BHARATH COLLEGE OF SCIENCE AND MANAGEMENT, Thanjavur-5

PG & Research Department of Bio Technology

SUBJECT: MICROBIAL BIOTECHNOLOGY

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UNIT I-

1. ISOLATION AND MAINTENANCE OF INDUSTRIALLY IMPORTANT MICROROGANISMS

2. MICROBIAL GROWTH AND DEATH KINETICS OF ORGANISMS

ISOLATION AND MAINTENANCE OF INDUSTRIALLY IMPORTANT MICROROGANISMS

Isolation of Microorganisms:

There are over a million species of micro-organisms widely distributed in nature. Less than 1% of the world's microorganisms have been studied. In fact, only a few hundred species are important for industrial use. The good sources for the isolation of microorganisms are soils, lakes and river muds. It is estimated that a gram of soil contains 106—108 bacteria, 104—106 actinomycete spores and 102—104 fungal spores.

Techniques employed for the isolation of microorganisms

1. Direct sponge of the soil

2. Soil dilution

3. Gradient plate method (Pour plate and streak plate technique)

4. Aerosol dilution

5. Flotation

6. Centrifugation.

Isolating microorganisms from soil or water source

i. The sample (soil or water) is diluted with sterile water to which an emulsifying agent (Tween) is added.

ii. Sample is thoroughly mixed and allowed to stand at room temperature.

iii. Supernatant is diluted, 10-1 to 10-10

iv. Various culture media are inoculated with diluted samples and incubated.

v. Colonies from the plates are isolated and identified.

vi. The required pure strains are maintained and preserved.

Methods for Isolation of Microorganisms:

The culture conditions can be appropriately modified to isolate certain types of microorganisms. The types of organisms that can be isolated by use of enrichment methods is given in Table 19.4. For instance, thermophiles can be isolated by using high temperature while acidophilus can be isolated in acidic pH. Enrichment methods are certainly useful for quick isolation of specific types of organisms.

Strains of Microorganisms from Unusual Environments:

Biotechnologists often prefer to isolate microorganisms from very extreme and unusual environments. This is done with a hope that such strains may be capable of producing new products of industrial importance. The unusual environments such as cold habitats, high altitudes, deserts, deep sea and petroleum fields are constantly being tried for this purpose. The enrichment methods described above (Table 19.4) will be very useful for isolating unusual strains.

TABLE 19.4 Types of microorganisms that can be isolated by enrichment methods

Type of organisms	*Enrichment method*
Thermophiles	High temperature (42–100°C)
Psychrotrophs	Low temperature (5–15°C)
Acidophiles	Low pH (2-4)
Halophiles	High NaCl concentration
Anaerobes	N_2 atmosphere
Actinoplanes	Pollen grains
Myxobacteria	Wood bark

Screening of Metabolites for Isolation of Microorganisms:

The microorganisms can be tested directly for the product formation, and isolated. In fact, the water or soil samples can be directly used or suitably diluted for metabolite screening. Agar plates can be used for screening metabolites formed from the microorganisms. For instance, if the required product is an antibiotic, then the test system consists of the strains of organisms which inhibit the zones, on the agar plates. The inhibitory activity indicates the possible presence of some antibiotic being produced by the microorganisms. Another example is the isolation of microorganisms producing amylases. When grown on agar plates containing starch, and then stained with iodine, amylase-producing organisms can be identified and isolated.

Screening for New Metabolites, and Isolation of Microorganisms:

Industrial microbiologists continue their search for newer metabolites produced by microorganisms. Research work is particularly directed for identifying chemotherapeutically important products for the treatment of tumors, bacterial diseases (newer antibiotics against

resistant strains) and viral diseases, besides several other substances (e.g. hormones, enzyme inhibitors). In addition, isolation of microorganisms for improvement of food industry, and for efficient degradation of the environmental pollutants and hazardous chemicals also assumes significance.

Preservation of Microorganisms:

There are distinct methods for preservation of microorganisms. The most important being storage by refrigeration, freezing and lyophilization.

MICROBIAL GROWTH AND DEATH KINETICS OF ORGANISMS

The growth of microorganisms is a highly complex and coordinated process, ultimately expressed by increase in cell number or cell mass. The process of growth depends on the availability of requisite nutrients and their transport into the cells, and the environmental factors such as aeration, O2 supply, temperature and pH. Doubling time refers to the time period required for doubling the weight of the biomass while generation time represents the period for doubling the cell numbers. In general, when all other conditions are kept ideal, growth of the microorganisms is dependent on the substrate (nutrient) supply. The microorganisms can be grown in batch, fed-batch, semi-continuous or continuous culture systems in a bioreactor.

In fed-batch fermentation, substrates are added at short time intervals during fermentation. In batch and fed-batch fermentation, the growth of the cells is quite comparable. And in both cases, growth medium is not removed until the end of fermentation process.In case of continuous fermentation, as the fermentation proceeds, fresh growth medium is added continuously. Simultaneously, an equal volume of spent medium containing suspended microorganisms is removed.

