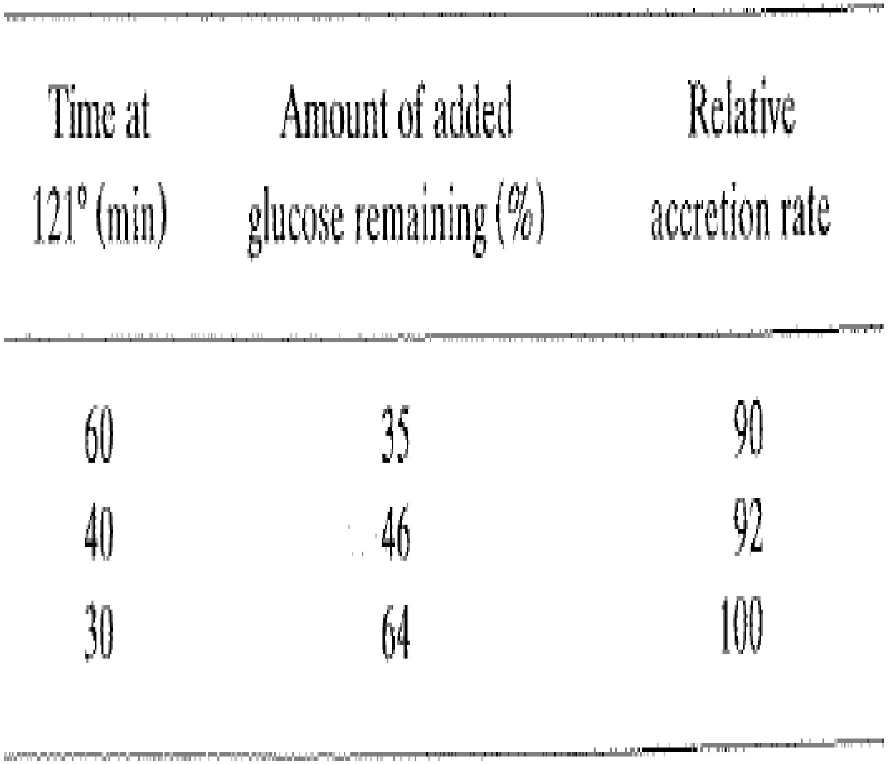
Sterilization

Two types of reaction contribute to the loss of nutrient quality during sterilization:

i. *Interactions between nutrient components of the medium.* A common occurrence during sterilization is the Maillard-type browning reaction which results in discoloration of the medium as well as loss of nutrient quality. These reactions are normally caused by the reaction of carbonyl groups, usually from reducing sugars, with the amino groups of amino acids and proteins. An example of the effect of sterilization time on the availability of glucose in a corn-steep liquor medium is shown in Table (in next slide). Problems of this type are normally resolved by sterilizing the sugar separately from the rest of the medium and recombining the two after cooling.

Sterilization

The effect of sterilization time on glucose concentration and product accretion rate in an antibiotic fermentation



Time at 121° (min)	Amount of added glucose remaining (%)	Relative accretion rate
60	35	90
40	46	92
30	64	100

ii. ***Degradation of heat labile components.*** **Certain vitamins, amino acids and proteins may be degraded during a steam sterilization regime. In extreme cases, such as the preparation of media for animal-cell culture, filtration may be used.**

Lecture 65-Sterilization

MEDIUM

Sterilization–6

The thermal destruction of essential media components conforms approximately with first order reaction kinetics and, therefore, may be described by equations similar to those derived for the destruction of bacteria:

$$x_t / x_0 = e^{-kt}$$

where x_t is the concentration of nutrient after a heat treatment period, t,

x_0 is the original concentration of nutrient at the onset of sterilization,

k is the reaction rate constant.

The effect of temperature on the reaction rate constant may be expressed by the Arrhenius equation:

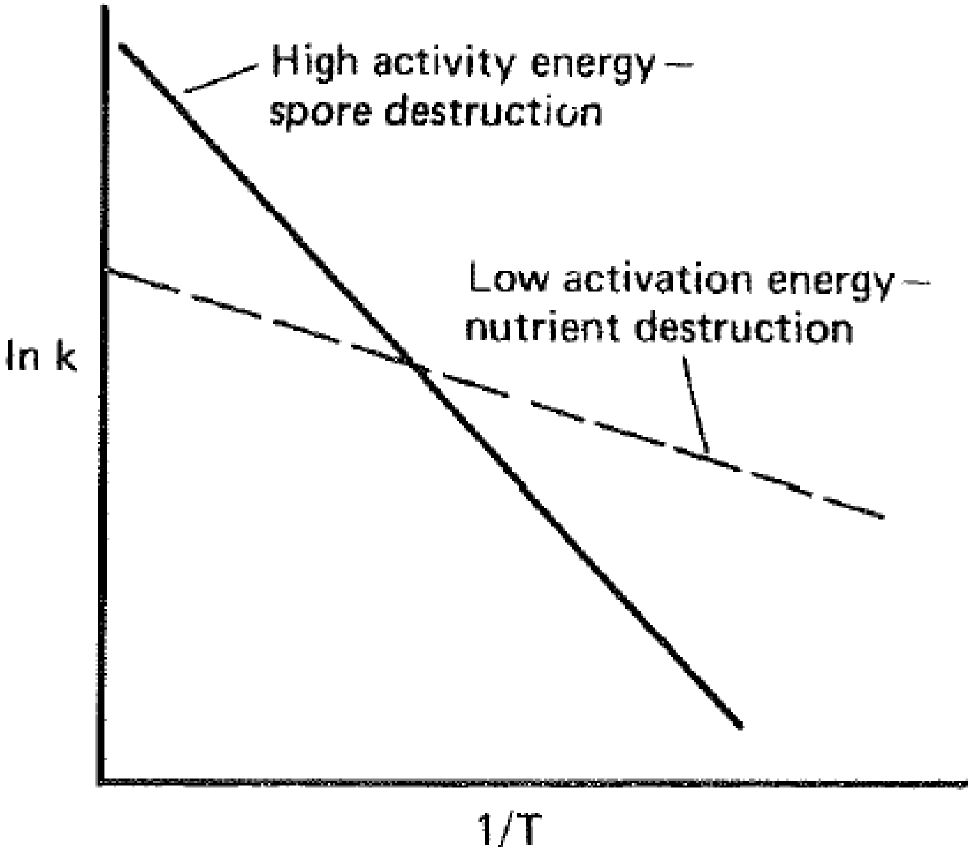
$$\ln k = \ln A - E / RT$$

Sterilization

Therefore, a plot of the natural logarithm of the reaction rate against *1/T* will give a straight line, slope -(*E/R*). As the value of *R*, the gas constant, is fixed the slope of the graph is determined by the value of the activation energy *(E).* The activation energy for the thermal destruction of *B. stearothermophilus* spores has been cited as 67.7 kcal mole^{-1}, whereas that for thermal destruction of nutrients is 10 to 30 kcal mole^{-1}.

Figure (in next slide) is an Arrhenius plot for two reactions - one with a lower activation energy than the other.

Sterilization



The effect of activation energy on spore and nutrient destruction.

From this plot it may be seen that as temperature is increased, the reaction rate rises more rapidly for the reaction with the higher activation energy.

Sterilization

Thus, considering the difference between activation energies for spore destruction and nutrient degradation, an increase in temperature would accelerate spore destruction more than medium denaturation.

Sterilization

Thus, it would appear to be advantageous to employ a high temperature for a short time to achieve the desired probability of sterility, yet causing minimum nutrient degradation.

Therefore, the ideal technique would be to heat the fermentation medium to a high temperature, at which it is held for a short period, before being cooled rapidly to the fermentation temperature.

However, it is obviously impossible to heat a batch of many thousands of litres of broth in a tank to a high temperature, hold for a short period and cool without the heating and cooling periods contributing considerably to the total sterilization time.

Sterilization

The only practical method of materializing the objective of a short-time, high-temperature treatment is to sterilize the medium in a continuous stream.

END

Lecture 66-Advances in Fermentation Technology

MEDIUM

Sterilization–7

The relative merits of batch and continuous sterilization may be summarized as follows:

❑ ***Advantages of continuous sterilization over batch sterilization***

i. **Superior maintenance of medium quality.**

ii. **Ease of scale-up - discussed later.**

iii. **Easier automatic control.**

iv. **The reduction of surge capacity for steam.**

v. **The reduction of sterilization cycle time.**

vi. **Under certain circumstances, the reduction fermenter corrosion.**

Sterilization

- ❑ ***Advantages of batch sterilization over continuous sterilization***

  i. **Lower capital equipment costs.**
  ii. **Lower risk of contamination - processes require the aseptic transfer of the sterile broth to the sterile vessel.**
  iii. **Easier manual control.**
  iv. **Easier to use with media containing a high proportion of solid matter.**

The early continuous sterilizers were constructed as plate heat exchangers and these were unsuitable on two accounts:

i. **Failure of the gaskets between the plates resulted in the mixing of sterile and unsterile streams.**
ii. **Particulate components in the media would block the heat exchangers.**

Sterilization

However, modern continuous sterilizers use double spiral heat exchangers in which the two streams are separated by a continuous steel division. Also, the spiral exchangers are far less susceptible to blockage.

But a major limitation to the adoption of continuous sterilization was the precision of control necessary for its success.

This precision has been achieved with the development of sophisticated computerized monitoring and control systems resulting in continuous sterilization being very widely used and it is now the method of choice.

Sterilization

Nevertheless, batch sterilization is still used in many fermentation plants; and will be discussed in later modules.

Lecture 67-Advances in Fermentation Technology

Sterilization:

THE DESIGN OF BATCH STERILIZATION PROCESSES

Although a batch sterilization process is less successful in avoiding the destruction of nutrients than a continuous one, the objective in designing a batch process is still to achieve the required probability of obtaining sterility with the minimum loss of nutritive quality. The highest temperature which appears to be feasible for batch sterilization is 121°C so the procedure should be designed such that exposure of the medium to this temperature is kept to a minimum.

This achieved by taking into account the contribution made to the sterilization by the heating and cooling periods of the batch treatment.

Sterilization

Deindoerfer and Humphrey (1959) presented a method to assess the contribution made by the heating and cooling periods. The following information must be available for the design of a batch sterilization process:

i. **A profile of the increase and decrease in the temperature of the fermentation medium during the heating and cooling periods of the sterilization cycle.**

ii. **The number of micro-organisms originally present in the medium.**

iii. **The thermal death characteristics of the 'design‘ organism. As explained earlier this may be *Bacillus stearothermophilus* or an alternative organism relevant to the particular fermentation.**

Sterilization

Knowing the original number of organisms present in the fermenter and the risk of contamination considered acceptable, the required Del factor may be calculated. A frequently adopted risk of contamination is 1 in 1000, which indicates that N_t should equal 10^{-3} of a viable cell. It is worth reinforcing at this stage that we are considering the total number of organisms present in the medium and not the concentration. If a specific case is considered where the unsterile broth was shown to contain 10^{11} viable organisms, then the Del factor may be calculated, thus:

$$
\begin{aligned}
\nabla &= \ln\left(10^{11}/10^{-3}\right) \\
\nabla &= \ln 10^{14} \\
&= 32.2
\end{aligned}
$$

Sterilization

Therefore, the overall Del factor required is 32.2. However, the destruction of cells occurs during the heating and cooling of the broth as well as during the period at 121°C, thus, the overall Del factor may be represented as:

$$\nabla_{\text{overall}} = \nabla_{\text{heating}} + \nabla_{\text{holding}} + \nabla_{\text{cooling}}$$

Knowing the temperature-time profile for the heating and cooling of the broth (prescribed by the characteristics of the available equipment) it is possible to determine the contribution made to the overall Del factor by these periods.

Sterilization

Thus, knowing the Del factors contributed by heating and cooling, the holding time may be calculated to give the required overall Del factor.

Lecture 68-Sterilization

Sterilization:

CALCULATION OF 'DEL FACTOR' DURING HEATING AND COOLING

The relationship between Del factor, the temperature and time is given by following equation:

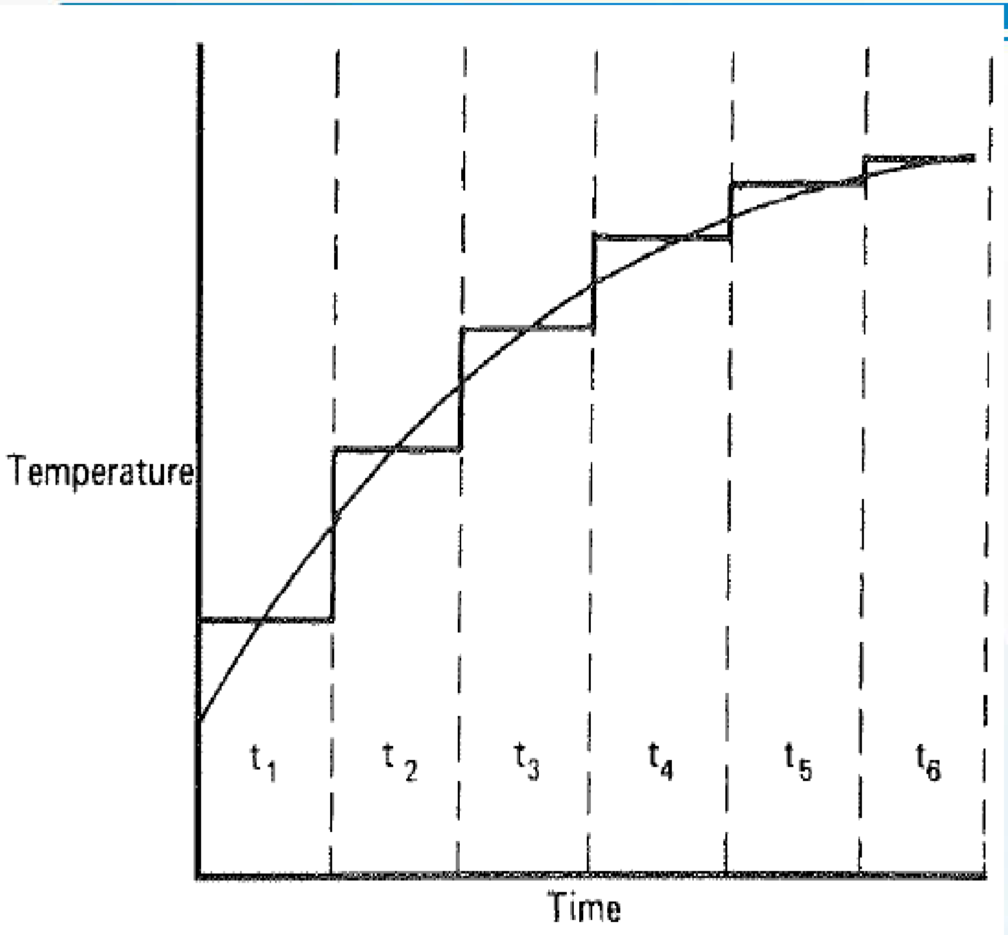
$$\nabla = A \cdot t \cdot e^{-(E/RT)}$$

However, during the heating and cooling periods the temperature ∇ is not constant and, therefore, the calculation of would require the integration of above equation for the time-temperature regime observed.

Deindoerfer and Humphrey (1959) produced integrated forms of the equation for a variety of temperature-time profiles, including linear, exponential and hyperbolic. However, the regime observed in practice is frequently difficult to classify, making the application of these complex equations problematical.

Sterilization

Richards (1968) demonstrated the use of a graphical method of integration and this is illustrated in Figure (on next slide). The time axis is divided into a number of equal increments, t_1, t_2 , t_3, etc., Richards suggesting 30 as a reasonable number.



The graphical integration method applied to the increase in temperature over a time period. t_1 , t_2 , *etc. represent equal time intervals*

Sterilization

For each increment, the temperature corresponding to the mid-point time is recorded. It may now be approximated that the total Del factor of the heating-up period is equivalent to the sum of the Del factors of the mid-point temperatures for each time increment.

The value of the specific death rate of *Bacillus stearothermophilus* spores at each mid-point temperature may be deduced from the Arrhenius equation using the thermal death characteristic published by Deindoerfer and Humphrey (1959).

The value of the Del factor corresponding to each time increment may then be calculated from the equations:

$$\nabla_1 = k_1 t, \quad \nabla_2 = k_2 t, \quad \nabla_3 = k_3 t, \quad \text{etc.}$$

Sterilization

The sum of the Del factors for all the increments will then equal the Del factor for the heating-up period.

The Del factor for the cooling-down period may be calculated in a similar fashion.

Lecture 69-Sterilization

Sterilization:

CALCULATION OF 'HOLDING TIME' AT CONSTANT TEMP.

From the previous calculations (in last module) the overall Del factor, as well as the Del factors of the heating and cooling parts of the cycle, have been determined. Therefore, the Del factor to be achieved during the holding time may be calculated by difference:

$$\nabla_{\text{holding}} = \nabla_{\text{overall}} - \nabla_{\text{heating}} - \nabla_{\text{cooling}}$$

Using our example where the overall Del factor is 32.2 and if it is taken that the heating Del factor was 9.8 and the cooling Del factor 10.1, the holding Del factor may be calculated:

$$\nabla_{\text{holding}} = 32.2 - 9.8 - 10.1,$$

$$\nabla_{\text{holding}} = 12.3.$$

Sterilization

But $\nabla = kt$, and from the data of Deindoerfer and Humphrey (1961) the specific death rate of *B. stearothermophilus* spores at 121°C is 2.54 min^{-1}.

$$\text{Therefore, } t = \nabla/k \text{ or } t = 12.3/2.54 = 4.84 \text{ min.}$$

If the contribution made by the heating and cooling parts of the cycle were ignored then the holding time would be given by the equation:

$$t = \nabla_{\text{overall}}/k = 32.2/2.54 = 12.68 \text{ min}$$

Sterilization

Thus, by considering the contribution made to the sterilization process by the heating and cooling parts of the cycle a considerable reduction in exposure achieved.

Lecture 70-Advances in Fermentation Technology

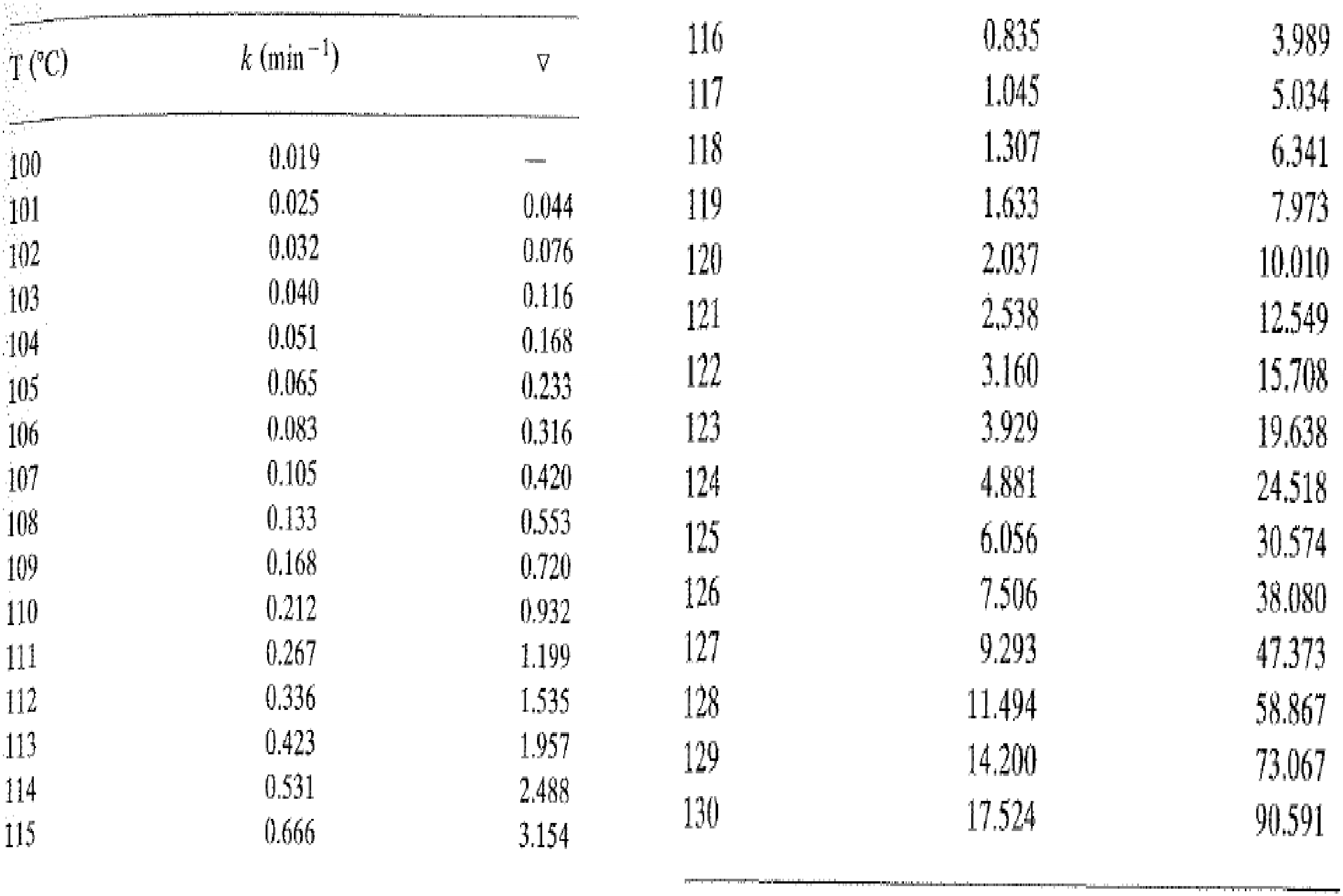
Sterilization:

Richards' RAPID METHOD FOR DESIGNING OF STERILIZATION CYCLES

Richards (1968) proposed a rapid method for the design of sterilization cycles avoiding the time consuming graphical integrations. The method assumes that all spore destruction occurs at temperatures above 100°C and that those parts of the heating and cooling cycle above 100°C are linear. Both these assumptions reasonably valid and the technique loses very little in accuracy and gains considerably in simplicity.

Furthermore, based on these assumptions, Richards has presented a table of Del factors for *B. stearothermophilus* spores which would be obtained in heating and cooling a broth up to (and down from) holding temperatures of 101-130°C, based on a temperature change of 1°C per minute. This information is presented in Table (on next slide), together with the specific death rates for *B. stearothermophilus* spores over the temperature range.

Sterilization



T (°C)	k (min^{-1})	∇
100	0.019	—
101	0.025	0.044
102	0.032	0.076
103	0.040	0.116
104	0.051	0.168
105	0.065	0.233
106	0.083	0.316
107	0.105	0.420
108	0.133	0.553
109	0.168	0.720
110	0.212	0.932
111	0.267	1.199
112	0.336	1.535
113	0.423	1.957
114	0.531	2.488
115	0.666	3.154
116	0.835	3.989
117	1.045	5.034
118	1.307	6.341
119	1.633	7.973
120	2.037	10.010
121	2.538	12.549
122	3.160	15.708
123	3.929	19.638
124	4.881	24.518
125	6.056	30.574
126	7.506	38.080
127	9.293	47.373
128	11.494	58.867
129	14.200	73.067
130	17.524	90.591

Sterilization

If the rate of temperature change is 1° per minute, the Del factors for heating and cooling may be read directly from the table; if the temperature change deviates from 1° per minute, the Del factors may be altered by simple proportion.

For example, if a fermentation broth were heated from 100° to 121°C in 30 minutes and cooled from 121° to 100° in 17 minutes, the Del factors for the heating and cooling cycles may be determined as follows:

From Table (in previous two slides), if the change in temperature had been 1° per minute, the Del factor for both the heating and cooling cycles would be 12.549. But the temperature change in the heating cycle was 21° in 30 minutes; therefore,

$$\mathrm{Del}_{\mathrm{heating}} = (12.549 \times 30)/21 = 17.93$$

Sterilization

and the temperature change in the cooling cycle was 21° in 17 minutes, therefore,

$$\mathrm{Del}_{\mathrm{cooling}} = (12.549 \times 17)/21 = 10.16.$$

Having calculated the Del factors for the heating and cooling periods the holding time at the constant temperature may be calculated as before.

Lecture 71-Advances in Fermentation Technology

Sterilization:

THE SCALE-UP OF BATCHSTERILIZATION PROCESSES

The use of the Del factor in the scale up of batch sterilization processes has been discussed by Banks (1979). It should be appreciated by this stage that the Del factor does not include a volume term, i.e. absolute numbers of contaminants and survivors are considered, not their concentration.

Thus, if the size of a fermenter is increased the initial number of spores in the medium will also be increased, but if the same probability of achieving sterility is required the final spore number should remain the same, resulting in an increase in the Del factor.

Sterilization

For example, if a pilot sterilization were carried out in a 1000 dm^3 vessel with a medium containing 10^6 organisms cm^{-3} requiring a probability of contamination of 1 in 1000, the Del factor would be:

$$\begin{aligned}\nabla &= \ln\left\{(10^6 \times 10^3 \times 10^3)/10^{-3}\right\}\\ &= \ln\left(10^{12}/10^{-3}\right)\\ &= \ln 10^{15} = 34.5.\end{aligned}$$

If the same probability of contamination were required in a 10,000 dm^3 vessel using the same medium the Del factor would be:

$$\begin{aligned}\nabla &= \ln\left\{(10^6 \times 10^3 \times 10^4)/10^{-3}\right\}\\ &= \ln\left(10^{13}/10^{-3}\right)\\ &= \ln 10^{16} = 36.8.\end{aligned}$$

Sterilization

Thus, the Del factor increases with an increase in the size of the fermenter volume. The holding time in the large vessel may be calculated by the graphical integration method or by the rapid method of Richards (1968), as discussed in last module, based on the temperature-time profile of the sterilization cycle in the large vessel.

However, it must be appreciated that extending the holding time on the larger scale (to achieve the increased ∇ factor) will result in increased nutrient degradation. Also, the contribution of the heating-up and cooling-down periods to nutrient destruction will be greater as scale increases.

Sterilization

Maintaining the same nutrient quality on a small and a large scale can be achieved in batch sterilization only by compromising the sterility of the vessel, which is totally unacceptable.

Thus, the decrease in the yield of a fermentation when it is scaled up is often due to problems of nutrient degradation during batch sterilization and the only way to eradicate the problem is to sterilize the medium continuously.

Lecture 72-Advances in Fermentation Technology

Sterilization:

METHODS OF BATCH STERILIZATION

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. The major advantages of a separate medium sterilization vessel may be summarized as:

i. 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.

ii. 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.

Sterilization

iii. In some fermentations, the medium is at its most viscous during sterilization and the power requirement for agitation is not alleviated by aeration as it would be during the fermentation proper. Thus, if the requirement for agitation during *in situ sterilization* were removed, the fermenter could be equipped with a less powerful motor. Obviously, the sterilization kettle would have to be equipped with a powerful motor, but this would provide sterile medium for several fermenters.

iv. The fermenter would be spared the corrosion which may occur with medium at high temperature.

Sterilization

The major disadvantages of a separate medium sterilization vessel may be summarized as:

i. The cost of constructing a batch medium sterilizer is much the same as that for the fermenter.

ii. If a cooker serves a large number of fermenters complex pipework would be necessary to transport the sterile medium, with the inherent dangers of contamination.

iii. Mechanical failure in a cooker supplying medium to several fermenters would render all the fermenters temporarily redundant. The provision of contingency equipment may be prohibitively costly.

Sterilization

Overall, the pressure to decrease the 'down time' between fermentations has tended to outweigh the perceived disadvantages of using separate sterilization vessels. Thus, sterilization in dedicated vessels is the method of choice for batch sterilization.