Batch Culture or Batch Fermentation:

A batch fermentation is regarded as a closed system. The sterile nutrient culture medium in the bioreactor is inoculated with microorganisms. The incubation is carried out under optimal physiological conditions (pH, temperature, O2 supply, agitation etc.). It may be necessary to add acid or alkali to maintain pH, and anti-foam agents to minimise foam. Under optimal conditions for growth, the following six typical phases of growth are observed in batch fermentation (Fig. 19.12).

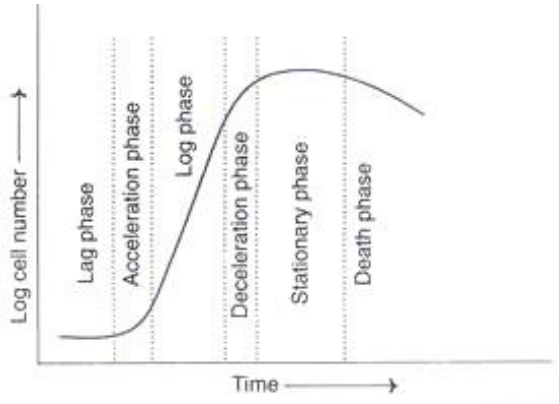


Fig. 19.12 : Pattern of microbial cell growth in batch culture or batch fermentation.

1. Lag phase

2. Acceleration phase

3. Logarithmic (log) phase (exponential phase)

4. Deceleration phase

5. Stationary phase

6. Death phase.

Lag phase:

The initial brief period of culturing after inoculation is referred to as lag phase. During the lag phase, the microorganisms adapt to the new environment—available nutrients, pH etc. There is no increase in the cell number, although the cellular weight may slightly increase.The length of the lag phase is variable and is mostly determined by the new set of physiological conditions, and the phase at which the micro-organisms were existing when inoculated. For instance, lag phase may not occur if the culture inoculated is at exponential phase (i.e., log phase), and growth may start immediately.

Acceleration phase:

This is a brief transient period during which cells start growing slowly. In fact, acceleration phase connects the lag phase and log phase.

Log phase:

The most active growth of microorganisms and multiplication occur during log phase. The cells undergo several doublings and the cell mass increases. When the number of cells or biomass is plotted against time on a semi logarithmic graph, a straight line is obtained, hence the term log phase. Growth rate of microbes in log phase is independent of substrate (nutrient supply) concentration as long as excess substrate is present, and there are no growth inhibitors in the medium. In general, the specific growth rate of microorganisms for simpler substrates is greater

than for long chain molecules. This is explained on the basis of extra energy needed to split long chain substrates. Two log phases are observed when a complex nutrient medium with two substrates is used in fermentation, and this phenomenon is referred to as diauxy. This happens since one of the substrates is preferentially metabolised first which represses the breakdown of second substrate. After the first substrate is completely degraded second lag phase occurs, during which period, the enzymes for the breakdown of second substrate the synthesized. Now a second log phase occurs.

Deceleration phase:

As the growth rate of microorganisms during log phase decreases, they enter the deceleration phase. This phase is usually very short-lived and may not be observable.

Stationary phase:

As the substrate in the growth medium gets depleted, and the metabolic end products that are formed inhibit the growth, the cells enter the stationary phase. The microbial growth may either slow down or completely stop. The biomass may remain almost constant during stationary phase. This phase, however, is frequently associated with dramatic changes in the metabolism of the cells which may produce compounds (secondary metabolites) of biotechnological importance e.g. production of antibiotics.

Death phase:

This phase is associated with cessation of metabolic activity and depletion of energy reserves. The cells die at an exponential rate (a straight line may be obtained when the number of surviving cells are plotted against time on a semi logarithmic plot). In the commercial and industrial fermentations, the growth of the microorganisms is halted at the end of the log phase or just before the death phase begins, and the cells are harvested.

Growth Kinetics of Microorganisms:

The different types of fermentation processes- batch, fed-batch, semi-continuous and continuous are described above. The kinetics of microbial growth with special reference to log phase of batch fermentation are briefly discussed here. After completion of lag phase, the cell enters log phase which is characterized by exponential growth (See Fig. 19.12). If the initial number of cells is N0, then

After 1st generation, the cell number will be $N0 \times 21$.

After 2nd generation, the cell number will be N0 x 22.

After 3rd generation, $N0 \times 23$ and so on. Thus, the number of cells after a given time (Nt) will be as follows:

$Nt = N0 \times 2n$

where n is the number of generations.

The term doubling time (td) or mean generation time (MGT) refers to the time taken for doubling the cell number or biomass. The specific growth rate constant expressed by μ, is the direct measure of rate of growth of the organism. If N is the number of cells at a given time, then the increase in the number of cells (growth rate) with time is given by the formula.

$dN/dt = \mu N$ (1)

If X is the biomass concentration at a given time, then the increase in the biomass (growth rate) with time is given by.

$dX/dt = \mu X$ (2)

In general, the specific growth rate (n) is a function of the concentration of limiting substrate (S), the maximum specific growth rate (μmax) and a substrate specific constant (Ks). Their relationship was expressed by Monond by the following equation

$\mu = \mu max\ S/\ Ks + S$ (3)

Both S and Ks are expressed as concentrations e.g., in moles or grams per liter.