Lecture 73-Advances in Fermentation Technology

Sterilization:

THE DESIGN OF CONTINUOUS STERILIZATION PROCESSES–1

The design of continuous sterilization cycles may be approached in exactly the same way as for batch sterilization systems. The continuous system includes a time period during which the medium is heated to the sterilization temperature, a holding time at the desired temperature and a cooling period to restore the medium to the fermentation temperature.

The temperature of the medium is elevated in a continuous heat exchanger and is then maintained in an insulated serpentine holding coil for the holding period.

The length of the holding period is dictated by the length of the coil and the flow rate of the medium.

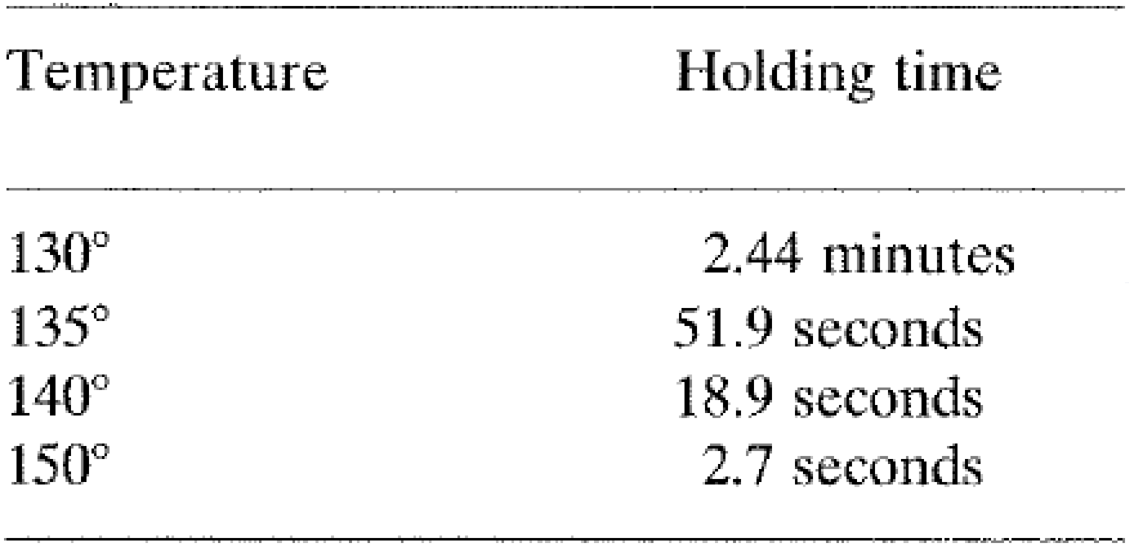
Sterilization

The hot medium is then cooled to the fermentation temperature using two sequential heat exchangers - the first utilizing the in-coming medium as the cooling source (thus conserving heat by heating-up the incoming medium) and the second using cooling water. The major advantage of the continuous process is that a much higher temperature may be utilized, thus reducing the holding time and reducing the degree of nutrient degradation.

The required Del factor may be achieved by the combination of temperature and holding time which gives acceptably small degree of nutrient decay.

Sterilization

The Del factor for the example sterilization was 45.7 and the following temperature time regimes were calculated to give the same Del factor:



Temperature	Holding time
130°	2.44 minutes
135°	51.9 seconds
140°	18.9 seconds
150°	2.7 seconds

Furthermore, because a continuous process involves treating small increments of medium the heating-up and cooling-down periods are very small compared with those in a batch system.

Sterilization

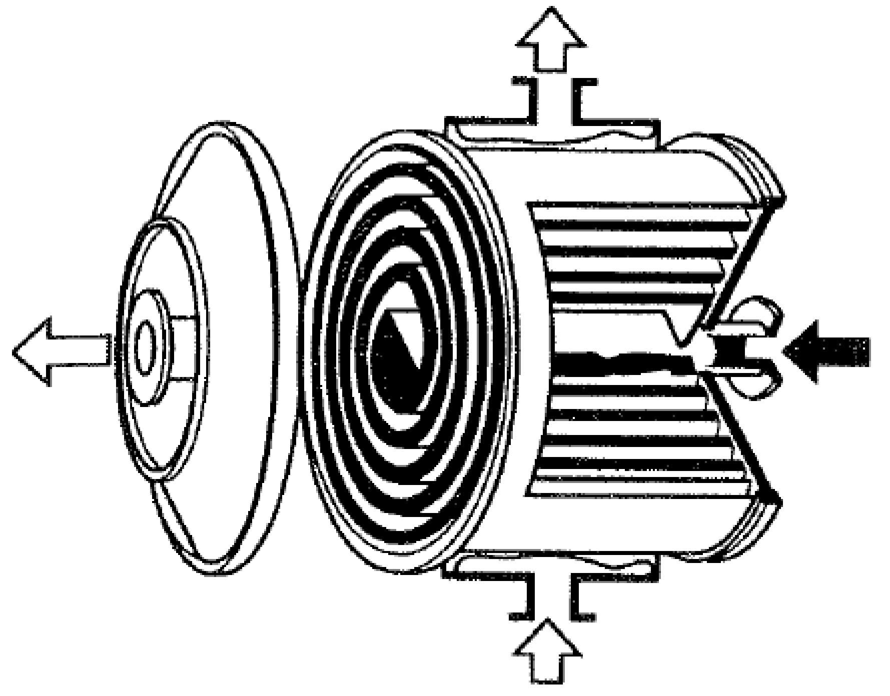
There are two types of continuous sterilizer which may be used for the treatment of fermentation media: the indirect heat exchanger and the direct heat exchanger (steam injector).

Lecture 74-Advances in Fermentation Technology

Sterilization:

THE DESIGN OF CONTINUOUS STERILIZATION PROCESSES–2

The most suitable indirect heat exchangers are of the double-spiral type which consists of two sheets of high-grade stainless steel which have been curved around a central axis to form a double spiral, as shown in following Figure. The ends of the spiral are sealed by covers.



Sterilization

To achieve sterilization temperatures steam is passed through one spiral and medium through the other in countercurrent streams. Spiral heat exchangers are also used to cool the medium after passing through the holding coil. Incoming unsterile medium is used as the cooling agent in the first cooler so that the incoming medium is partially heated before it reaches the sterilizer and, thus, heat is conserved. The major advantages of the spiral heat exchanger are:

i. The two streams of medium and cooling liquid, or medium and steam, are separated by a continuous stainless steel barrier with gasket seals being confined to the joints with the end plates. This makes cross contamination between the two streams unlikely.

Sterilization

ii. The spiral route traversed by the medium allows sufficient clearances to be incorporated for the system to cope with suspended solids. The exchanger tends to be self-cleaning which reduces the risk of sedimentation, fouling and 'burning-on'.

Lecture 75-Advances in Fermentation Technology

Sterilization:

THE DESIGN OF CONTINUOUS STERILIZATION PROCESSES-3

Indirect plate heat exchangers consist of alternating plates through which the countercurrent streams are circulated. The plates are separated by gaskets and failure of these gaskets can cause cross-contamination between the two streams. Also, the clearances between the plates are such that suspended solids in the medium may block the exchanger and, thus, the system is only useful in sterilizing completely soluble media. However, the plate exchanger is more adaptable than the spiral system in that extra plates may be added to increase its capacity.

The continuous steam injector injects steam directly into the unsterile broth. The advantages and disadvantages of the system have been summarized by Banks (1979):

Sterilization

i. Very short (almost instantaneous) heating up times.
ii. It may be used for media containing suspended solids.
iii. Low capital cost.
iv. Easy cleaning and maintenance.
v. High steam utilization efficiency.

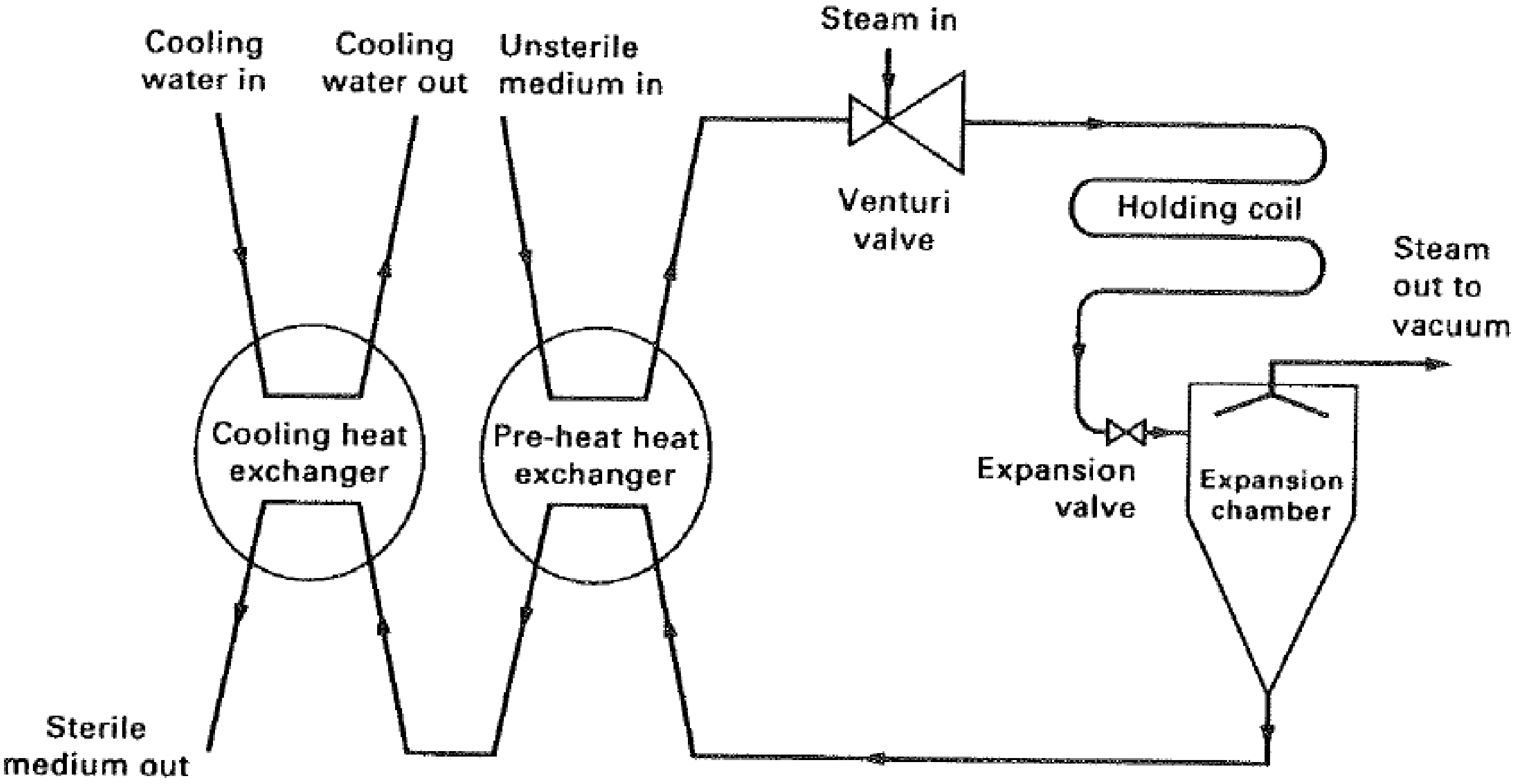
However, the disadvantages are:

i. Foaming may occur during heating.
ii. Direct contact of the medium with steam requires that allowance be made for condense dilution and requires 'clean' steam, free from anticorrosion additives.

In some cases the injection system is combined with flash cooling, where the sterilized medium is cooled by passing it through an expansion valve into a vacuum chamber. Cooling then occurs virtually instantly.

Sterilization

A flow chart of a continuous sterilization system using direct steam injection is shown in following Figure:



Flow diagram of a typical continuous injector-flash cooler sterilizer

Sterilization

In some cases a combination of direct and indirect heat exchangers may be used. This is especially true for starch-containing broths when steam injection is used for the pre-heating step.

By raising the temperature virtually instantaneously the critical gelatinization temperature of the starch is passed through very quickly and the increase in viscosity normally associated with heated starch colloids can be reduced.

Lecture 76-Advances in Fermentation Technology

Sterilization

of the FERMENTER,FEEDS, and of LIQUID WASTES

❑Sterilization of the Fermenter

If the medium is sterilized in a separate batch cooker, or is sterilized continuously, then the fermenter has to be sterilized separately before the sterile medium is added to it. This is normally achieved by heating the jacket or coils of the fermenter with steam and sparging steam into the vessel through all entries, apart from the air outlet from which steam is allowed to exit slowly. Steam pressure is held at 15 psi in the vessel for approximately 20 minutes. It is essential that sterile air is sparged into the fermenter after the cycle is complete and a positive pressure is maintained; otherwise a vacuum may develop and unsterile air be drawn into the vessel.

Sterilization

❑Sterilization of the Feeds

A variety of additives may be administered to a fermentation during the process and it is essential that these materials are sterile. The sterilization method depends on the nature of the additive, and the volume and feed rate at which it is administered. If the additive is fed in large quantities then continuous sterilization may be desirable. Batch sterilization of feed liquids normally involves steam injection into the material held in storage vessels. Whatever the sterilization system employed it is essential that all ancillary equipment and feed pipework associated with the additions are sterilizable.

Sterilization

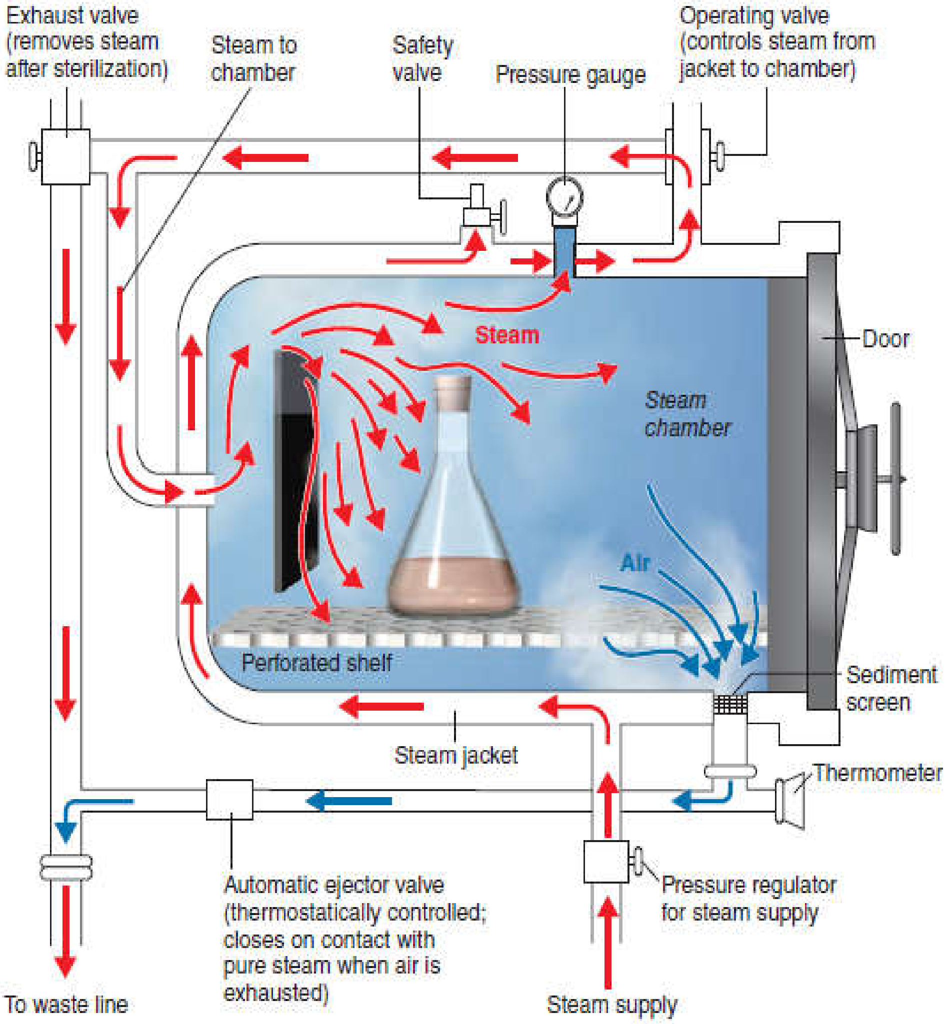
❑Sterilization of the Liquid Wastes

Process organisms which have been engineered to produce 'foreign' products and therefore contain heterologous genes are subject to strict containment regulations. Thus, waste biomass of such organisms must be sterilized before disposal.

Sterilization may be achieved by either batch or continuous means but the whole process must be carried out under contained conditions.

Batch sterilization involves the sparging of steam into holding tanks, whereas continuous processes would employ the type of heat exchangers.

Sterilization



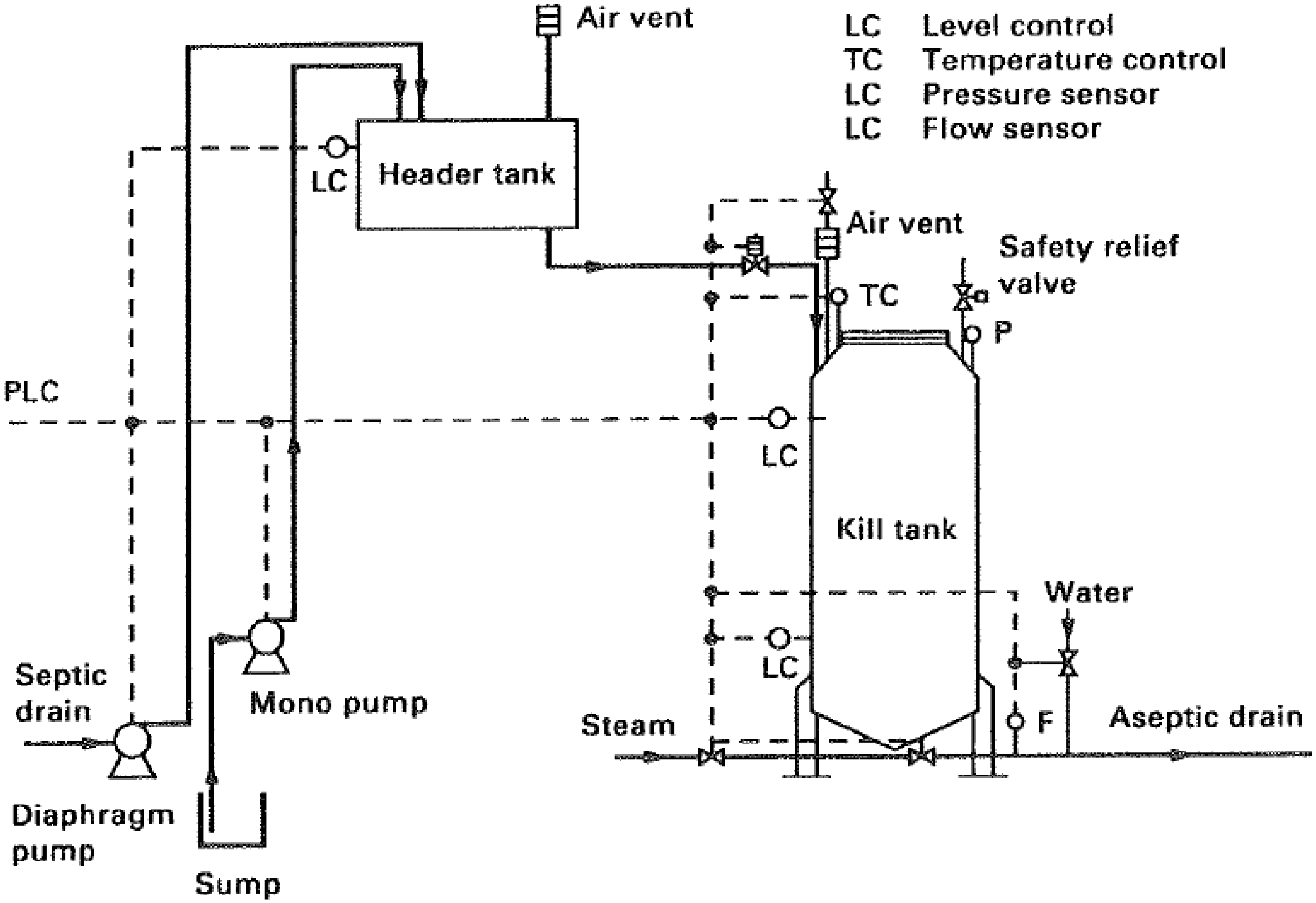
An autoclave. The entering steam forces the air out of the bottom (blue arrows). The automatic ejector valve remains open as long as an air-steam mixture is passing out of the waste line. When all the air has been ejected, the higher temperature of the pure steam closes the valve, and the pressure in the chamber increases.

Sterilization

A holding vessel for the batch sterilization of waste is shown in Figure on next slide. Whichever method is employed the effluent must be cooled to below 60°C before it is discharged to waste. The sterilization processes have to be validated and are designed using the Del factor approach.

However, the kinetic characteristics used in the calculations would be those of the process organism rather than of *B. stearothermophilus.* Also, the N_t value used in the design calculations would be smaller than 10^{-3} which is used for medium sterilization and would depend on the assessment of the hazard involved should the organism survive the decontamination process.

Sterilization



A vessel for the batch sterilization of liquid waste from a contained fermentation

Sterilization

Thus, the sterilization regime used for destruction of the process organism will be different from that used in sterilizing the medium.

FILTER

Sterilization

Suspended solids maybe separated from a fluid during filtration by the following mechanisms:

i. ***Inertial impaction.***

ii. ***Diffusion.***

iii. ***Electrostatic attraction.***

iv. ***Interception.***

Sterilization

❑Inertial Impaction

Suspended particles in a fluid stream have momentum. The fluid in which the particles are suspended will flow through the filter by the route of least resistance. However, the particles, because of their momentum, tend to travel in straight lines and may therefore become impacted upon the fibers where they may then remain. Inertial impaction is more significant in the filtration of gases than in the filtration of liquids.

❑Diffusion

Extremely small particles suspended in a fluid are subject to Brownian motion which is random movement due to collisions with fluid molecules. Thus, such small particles tend to deviate from the fluid flow pattern and may be come impacted upon the filter fibers. Diffusion is more significant in the filtration of gases than in the filtration of liquids.

Sterilization

❑Electrostatic attraction

Charged particles may be attracted by opposite charges on the surface of the filtration medium.

❑Interception

The fibers comprising a filter are interwoven to define openings of various sizes. Particles which are larger than the filter pores are removed by direct interception. However, a significant number of particles which are smaller than the filter pores are also retained by interception. This may occur by several mechanisms – more than one particle may arrive at a pore simultaneously, an irregularly shaped particle may bridge a pore, once a particle has been trapped by a mechanism other than interception the pore may be partially occluded enabling the entrapment of smaller particles. Interception is equally important a mechanism in the filtration of gases and liquids.

Sterilization

Filters have been classified into two types – those in which the pores in the filter are smaller than the particles which are to be removed and those in which the pores are larger than the particles which are to be removed.

Lecture 78-Advances in Fermentation Technology

FILTER

Sterilization OF FERMENTATION MEDIA

Media for animal-cell culture cannot be sterilized by steam because they contain heat-labile proteins. Thus, filtration is the method of choice and fixed pore or absolute filtration is the better system to use. An ideal filtration system for the sterilization of animal cell culture media must fulfill the following criteria:

i. ***The filtered medium must be free of fungal, bacterial and mycoplasma contamination.***
ii. ***There should be minimal adsorption of protein to the filter surface.***
iii. ***The filtered medium should be free of viruses.***
iv. ***The filtered medium should be free of bacterial endotoxins.***

Sterilization

Several filter manufacturers now supply absolute filtration systems for the sterilization of animal cell culture medium. Such systems consist of membrane cartridges which are fitted into stainless steel, steam sterilizable modules. The membranes for media filtration are constructed from steam sterilizable hydrophilic material and are treated to produce a filtrate of particular quality. For example, if minimal protein adsorption is a major criterion then a specially coated filter membrane is used. It would be very difficult to construct a single filtration membrane which would fulfill all four criteria cited above. Thus, a series of filters are used to achieve the desired result.

Sterilization

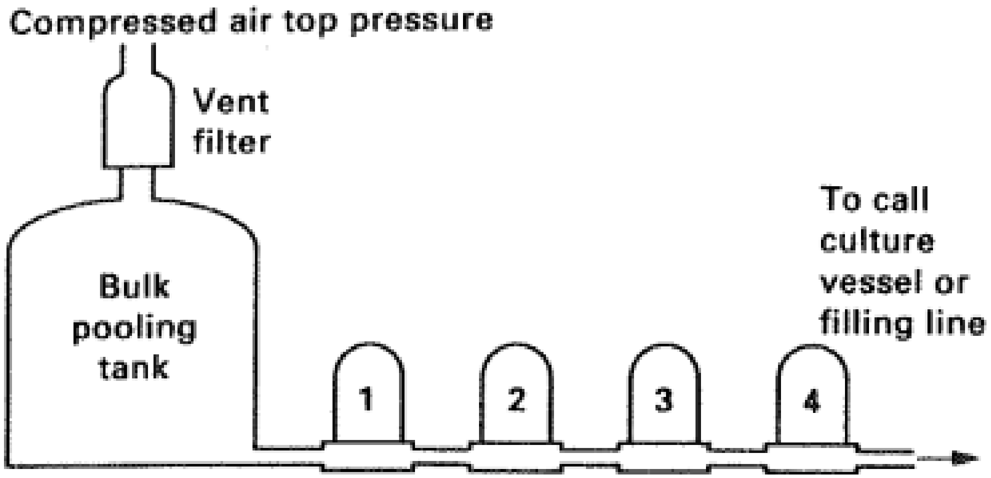


Figure representing the Filtration system for the provision of sterile, Mycoplasma free serum (Pall Process Filtration Ltd.,Portsmouth, U.K.).

Filter 1.	**5µm absolute rated pre-filter for removal of coarse precipitates.**
Filter 2.	**0.5µm absolute rated pre-filter for bulk bioburden removal.**
Filter 3.	**0.1µm absolute rated single layer pre-filter for further bioburden and endotoxin removal.**
Filter 4.	**0.1um absolute rated double layer final filter for absolute sterility, mycoplasma removal and further endotoxin control.**

Sterilization

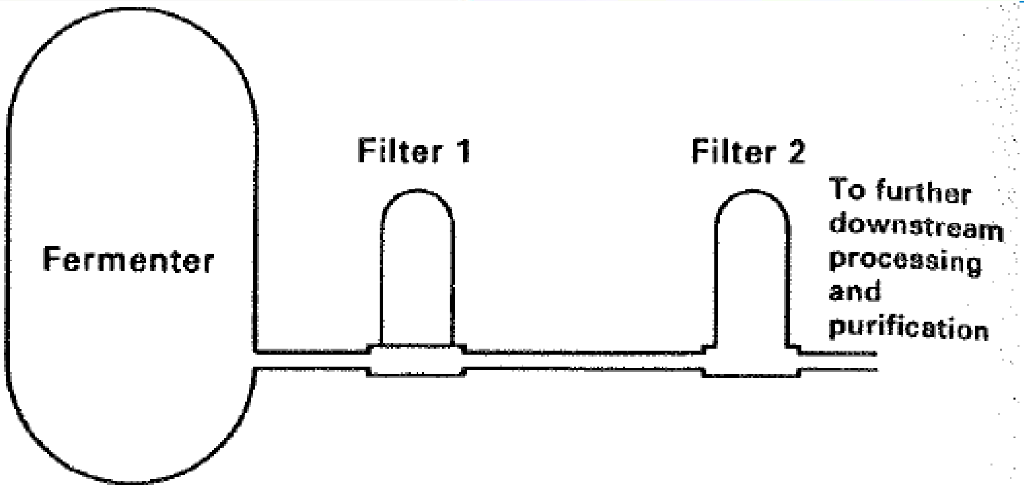


Figure representing the Filtration system for the removal of cells and cell debris form an animal cell culture fermentation.(Pall Process Filtration Ltd., Portsmouth, U.K.)*.*

Filter 1.	**1.0µm absolute rated prefilter for bulk cell and cell debris removal.**
Filter 2.	**O.2µm absolute rated single layer 'Bio-Inert' filter for final bioburden removal.**

Sterilization

The pre-filter used in figure shown in previous slide is a polypropylene 1.0µm rated filter to remove the bulk of the cells and debris and the second filter is an hydroxyl modified nylon/polyester 0.2-µm rated filter giving absolute cell removal with minimal protein adsorption.