The growth rate (μ) of an organism is not fixed but it is variable depending on the environmental conditions such as concentration of substrate and temperature. At a low concentration, the substrate is the limiting factor for growth (Fig. 19.14A). The Fig. 19.14B represents the growth rate for a given substrate concentration (by plotting against S).

Growth curves for unicellular organism in batch culture

In batch culture, the substrate is initially present at a higher concentration i.e. (S) > Ks, hence the equation (3) is approximately 1.

$S/\ Ks + S = 1$

Thus, $\mu = \mu max$.

When the substrate concentration is low, as usually occurs at the end of growth phase, then,

$S/\ Ks + S < 1$

Hence $\mu < \mu max$.

UNIT II- FERMENTATAION PROCESS

Bioreactor

A bioreactor helps to produce a large volume of culture. The bioreactor is a large vessel where the different cells such as human or plant, or animal cell are used to culture new biological products. It provides optimum conditions like temperature, pH, substrate, oxygen, etc required

for the culturing of desired products. Simple stirred-tank bioreactor and sparged stirred-tank bioreactor are the two types bioreactors used for this purpose.

1. Continuous Stirred Tank Bioreactors:

A continuous stirred tank bioreactor consists of a cylindrical vessel with motor driven central shaft that supports one or more agitators (impellers). The shaft is fitted at the bottom of the bioreactor (Fig. 19.1 A). The number of impellers is variable and depends on the size of the bioreactor i.e., height to diameter ratio, referred to as aspect ratio.

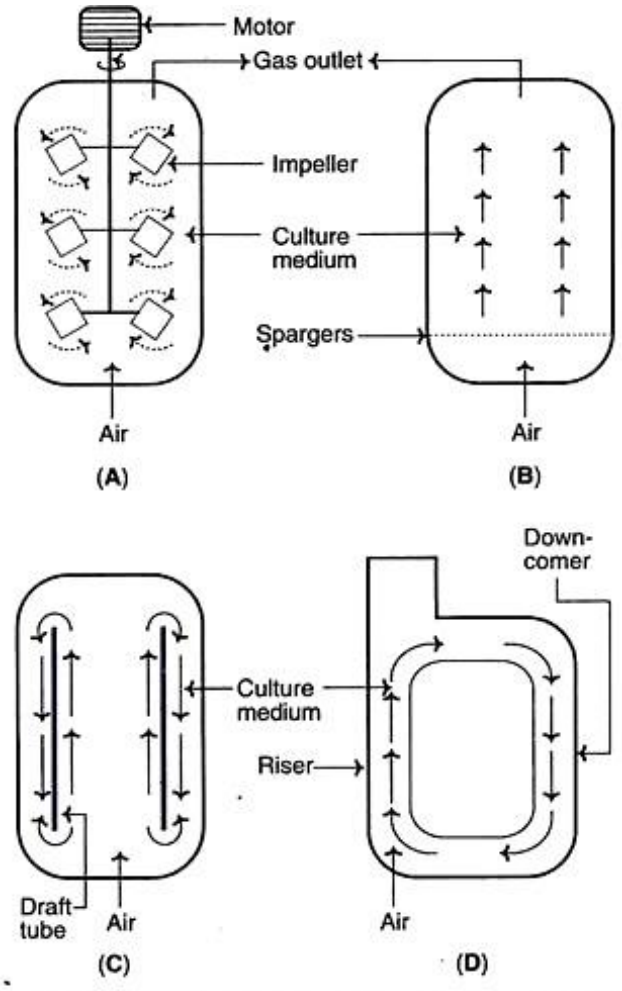


Fig. 19.1 : Types of bioreactors (A) Continuous stirred tank bioreactor (B) Bubble column bioreactor (C) Internal-loop airlift bioreactor (D) External-loop airlift bioreactor.

Airlift Bioreactors:

In the airlift bioreactors, the medium of the vessel is divided into two interconnected zones by means of a baffle or draft tube. In one of the two zones referred to a riser, the air/gas is pumped.

The other zone that receives no gas is the down comer. The dispersion flows up the riser zone while the down flow occurs in the down comer. There are two types of airlift bioreactors.Internal-loop airlift bioreactor (Fig. 11.1C) has a single container with a central draft tube that creates interior liquid circulation channels. These bioreactors are simple in design, with volume and circulation at a fixed rate for fermentation.

External loop airlift bioreactor (Fig. 19.1D) possesses an external loop so that the liquid circulates through separate independent channels. These reactors can be suitably modified to suit the requirements of different fermentations. In general, the airlift bioreactors are more efficient than bubble columns, particularly for more denser suspensions of microorganisms. This is mainly because in these bioreactors, the mixing of the contents is better compared to bubble columns.Airlift bioreactors are commonly employed for aerobic bioprocessing technology. They ensure a controlled liquid flow in a recycle system by pumping. Due to high efficiency, airlift bioreactors are sometimes preferred e.g., methanol production, waste water treatment, single-cell protein production. In general, the performance of the airlift bioreactors is dependent on the pumping (injection) of air and the liquid circulation.

Two-stage airlift bioreactors:

Two-stage airlift bioreactors are used for the temperature dependent formation of products. Growing cells from one bioreactor (maintained at temperature 30°C) are pumped into another bioreactor (at temperature 42°C). There is a necessity for the two-stage airlift bioreactor, since it is very difficult to raise the temperature quickly from 30°C to 42°C in the same vessel. Each one of the bioreactors is fitted with valves and they are connected by a transfer tube and pump (Fig. 19.2A). The cells are grown in the first bioreactor and the bioprocess proper takes place in the second reactor.

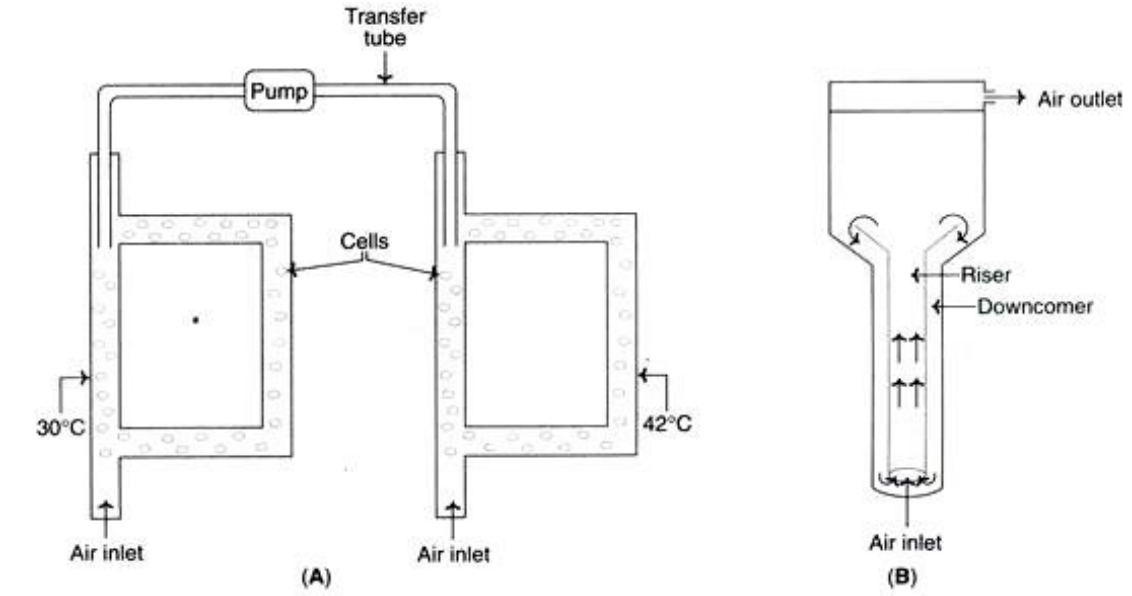


Fig. 19.2 : Types of bioreactors (A) Two–stage airlift bioreactor (B) Tower bioreactor.

Tower bioreactors:

A pressure-cycle fermenter with large dimensions constitutes a tower bioreactor (Fig. 19.2B). A high hydrostatic pressure generated at the bottom of the reactor increases the solubility of O2 in the medium. At the top of the riser, (with expanded top) reduces pressure and facilitates expulsion of CO2. The medium flows back in the down comer and completes the cycle. The advantage with tower bioreactor is that it has high aeration capacities without having moving parts.

Fluidized Bed Bioreactors:

Fluidized bed bioreactor is comparable to bubble column bioreactor except the top position is expanded to reduce the velocity of the fluid. The design of the fluidized bioreactors (expanded top and narrow reaction column) is such that the solids are retained in the reactor while the liquid flows out (Fig. 19.3A). These bioreactors are suitable for use to carry out reactions involving fluid suspended biocatalysts such as immobilized enzymes, immobilized cells, and microbial flocs.

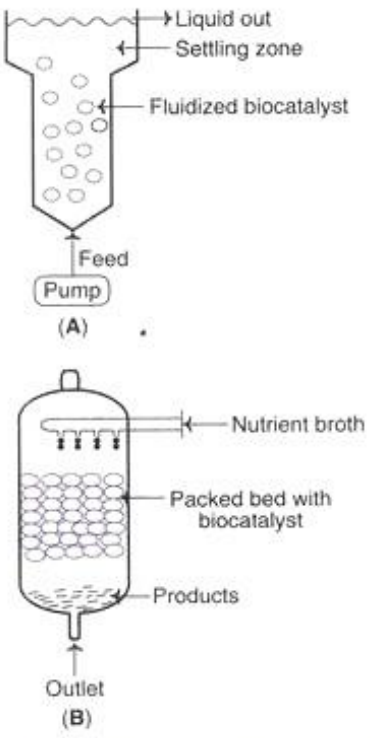


*Fig. 19.3 : Types of bioreactors (**A**) Fluidized bed bioreactor (**B**) Packed bed bioreactor.*

For an efficient operation of fluidized beds, gas is spared to create a suitable gas-liquid-solid fluid bed. It is also necessary to ensure that the suspended solid particles are not too light or too dense (too light ones may float whereas to dense ones may settle at the bottom), and they are in a good suspended state. Recycling of the liquid is important to maintain continuous contact between the reaction contents and biocatalysts. This enable good efficiency of bioprocessing.