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FILTER

Sterilization OF FERMENTATION AIR

Aerobic fermentations require the continuous addition of considerable quantities of sterile air. Although it is possible to sterilize air by heat treatment, the most commonly used sterilization process is filtration. Fixed pore filters (which have an absolute rating) are very widely used in the fermentation industry and several manufacturers produce filtration systems for air sterilization. These systems, like those for the sterilization of liquids, consist of pleated membrane cartridges designed to be accommodated in stainless steel modules. The most common construction material used for the pleated membranes for air sterilization is Polytetrafluoroethylene (PTFE), which is hydrophobic and is therefore resistant to wetting.

Sterilization

Also, PTFE filters may be steam sterilized and are resistant to ammonia which may be injected into the air stream, prior to the filter, for pH control. As for the filter sterilization of liquids it is essential that a prefilter is incorporated up-stream of the absolute filter. The prefilter traps large particles such as dust, oil and carbon (from the compressor) and pipescale and rust (from the pipework). The use of a coalescing prefilter also ensures the removal of water from the air; entrained water is coalesced in the filter (air flow being from the inside of the filter to the outside) and is discharged via an automatic drain.

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Sterilization OF FERMENTER EXHAUST AIR

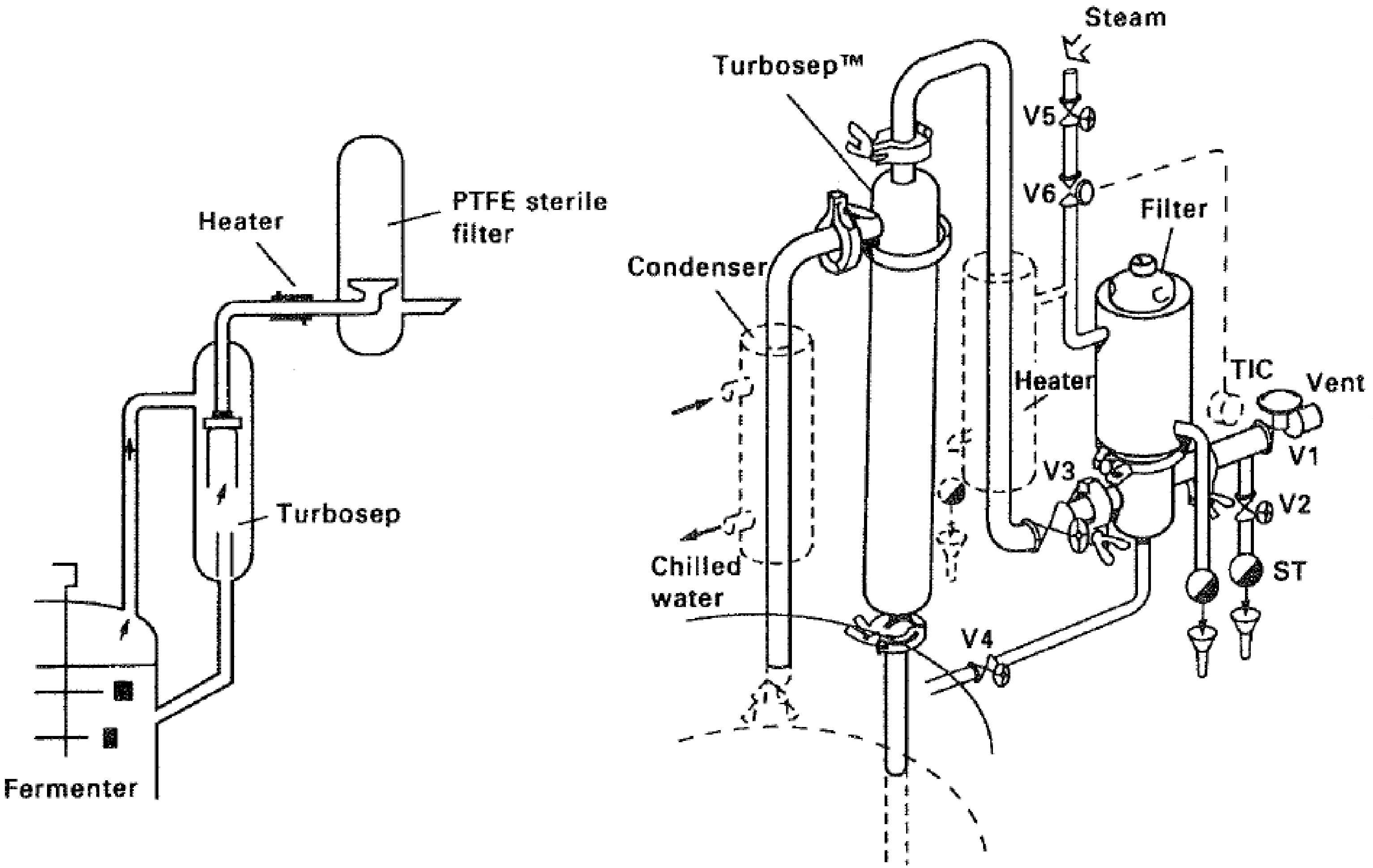
In many traditional fermentations the exhaust gas from the fermenter was vented without sterilization or vented through relatively inefficient depth filters. With the advent of the use of recombinant organisms and a greater awareness of safety and emission levels of allergic compounds the containment of exhaust air is more common (and in the case of recombinant organisms, compulsory). Fixed pore membrane modules are also used for this application but the system must be able to cope with the sterilization of water saturated air, at a relatively high temperature and carrying a large contamination level. Also, foam may overflow from the fermenter into the air exhaust line.

Sterilization

Thus, some form of pretreatment of the exhaust gas is necessary before it enters the absolute filter. This pretreatment may be a hydrophobic prefilter or a mechanical separator to remove water, aerosol particles and foam.

The pretreated air is then fed to a 0.2µm hydrophobic filter. Again, it is important to appreciate that the filtration system must be steam sterilizable. Figures in next slides illustrate the prefilter and mechanical separator systems respectively.

Sterilization



A mechanical separator and hydrophobic filter system for the sterilization of off-gas from a fermenter. Left. Cut-away diagram. Right. Equipment arrangement, showing steam supply. VI-V6, *valves; O, steam, traps*

Sterilization

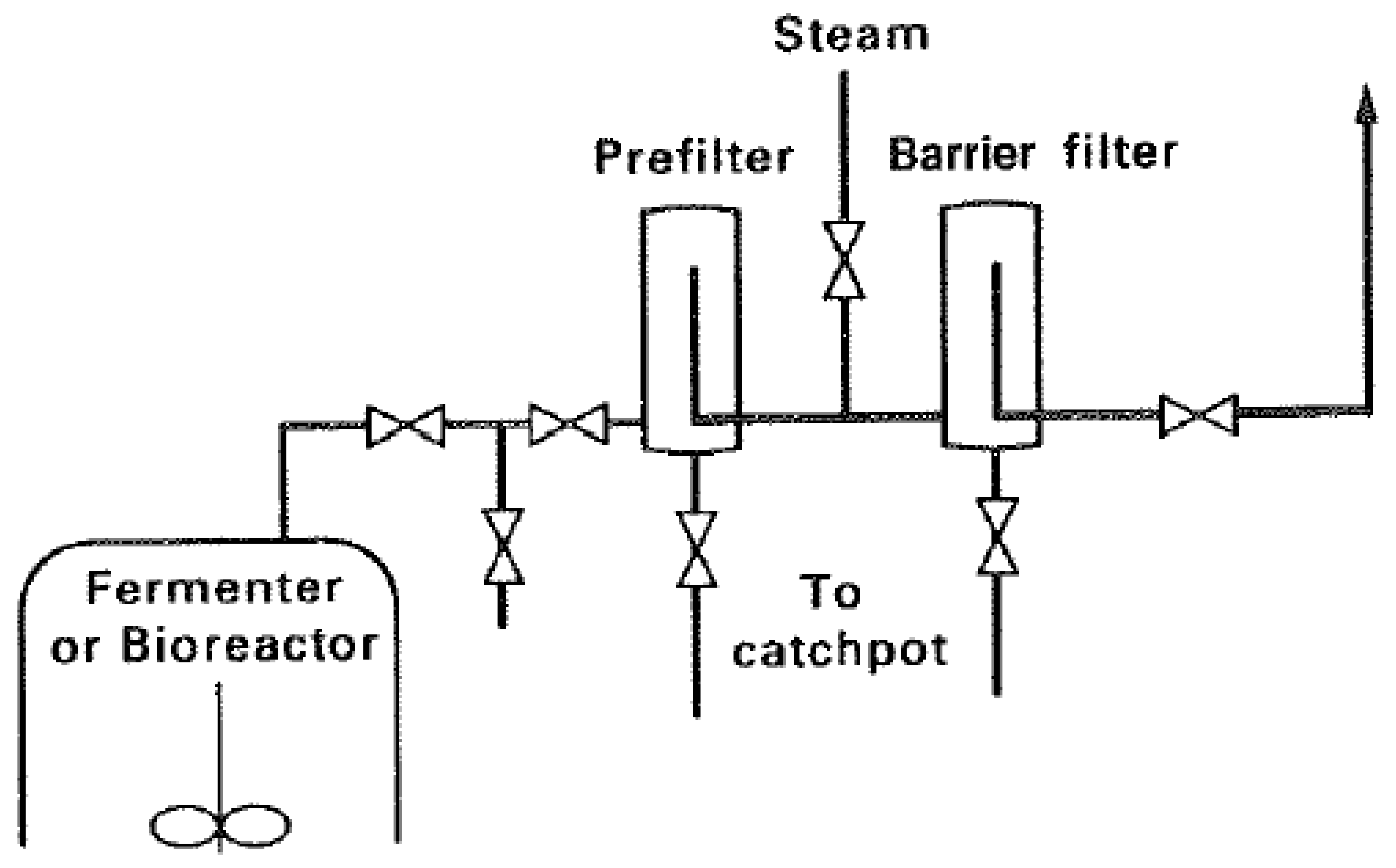


Figure showing Dual hydrophobic filter system for the sterilization of off-gas from a fermenter.

Lecture 81 Advances in Fermentation Technology

Recovery and Purification of Fermentation Products-1

- The extraction and purification of fermentation products may be difficult and costly. Ideally, one tries to obtain a high-quality product as quickly as possible at an efficient recovery rate using minimum plant investment operated at minimal costs.

- Unfortunately, recovery costs of microbial products may vary from as low as 15% to as high as 70% of the total manufacturing costs.

Recovery & Purification of Fermentation Products

- If a fermentation broth is analysed at the time of harvesting it will be discovered that the specific product may be present at a low concentration in an aqueous solution that contains intact micro-organisms, cell fragments, soluble and insoluble medium components and other metabolic products.

- The product may also be intracellular, heat labile and easily broken down by contaminating micro-organisms. All these factors tend to increase the difficulties of product recovery.

Recovery & Purification of Fermentation Products

To ensure good recovery or purification, speed of operation may be the over-riding factor because of the labile nature of a product.

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Recovery and Purification of FermentationProducts–2

The processing equipment must therefore be of the correct type and also the correct size to ensure that the harvested broth can be processed within a satisfactory time limit.

The choice of recovery process is based on the following criteria:

1. The intracellular or extracellular location of the product.
2. The concentration of the product in the fermentation broth.
3. The physical and chemical properties of the desired product (as an aid to selecting separation procedures).
4. The intended use of the product.
5. The minimal acceptable standard of purity.

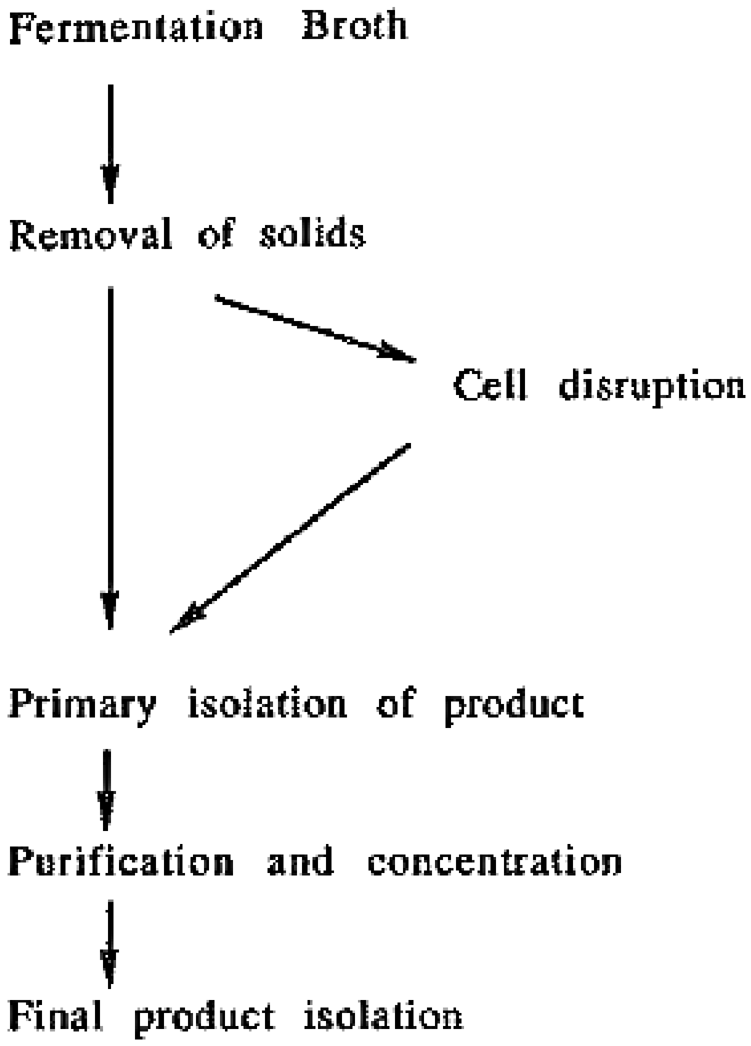
Recovery & Purification of Fermentation Products

6. The magnitude of bio-hazard of the product or broth.
7. The impurities in the fermenter broth.
8. The marketable price for the product.

The main objective of the first stage for the recovery of an extracellular product is the removal of large solid particles and microbial cells usually by centrifugation or filtration (as shown in Fig. on next slide).

Recovery & Purification of Fermentation Products

Stages in the recovery of product from a harvested fermentation broth.



Fermentation Broth

Removal of solids

Cell disruption

Primary isolation of product

Purification and concentration

Final product isolation

Recovery & Purification of Fermentation Products

In the next stage, the broth is fractionated or extracted into major fractions using ultrafiltration, reverse osmosis, adsorption/ ion-exchange/gel filtration or affinity chromatography, liquid-liquid extraction, two phase aqueous extraction or precipitation.

Afterwards, the product-containing fraction is purified by fractional precipitation, further more precise chromatographic techniques and crystallization to obtain a product which is highly concentrated and essentially free from impurities. Other products are isolated using modifications of this flow-stream.

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Recovery and Purification of FermentationProducts–3

It may be possible to modify the handling characteristics of the broth so that it can be handled faster with simpler equipment making use of a number techniques:

1. Selection of a micro-organism which produce pigments or undesirable metabolites.
2. Modification of the fermentation reduce the production of metabolites.
3. Precise timing of harvesting.
4. pH control after harvesting.
5. Temperature treatment after harvesting.
6. Addition of flocculating agents.
7. Use of enzymes to attack cell walls.

Recovery & Purification of Fermentation Products

The recovery and purification of many compounds may be achieved by a number of alternative routes. The decision to follow a particular route involves comparing the following factors to determine the most appropriate under a given set of circumstances (some of them are given below):

1. Capital costs
2. Processing costs
3. Throughput requirements
4. Yield potential
5. Product quality
6. Technical expertise available

Recovery & Purification of Fermentation Products

Micro-organisms and other similar sized particles can be removed from a broth by using a centrifuge when filtration is not a satisfactory separation method. Although a centrifuge may be expensive when compared with a filter it may be essential when:

1. **Filtration is slow and difficult.**
2. **The cells or other suspended matter must be obtained free of filter aids.**
3. **Continuous separation to a high standard of hygiene is required.**

Recovery & Purification of Fermentation Products

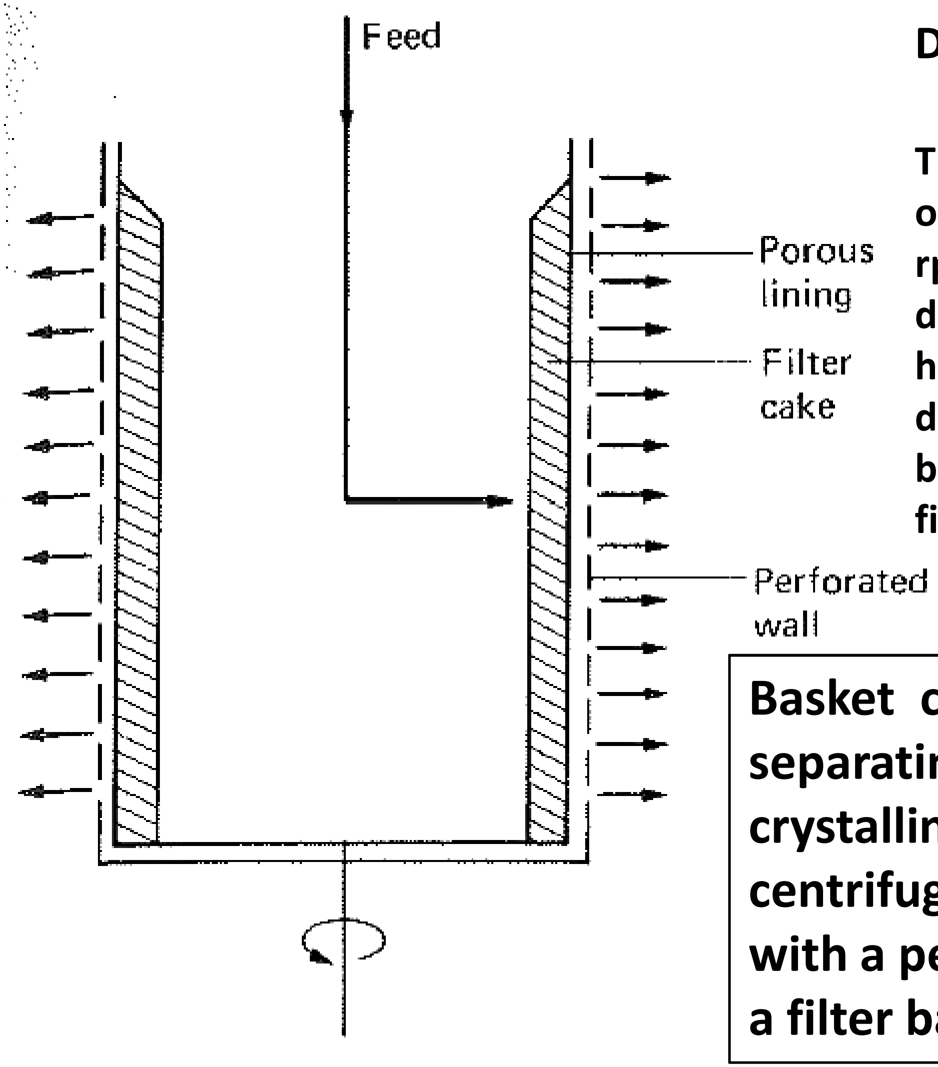


Diagram of Basket Centrifuge

These centrifuges are normally operated at speeds of up to 4000 rpm for feed rates of 50 to 300 dm^3 min^{-1} and have a solids holding capacity of 30 to 500 dm^3. The basket centrifuge may be considered to be a centrifugal filter.

Basket centrifuges are useful for separating mould mycelia or crystalline compounds. The centrifuge is most commonly used with a perforated bowl lined with a filter bag of nylon, cotton, etc.

Recovery & Purification of Fermentation Products

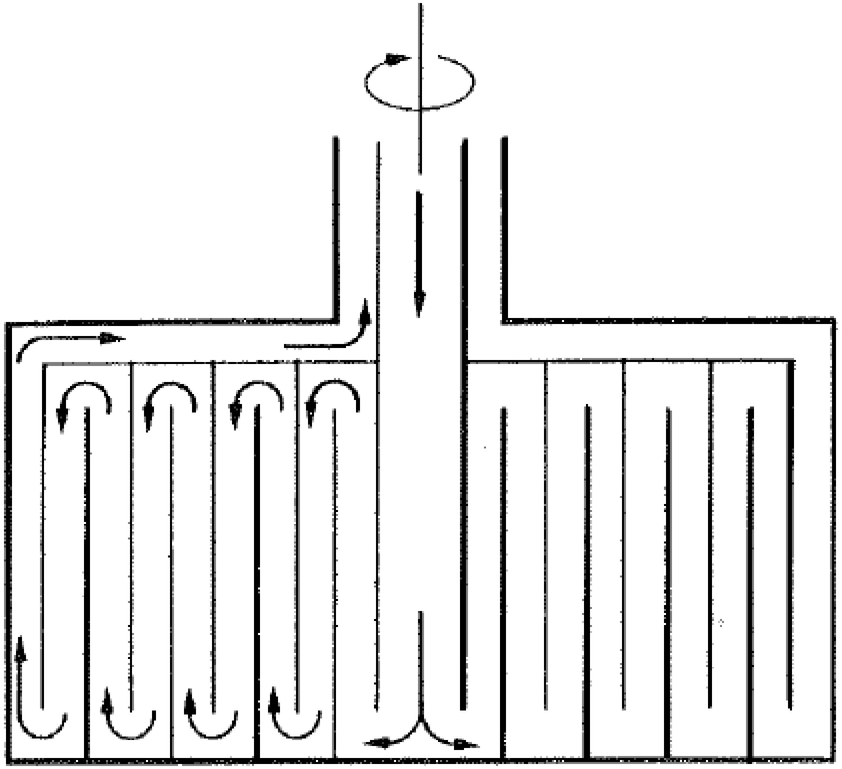


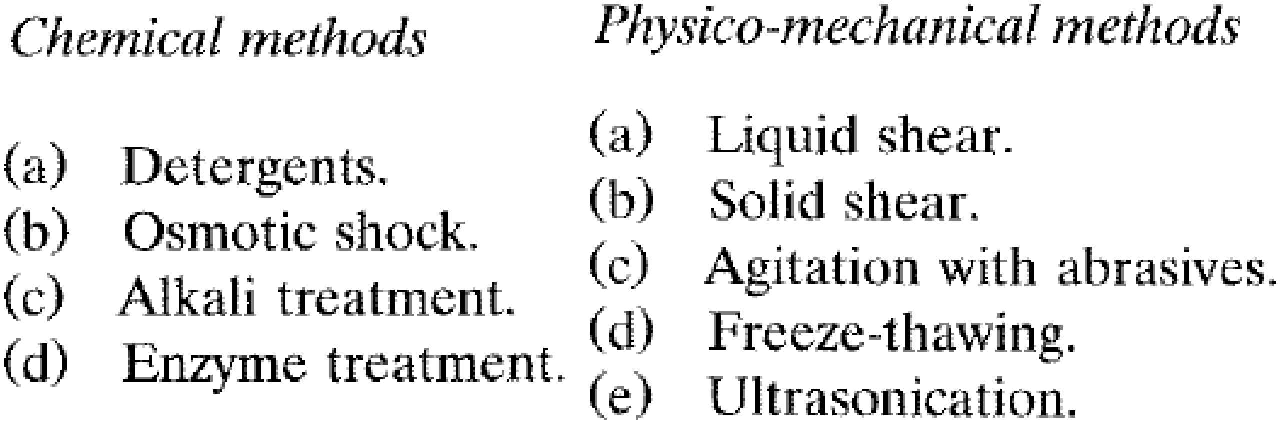
Diagram of a Multi-chamber Centrifuge

This centrifuge is used for a slurry of up to 5% solids of particle size 0.1 to 200 µm diameter. A series of concentric chambers are mounted within the rotor chamber. The broth enters via the central spindle and then takes a circuitous route through the chambers. Solids collect on the outer faces of each chamber. The smaller particles are collected in outer chambers where they are subjected to greater centrifugal forces (the greater the radial position of a particle, the greater the rate of sedimentation).

Recovery & Purification of Fermentation Products

❑ Cell Disruption

Micro-organisms are protected by extremely tough cell walls. In order to release their cellular contents a number of methods for cell disintegration have been developed. Any potential method of disruption must ensure that labile materials are not denatured by the process or hydrolysed by enzymes present in the cell. Methods available fall into two major categories:



Chemical methods

(a) Detergents.
(b) Osmotic shock.
(c) Alkali treatment.
(d) Enzyme treatment.

Physico-mechanical methods

(a) Liquid shear.
(b) Solid shear.
(c) Agitation with abrasives.
(d) Freeze-thawing.
(e) Ultrasonication.

Recovery & Purification of Fermentation Products

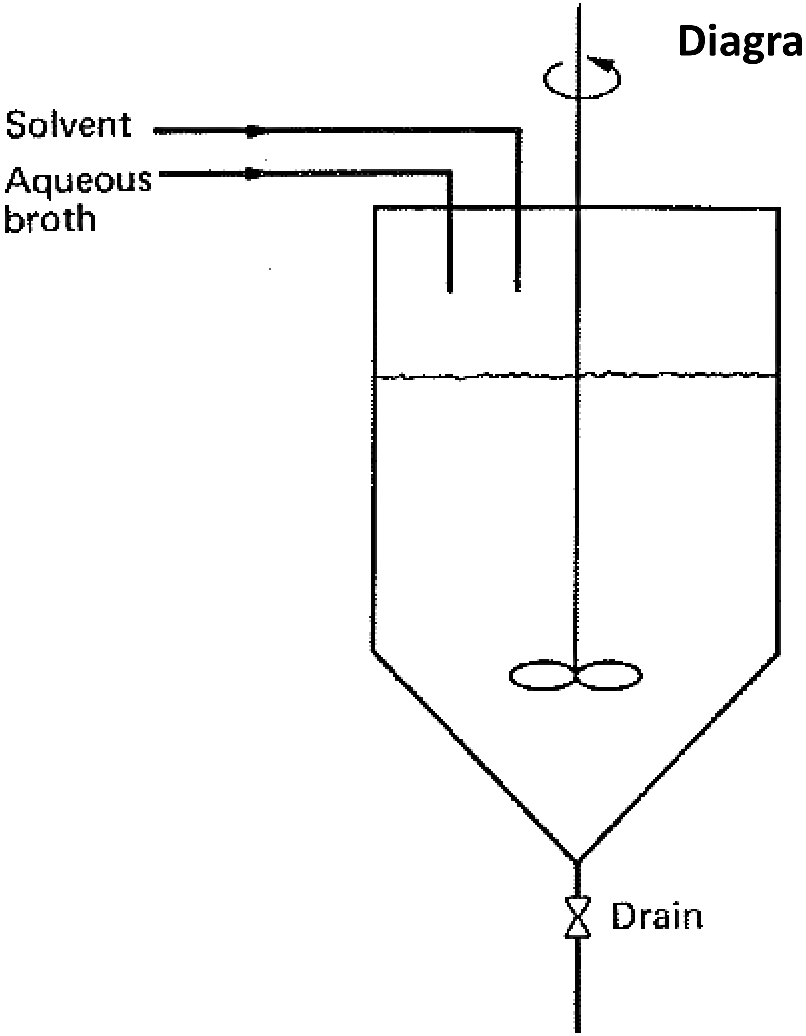


Diagram of a single-stage extraction unit

The separation of a component from a liquid mixture by treatment with a solvent in which the desired component is preferentially soluble is known as liquid-liquid extraction. The specific requirement is that a high percentage extraction of product must be obtained but concentrated in a smaller volume of solvent.