Packed Bed Bioreactors:

A bed of solid particles, with biocatalysts on or within the matrix of solids, packed in a column constitutes a packed bed bioreactor (Fig. 19.3B). The solids used may be porous or non-porous gels, and they may be compressible or rigid in nature. A nutrient broth flows continuously over the immobilised biocatalyst. The products obtained in the packed bed bioreactor are released into the fluid and removed. While the flow of the fluid can be upward or downward, down flow under gravity is preferred. The concentration of the nutrients (and therefore the products formed) can be increased by increasing the flow rate of the nutrient broth. Because of poor mixing, it is rather difficult to control the pH of packed bed bioreactors by the addition of acid or alkali. However, these bioreactors are preferred for bioprocessing technology involving product-inhibited reactions. The packed bed bioreactors do not allow accumulation of the products to any significant extent.

UNIT III- UPSTREAM PROCESSING

Upstream processes, include selection of a microbial strain characterized by the ability to synthesize a specific product having the desired commercial value. This strain then is subjected to improvement protocols to maximize the ability of the strain to synthesize economical amounts of the product. Included in the upstream phase is the fermentation process itself which usually is carried out in large tanks known as fermenters or bioreactors. In addition to mechanical parts which provide proper conditions inside the tank such as aeration, cooling, agitation, etc., the tank is usually also equipped with complex sets of monitors and control devices in order to run the microbial growth and product synthesis under optimized conditions. The processing of the fermentation reactions inside the fermenter can be done using many modifications of engineering technologies. One of the most commonly used fermenter types is the stirred-tank fermenter which utilizes mechanical agitation principles, mainly using radial-flow impellers, during the fermentation process.

Overview of Upstream Processing

Upstream processing normally deals with three important points.

The first relates to fermentation media, especially the selection of suitable cost effective carbon and energy sources, along with other essential nutrients. The media optimization is a vital aspect of process development to ensure maximization of yield and profit.

The second aspect involves aspects associated with the producer microorganism. They include the strategy for initially obtaining a suitable microorganism, industrial stain improvement to enhance productivity and yield, maintenance of strain purity, preparation of a suitable inoculums and continuing development of selected strains to increase the economic efficiency of the process.

The third component relates to the fermentation which is usually performed under rigorously controlled conditions developed to optimize the growth of the organism or the production of a target microbial product.

Fermentation medium

The medium used for fermentation may be classified as defined, complex or technical medium. Defined medium consists only of precisely chemically defined substrates. Complex medium is composed of substrates with undefined composition, such as extracts or hydrolysates from waste products, which are cheap substrates commonly used in industrial production. Relatively expensive substrates, such as yeast extract, brain heart infusion, peptone, and tryptone are often used for complex medium. Technical media are used on an industrial scale and are cheaper. The substrate sources can also be derived from industrial waste, and are often highly impure mixtures, requiring pretreatment before they could be used for a fermentation process. Examples

are soy meal, whey, fishmeal, malt extract, and sulfite waste liquor. Wastewater from monosodium glutamate production, which contains high levels of chemical oxygen demand (COD), sulphate, and ammoniacal nitrogen at a low pH, has been used as the nitrogen and water source, with sugar beet pulp as the carbon source, for the production of pectinase.

Media sterilization is necessary to ensure that only the desired microorganism is present to carry out the fermentation, that products are made of predicted quality, that the environment is protected from undesirable contamination, and that deterioration (microbial spoilage) of products is prevented. Sterilization by high temperature achieved by direct or indirect steam or electric heating, membrane filtration, microwave irradiation, high voltage pulses and photosemiconductor powders which involve the rupture of the cell membrane by increasing the transmembrane electric field strength beyond a certain threshold.

Inoculation is the transfer of seed material or inoculum into the fermentor. Inoculation of a laboratory fermentor is generally done using presterilized tubing and a peristaltic pump. However, on a larger scale, inoculum transfer is done by applying a positive pressure on the inoculum fermentor and connecting it aseptically to the production fermentor. The connecting lines are sterilized before being used for transfer of inoculum. Heat susceptible substances such as amino acids and some vitamins must be dissolved in small volumes of water, sterilized by filtration and added separately to the final medium aseptically.

Fermentation systems

A fermentation system is usually operated in one of the following modes: batch, fed batch, or continuous fermentation. The choice of the fermentation mode is dependent on the relation of consumption of substrate to biomass and products.

Innoculum

Upstream processing of proteins using bioreactors and cells usually begins with the preparation of the inoculum which proceeds in scale-up steps until enough inoculum is made to aseptically inoculate the final, sterile, media-filled bioreactor.During the culture period samples are removed, aseptically, and various parameters are measured by fermentation technicians or operators including optical density (OD) and live cell count. Samples are also brought to quality control where other parameters may be measured such as the levels of glucose, lactate and ammonia, as well as the identity and concentration of the human protein that the cells are producing. Also part of upstream processing are the initial purification steps which could include centrifugation and/or filtration in order to separate cells from media. The cells or the media would be discarded to the kill tank, depending on where the protein was located. In this course we are using glass bioreactors and representative of three types of cells used in upstream processing of human protein pharmaceuticals: bacterial, animal, and fungal cells. In bacteria, such as biotechnology's workhorse, Escherichia coli , Pichia pastoris , proteins are secreted into

the media so the media is saved for later isolation and purification of the protein of interest in downstream processing.

UNIT IV- DOWNSTREAM PROCESSING

DOWN STREAM PROCESSING:

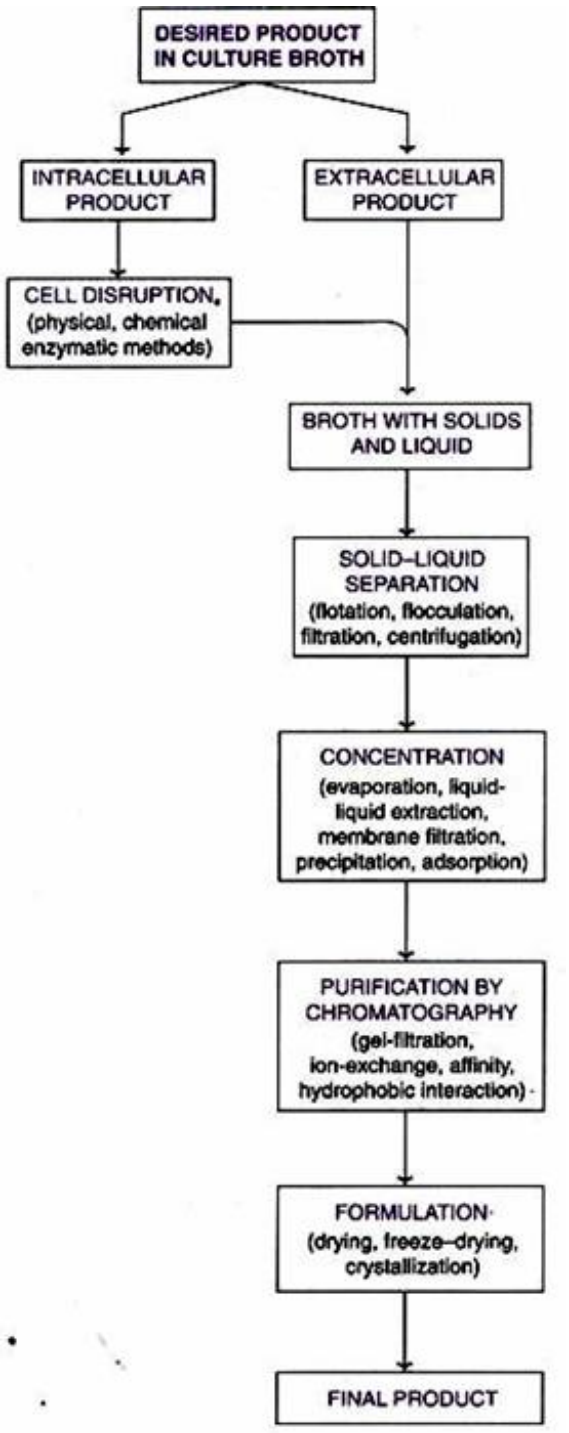


Fig. 20.1 : A summary of the major steps in downstream processing.

1. Solid-Liquid Separation:

The first step in product recovery is the separation of whole cells (cell biomass) and other insoluble ingredients from the culture broth (Note: If the desired product is an intracellular metabolite, it must be released from the cells before subjecting to solid-liquid separation). Some authors use the term harvesting of microbial cells for the separation of cells from the culture medium. Several methods are in use for solid-liquid separation. These include flotation, flocculation, filtration and centrifugation.

2.Flotation:

When a gas is introduced into the liquid broth, it forms bubbles. The cells and other solid particles get adsorbed on gas bubbles. These bubbles rise to the foam layer which can be collected and removed. The presence of certain substances, referred to as collector substances, facilitates stable foam formation e.g., long chain fatty acids, amines.

3.Flocculation:

In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

4.Filtration:

Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors— the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters such as depth filters, absolute filters, rotary drum vacuum filters and membrane filters are in use.

Depth Filters:

They are composed of a filamentous matrix such as glass wool, asbestos or filter paper. The particles are trapped within the matrix and the fluid passes out. Filamentous fungi can be removed by using depth filters.

Absolute Filters:

These filters are with specific pore sizes that are smaller than the particles to be removed. Bacteria from culture medium can be removed by absolute filters.