Recovery & Purification of Fermentation Products

A major item of equipment in an extraction process is the solvent-recovery plant which is usually a distillation unit. It is not normally essential to remove all the raffinate (product which has component removed) from the solvent as this will be recycled through the system.

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Recovery and Purification of

FermentationProducts–4

Drying of any product (including biological products) is often the last stage of a manufacturing process. It involves the final removal of water from a heat-sensitive material ensuring that there is minimum loss in viability, activity or nutritional value. Drying is undertaken because:

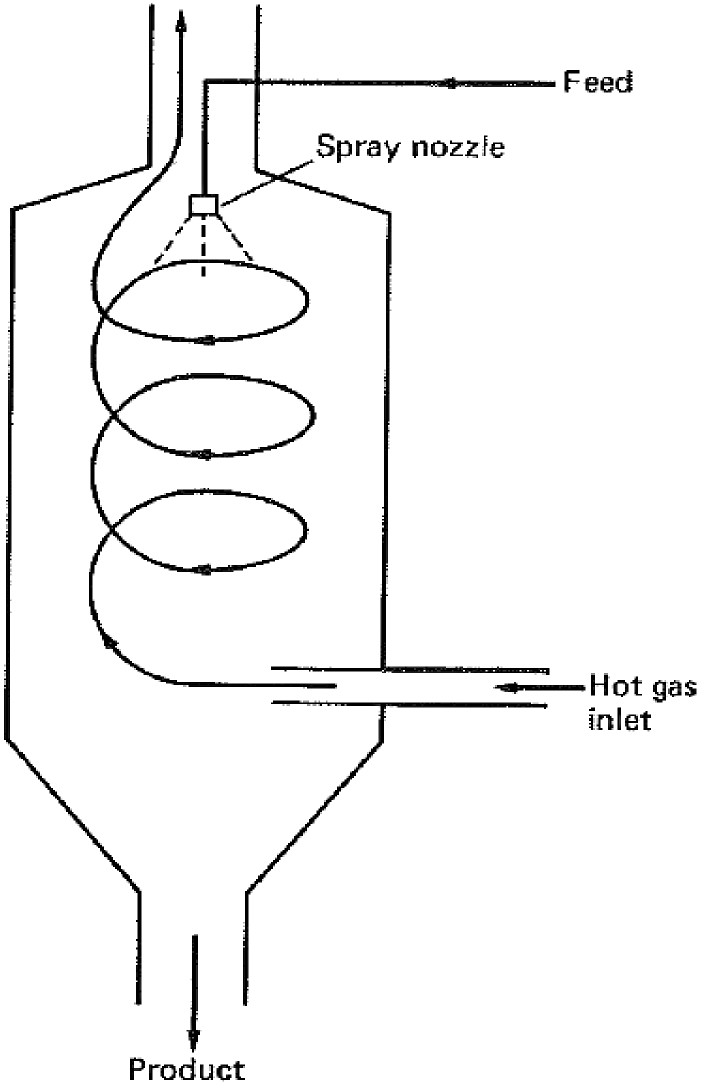
i. The cost of transport can be reduced.
ii. The material is easier to handle and package.
iii. The material can be stored more conveniently in the dry state.

It is important that as much water as possible is removed initially by centrifugation or in a filter press to minimize heating costs in the drying process.

Recovery & Purification of Fermentation Products

Diagram of Counter-current spray drier

A spray drier is most widely used for drying of biological materials when the starting material is in the form of a liquid or paste. The material to be dried does not come into contact with the heating surfaces, instead, it is atomized into small droplets through for example a nozzle or by contact with a rotating disc. Spray driers are the most economical available handling large volumes, and it is only at feed below 6 kg min^{-1} that drum driers become economic.



Recovery & Purification of Fermentation Products

Freeze drying is an important operation in the production of many biologicals and pharmaceuticals. Material is first frozen and then dried by sublimation in a high vacuum. The great benefit of this technique is that it does not harm heat sensitive materials.

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Recovery and Purification of FermentationProducts–5

Crystallization is an established method used in the initial recovery of organic acids and amino acids, and more widely used for final purification of a diverse range of compounds. In citric acid production, the filtered broth is treated with $Ca(OH)_2$ so that the relatively insoluble calcium crystals will be precipitated from solution.

The concept of recovering a metabolite directly from an unfiltered fermentation broth is of considerable interest because of its simplicity, the reduction in process stages and the potential cost savings. It may also be possible to remove the desired fermentation product continuously from a broth during fermentation so that inhibitory effects due to product formation and product degradation can be minimized throughout the production phase.

Recovery & Purification of Fermentation Products

Roffler *et al.* (1984) reviewed the use of a number of techniques for the *in-situ* recovery of fermentation products:

1. Vacuum and flash fermentations for the recovery of ethanol from fermentation broths.
2. Extractive fermentation (liquid-liquid and phase aqueous) for the recovery of organic acids and toxin produced by *Clostridium tetani*.
3. Adsorption for the recovery of ethanol and cycloheximide.
4. Ion-exchange in the extraction of salicylic acid and antibiotics.

5. Dialysis fermentation in the selective recovery of lactic acid, salicylic acid and cycloheximide.

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Effluent Treatment–1

Every fermentation plant utilizes raw materials which are converted to a variety of products. Depending on the individual process, varying amounts of a range of waste materials are produced. Typical wastes might include unconsumed inorganic and organic media components, microbial cells and other suspended solids, filter aids, waste wash water from cleansing operations, cooling water, water containing traces of solvents, acids, alkalis, human sewage, etc.

Historically, it was possible to dispose of wastes directly to a convenient area of land or into a nearby watercourse. This cheap and simple method of disposal is now very rarely possible, nor is it environmentally desirable.

Effluent Treatment

Water authoritiesand similar bodies have become more active in combating pollution caused by domestic and industrial wastes. Legislation in all developed countries now regulates the discharge of wastes.

With liquid wastes, it may be possible to dispose of untreated effluents to a municipal sewage treatment works (STW). Obviously, much will depend on the composition, strength and volumetric flow rate of the effluent. STWs are planned to operate with an effluent of a reasonably constant composition at a steady flow rate. Thus, if the discharge from an industrial process is large in volume and intermittently produced it may be necessary to install storage tanks on site to regulate the effluent flow.

Normally, fermentation effluents do not contain toxic materials which directly affect the aquatic flora or fauna.

Effluent Treatment

Unfortunately, most of the effluents do contain high levels of organic matter which are readily oxidized by microbial attack and so drastically deplete the dissolved oxygen concentration in the receiving water unless there is a large dilution factor.

Effluents may be treated in a variety of ways by a number of processes it may be possible to recover waste organic material as a solid and sell it as a by-product which may be an animal feed supplement or a nutrient to use in fermentation media. The marketable by-product helps to offset the cost of the treatment process.

It is now recognized that water is no longer a cheap raw material, hence there are considerable advantages in reducing the quantities used and in recycling whenever it is feasible.

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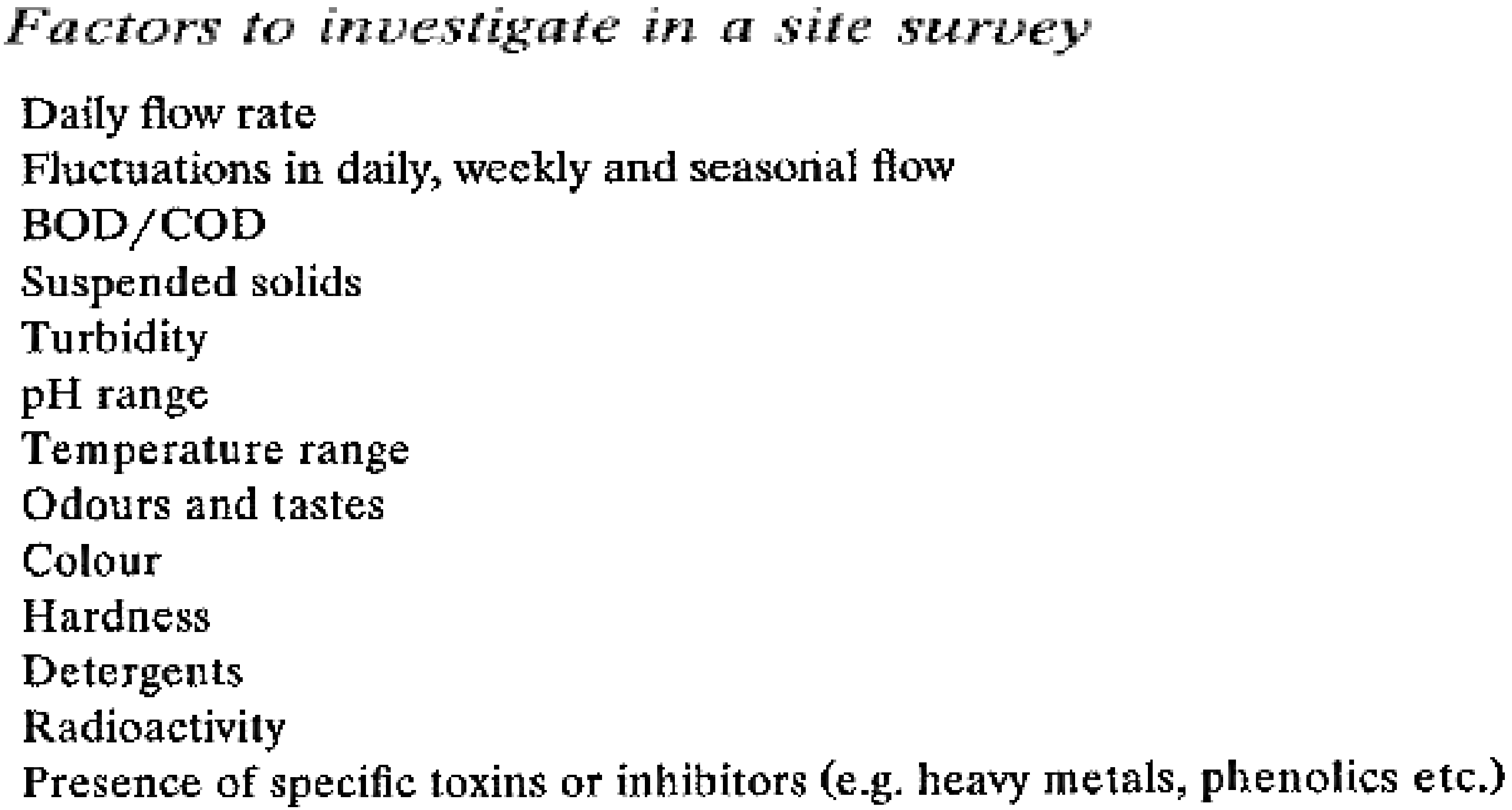
Effluent Treatment–2

Since oxygen is essential for the survival of most organisms, it is important to ensure that there are adequate levels of dissolved oxygen in rivers, lakes, reservoirs, etc., if they are to be managed satisfactorily.

It is therefore important to know how effluents containing soluble and particulate organic matter can influence the dissolved oxygen concentration. One widely used method of assessment is the 'biochemical oxygen demand' (BOD), which is a measure of the quantity of oxygen required for the oxidation of organic matter in water, by micro-organisms present, in a given time interval at a given temperature.

Effluent Treatment

A complete survey of industrial operations is essential for any individual site before an economical waste treatment programme can be planned.



Factors to investigate in a site survey

Daily flow rate
Fluctuations in daily, weekly and seasonal flow
BOD/COD
Suspended solids
Turbidity
pH range
Temperature range
Odours and tastes
Colour
Hardness
Detergents
Radioactivity
Presence of specific toxins or inhibitors (e.g. heavy metals, phenolics etc.)

Effluent Treatment

If the survey is comprehensive it should be possible to plan an overall treatment programme for a site and to establish:

1. **Water sources which can be combined or reused.**
2. **Concentrated waste streams which contain valuable wastes to be recovered as food, animal feed, fertilizer or fuel.**
3. **Toxic effluents needing special treatment, or acids or alkalis needing neutralization.**
4. **The effluent loading expected under maximum production conditions.**
5. **The effluent(s) which might be discharged into municipal sewers.**
6. **The effluent(s) which might be discharged directly, without treatment, on to land or to a watercourse and not cause any pollution.**

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Effluent

Treatment–3

The effluent disposal procedure which is finally adopted by a particular manufacturer is obviously determined by a number of factors, of which the most important is the control exercised by the relevant authorities in many countries on the quantity and quality of the waste discharge and the way in which it might be done. The range of effluent-disposal methods which can be considered is:

1. The effluent is discharged to land, river or sea in an untreated state.
2. The effluent is removed and disposed of in a landfill site or is incinerated.

Effluent Treatment

3. The effluent is partially treated on site prior to further treatment or disposal by one of the other routes indicated.
4. Part of the effluent is untreated and discharged as in 1 or 2, the remainder is treated at a sewage works or at the site before discharge.
5. All of the effluent is sent to the sewage works for treatment, although there might be reluctance by the sewage works to accept it, possibly resulting in some preliminary on-site treatment being required, and discharge rates and effluent composition defined.
6. All the effluent is treated at the factory before discharge.

Effluent Treatment

❑ Disposal of Effluents to Sewers

Municipal authorities and water treatment companies which accept trade effluents into their sewage systems will want to be sure that:

1. The sewage works has the capacity to cope with the estimated volume of effluent.
2. The effluent will not interfere with the treatment processes used at the sewage works.
3. There are no compounds present in the effluent which will pass through the sewage works unchanged and then cause problems when discharged into a watercourse.

Effluent Treatment

Fermentation wastes may be treated on-site or at an STW by any or all of the three following methods:

- **A. Physical treatment**
- **B. Chemical treatment**
- **C. Biological treatment**

The final choice of treatment and disposal processes used in each individual factory will depend on local circumstances.