Rotary Drum Vacuum Filters:

These filters are frequently used for separation of broth containing 10-40% solids (by volume) and particles in the size of 0.5-10µm. Rotary drum vacuum filters have been successfully used for filtration of yeast cells and filamentous fungi. The equipment is simple with low power consumption and is easy to operate. The filtration unit consists of a rotating drum partially immersed in a tank of broth (Fig. 20.2). As the drum rotates, it picks up the biomass which gets deposited as a cake on the drum surface. This filter cake can be easily removed.

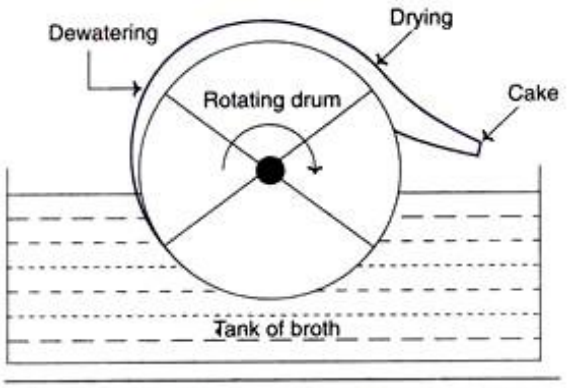


Fig. 20.2 : Diagrammatic representation of a rotary drum vacuum filter.

Membrane Filters:

In this type of filtration, membranes with specific pore sizes can be used. However, clogging of filters is a major limitation. There are two types of membrane filtrations—static filtration and cross-flow filtration . In cross-flow filtration, the culture broth is pumped in a crosswise fashion across the membrane. This reduces the clogging process and hence better than the static filtration.

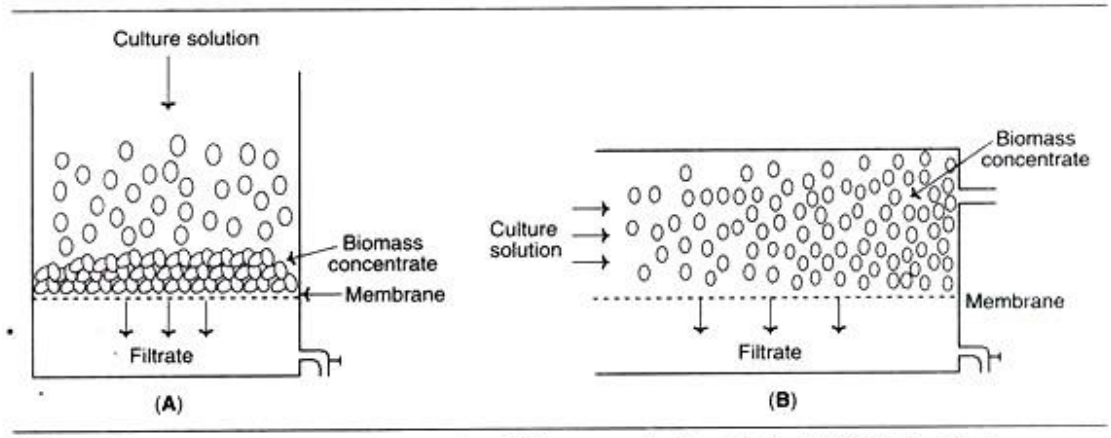


Fig. 20.3 : Filter systems for separation of biomass and culture filtrate (A) Static–flow filtration (B) Cross–flow filtration.

There are 3 major types of filtrations based on the particle sizes and other characters (Table 20.1). These are microfiltration, ultrafiltration and reverse osmosis.

Table 20.1 Major types of filtration processes with characteristic features

	Type	Sizes of particles separated	Compound or particle separated
1.	Microfiltration	0.1–10 μm	Cells or cell fractions, viruses.
2.	Ultrafiltration	0.001–0.1 μm	Compounds with molecular weights greater than 1000 (e.g. enzymes).
3.	Reverse osmosis (hyperfiltration)	0.0001–0.001 μm	Compounds with molecular weights less than 1000 (e.g. lactose).

Centrifugation:

The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. Thus, centrifugation is mostly used for separating solid particles from liquid phase (fluid/particle separation). Unlike the centrifugation that is conveniently carried out in the laboratory scale, there are certain limitations for large scale industrial centrifugation. However, in recent years, continuous flow industrial centrifuges have been developed. There is a continuous feeding of the slurry and collection of clarified fluid, while the solids deposited can be removed intermittently. Centrifugation Commonly Used in Downstream ProcessingTubular bowl centrifuge .