Treatment processes may also be described in the following manner:

1. **Primary treatment; physical and chemical methods, e.g. sedimentation, coagulation etc.**

Effluent Treatment

2. Secondary treatment; biological methods (e.g. activated sludge) conducted after primary treatment.
3. Tertiary treatment; physical, chemical or biological methods (e.g. microstrainers, sand filters and grass plot irrigation) used to improve the quality of liquor from previous stages.
4. Sludge conditioning and disposal; physical, chemical and biological methods. Anaerobic digestion is often used to condition (make it more amenable to dewatering) the sludge produced in previous stages. Following dewatering (e.g. by centrifugation using a decanter centrifuge) the sludge can then be disposed of by incineration, landfilling, etc.

Effluent Treatment

Most organic-waste materials may be degraded biologically by aerobic or anaerobic processes. The most widely used aerobic processes are trickling filters, rotating disc contactors, activated sludge processes and their modifications.

The anaerobic processes (digestion, filtration and sludge blankets) are used both in the treatment of specific wastewaters and in sludge conditioning.

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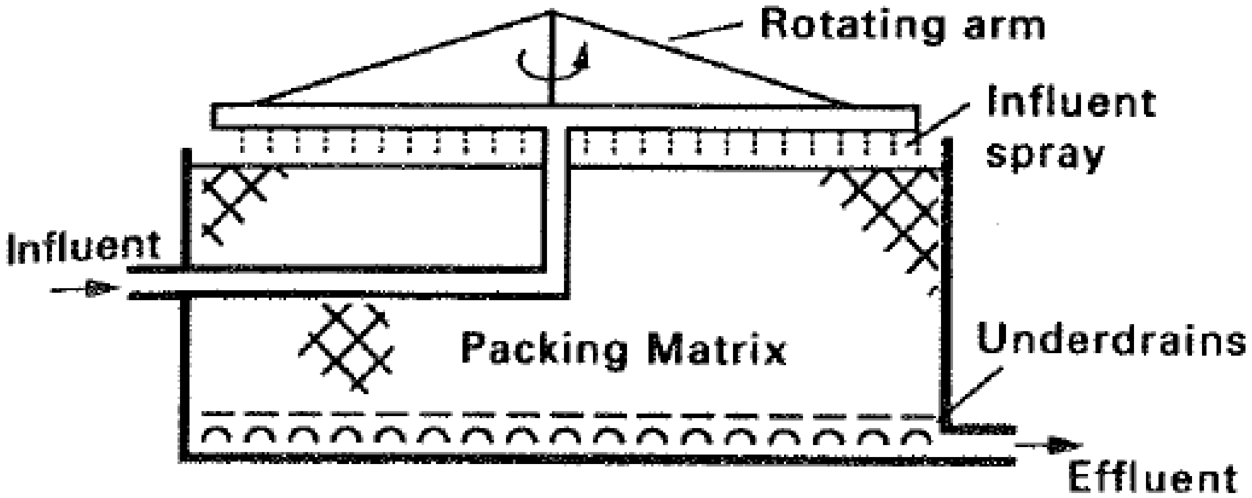
Effluent Treatment–4

☐ Trickling Filters

The term filter in this unit operation is a misnomer, as the action of a trickling filter is not one of filtration, but rather it is a fixed film bio-reactor. Settled effluent to be treated is passed down through a packed bed counter-current to a flow of air. Micro-organisms adhering to the packing matrix adsorb oxygen from the upflowing air and organic matter from the downflowing effluent; the latter is then metabolized and the effluent stream's BOD reduced. The effluent trickles gradually through the bed and a slime layer of biologically active material (bacteria, fungi, algae, protozoa and nematodes) forms on the surface of the support material.

Effluent Treatment



Schematic diagram of a trickling filter

Because trickling filters do not have both a high specific area and a high voidage, they are less suitable for the treatment of large volumes of strong industrial effluents.

Effluent Treatment

❑ Biologically Aerated Filters (BAFs)

Biologically aerated filters are a relatively recent development based on the trickling filter. They consist of a packed bed which provides sites for microbial growth through which air is passed but, unlike trickling filters, the reactor volume is flooded with the effluent to be treated which is passed upwards or downwards through the reactor.

The combination of aeration and filtration allows high rates of BOD and ammonia removal together with solids capture, so that sedimentation tanks may not be required.

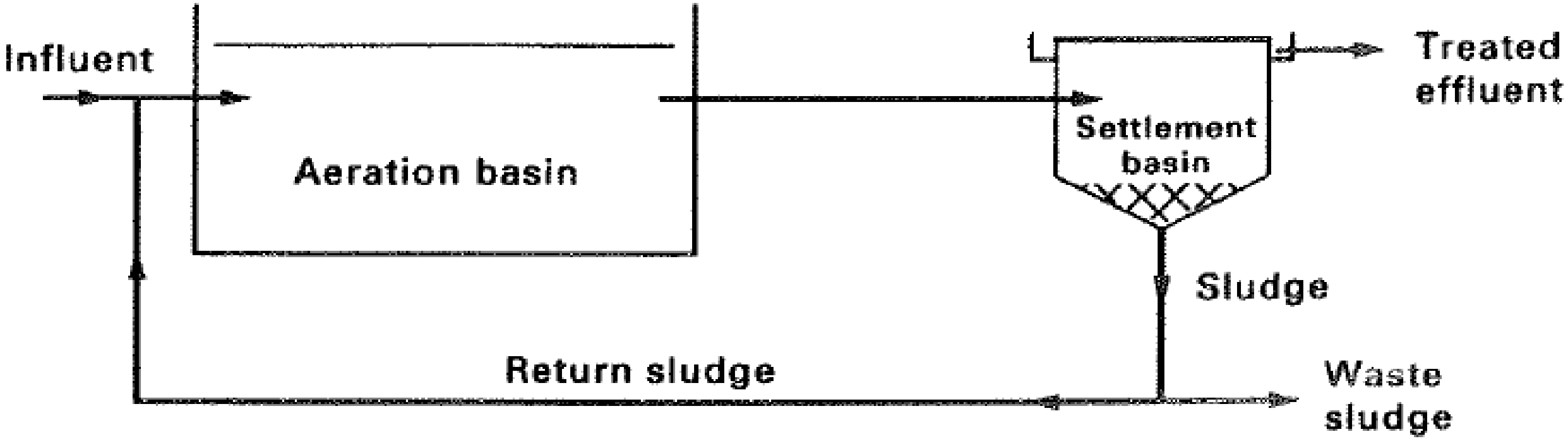
Effluent Treatment

❑ Activated Sludge Processes

The basic activated-sludge process (Fig. on next slide) consists of aerating and agitating the effluent in the presence of a flocculated suspension of micro-organisms on particulate organic matter - the activated sludge. This process in most widely used biological treatment process for both domestic and industrial wastewaters.

A number of modifications of the basic process can be used to improve treatment efficiency, or for a more specific purpose such as denitrification. Tapered aeration and stepped feed aeration are used to balance oxygen demand with the amount of oxygen supplied.

Effluent Treatment



Simplified cross-section of an activated sludge process.

Contact stabilization exploits biosorption processes and thereby allows considerable reduction in basin capacity (50%) for a given wastewater throughput. Denitrification can be accomplished in an activated-sludge plant when the first part of the basin is not aerated.

In advanced activated-sludge systems the amount of dissolved oxygen available for biological activity is increased to improve treatment rate.

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Effluent

Treatment–5

Anaerobic treatment of waste organic materials originated with the use of septic tanks, which have now been replaced by a variety of high-rate digesters. Loehr (1968) has listed the following reasons for using anaerobic processes for waste treatment:

1. **Higher loading rates can be achieved than are possible for aerobic treatment techniques.**
2. **Lower power requirements may be needed per unit of BOD treated.**
3. **Useful end-products such as digested sludge and/or combustible gases may be produced.**
4. **Organic matter is metabolized to a stable form.**

Effluent Treatment

5. There is an alteration of water-binding characteristics to permit rapid sludge dewatering.
6. The reduced amount of microbial biomass leads to easier handling of sludge.
7. Low levels of microbial growth will decrease the possible need for supplementary.

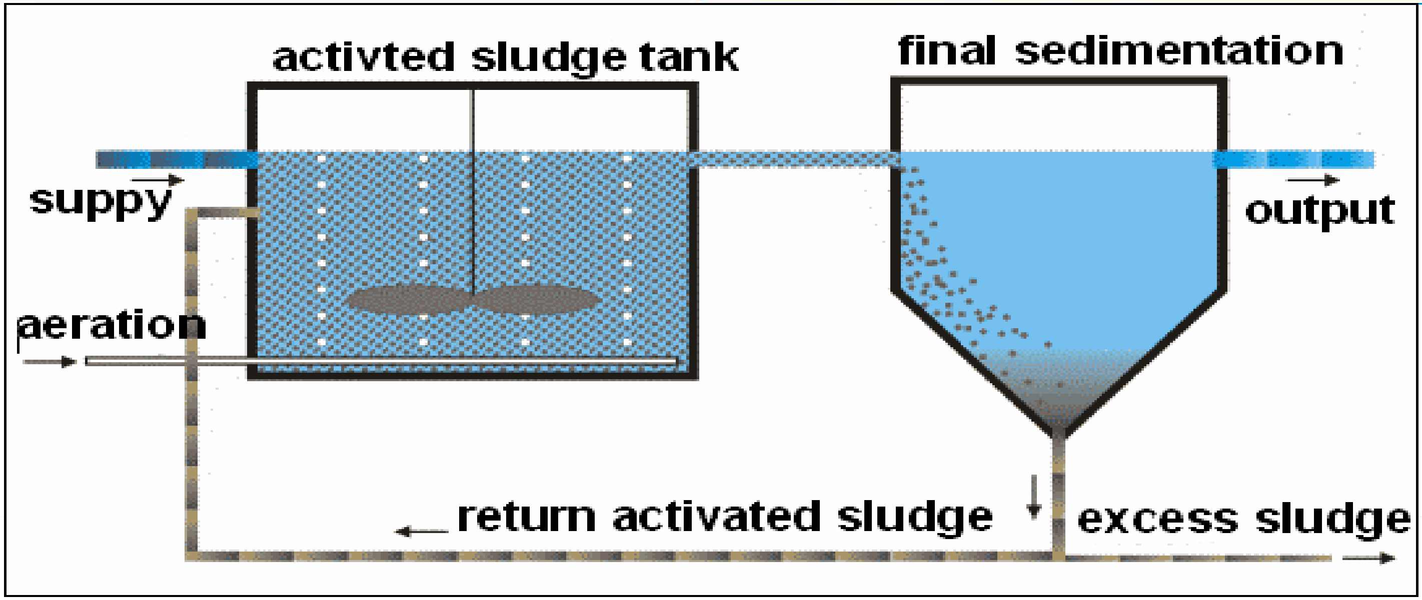
Large volumes of wet sludge which are produced in primary and secondary sedimentation tanks may have to be reduced in volume before disposal. This volume of sludge can be reduced by anaerobic digestion. In sludges containing 20,000 - 60,000 mg dm^{-3} solid matter, 80% of the degradable matter may be digested, which will reduce the solids content by 50%.

Effluent Treatment

During anaerobic digestion acid fermenting bacteria degrade the waste to free volatile fatty acids, mainly acetic and propionic acid, which are then converted to methane (60%) and carbon dioxide (40%).

The gas produced (biogas) is a very useful by-product, and can be burnt as a heating fuel, fed to gas engines to generate electricity or used as a vehicle fuel. As well as being used in sludge digestion and conditioning, anaerobic digesters are also used directly in the treatment of many high strength wastewaters, for example from the food and agricultural industries.

Effluent Treatment



Aeration and Mixing mechanical devices consist of equipment that allow a deeper contact between air and mixed liquor into the tank. Basically, mechanical aerators can be classified into two types: *Mechanical aerators with vertical axis* & *Mechanical aerators with horizontal axis.*

Significant amounts of fossil fuel can be saved in the distillery by generating power and heat from the methane produced during wastewater treatment in cogeneration units and using the generated form of energy within the alcohol production process.

Lecture 91-Advances in Fermentation Technology

Fermentation

Economics

If a fermentation process is to yield a product at a competitive price, the chosen micro-organism or animal cell culture should give the desired end-product in predictable, and economically adequate, quantities. A number of basic objectives are commonly used in developing a successful process which will be economically viable.

1. The capital investment in the fermenter and ancillary equipment should be confined to a minimum, provided that the equipment is reliable and may be used in a range of fermentation processes.

Fermentation Economics

2. Raw materials should be as cheap as possible and utilized efficiently. A search for possible alternative materials might be made, even when a process is operational.
3. The highest-yielding strain of micro-organism or animal cell culture should be used.
4. There should be a saving in labour whenever possible and automation should be used where it is feasible.
5. When a batch process is operated, the growth cycle should be as short as possible to obtain the highest yield of product and allow for maximum utilization of equipment. To achieve this objective it may be possible to use fed-batch culture.

Fermentation Economics

6. Recovery and purification procedures should be as simple and rapid as possible.
7. The effluent discharge should be kept to a minimum.
8. Heat and power should be used efficiently.
9. Space requirements should be kept to a minimum, but there should be some allowance for potential expansion in production capacity.
10. All the above must comply with safety guidelines and regulations.

The consideration of so many criteria means that there may have to be a compromise for the particular set of circumstances relating to an individual process.

Fermentation Economics

The fermentation technologist should be aware of the problem of assessing market potential, although he/she may not be primarily involved in collating or assessing the necessary data. It is necessary to estimate the size of the present and potential market and the increase in demand for a compound.

Hepner (1978) has examined the factors that determine the feasibility of large-scale ethanol production by fermentation. He considered that ethanol produced by fermentation would only be competitive with synthetic ethanol from crude oil if the fermentation plant was in an area where cheap supplies of carbohydrate were available.

Fermentation Economics

Stowell and Bateson (1984) identified a number of factors contributing to these costs:

1. **Yield losses, even if only modest, are certain to occur at each stage of the recovery process.**
2. **High energy and maintenance costs associated with running filtration and centrifugation equipment.**
3. **High costs of solvents and other raw materials used in recovery and refining of products.**