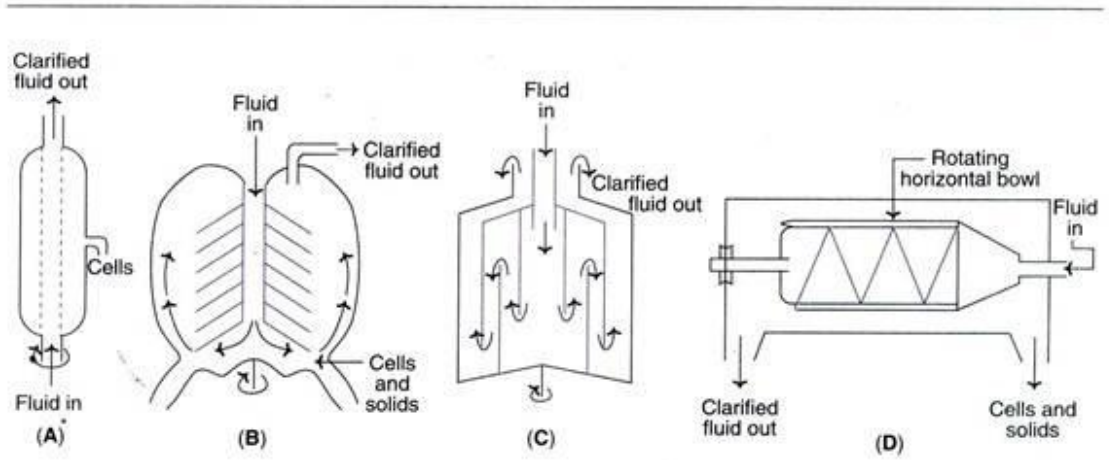


Fig. 20.4 : Centrifuges commonly used in downstream processing (A) Tubular bowl centrifuge (B) Disc centrifuge (C) Multichamber centrifuge (D) Scroll centrifuge (decanter).

Tubular bowl centrifuge (Fig. 20.4A):

This is a simple and a small centrifuge, commonly used in pilot plants. Tubular bowl centrifuge can be operated at a high centrifugal speed, and can be run in both batch or continuous mode. The solids are removed manually.

Disc centrifuge (Fig. 20.4B):

It consists of several discs that separate the bowl into settling zones. The feed/slurry is fed through a central tube. The clarified fluid moves upwards while the solids settle at the lower surface.

Multi-chamber centrifuge (Fig. 20.4C):

This is basically a modification of tubular bowl type of centrifuge. It consists of several chambers connected in such a way that the feed flows in a zigzag fashion. There is a variation in the centrifugal force in different chambers. The force is much higher in the periphery chambers, as a result smallest particles settle down in the outermost chamber.

Scroll centrifuge or decanter (Fig. 20.4D):

It is composed of a rotating horizontal bowl tapered at one end. The decanter is generally used to concentrate fluids with high solid concentration (biomass content 5-80%). The solids are deposited on the wall of the bowl which can be scrapped and removed from the narrow end.

Release of Intracellular Products:

As already stated, there are several biotechnological products (vitamins, enzymes) which are located within the cells. Such compounds have to be first released (maximally and in an active form) for their further processing and final isolation. The microorganisms or other cells can be disintegrated or disrupted by physical, chemical or enzymatic methods. The outline of different techniques used for breakage of cells is given in Fig. 20.5

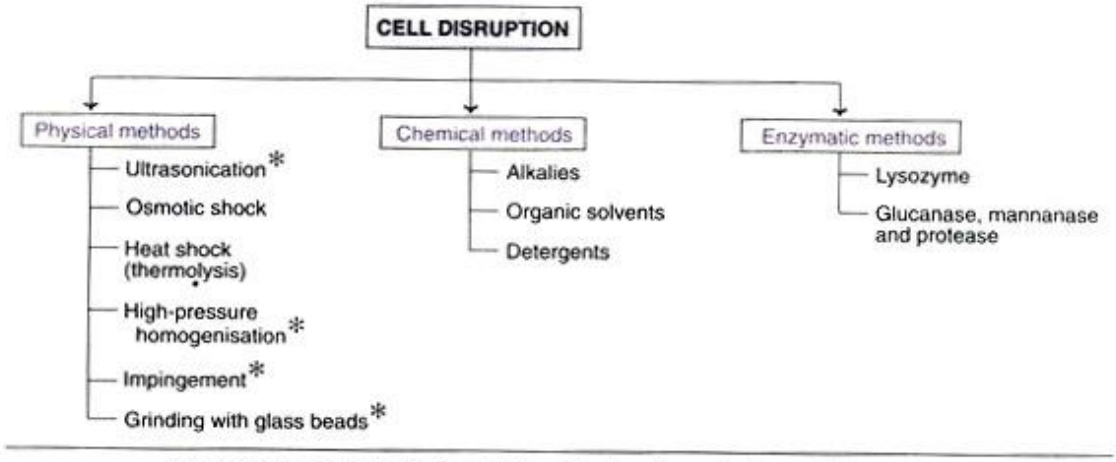


Fig. 20.5 : Major methods for cell disruption to release the intracellular products (indicate mechanical methods while all the remaining are non-mechanical).*

The selection of a particular method depends on the nature of the cells, since there is a wide variation in the property of cell disruption or breakage. For instance, Gram-negative bacteria and filamentous fungi can be more easily broken compared to Gram-positive bacteria arid yeasts.

Cell Disruption:

Physical methods of cell disruption:

The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.

Ultra sonication:

Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

Osmotic shock:

This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis):

Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

High pressure homogenization:

This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.

Impingement:

In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

Grinding with glass beads:

The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells. A diagrammatic representation of a cell disrupter employing glass beeds is shown in Fig. 20.6. It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

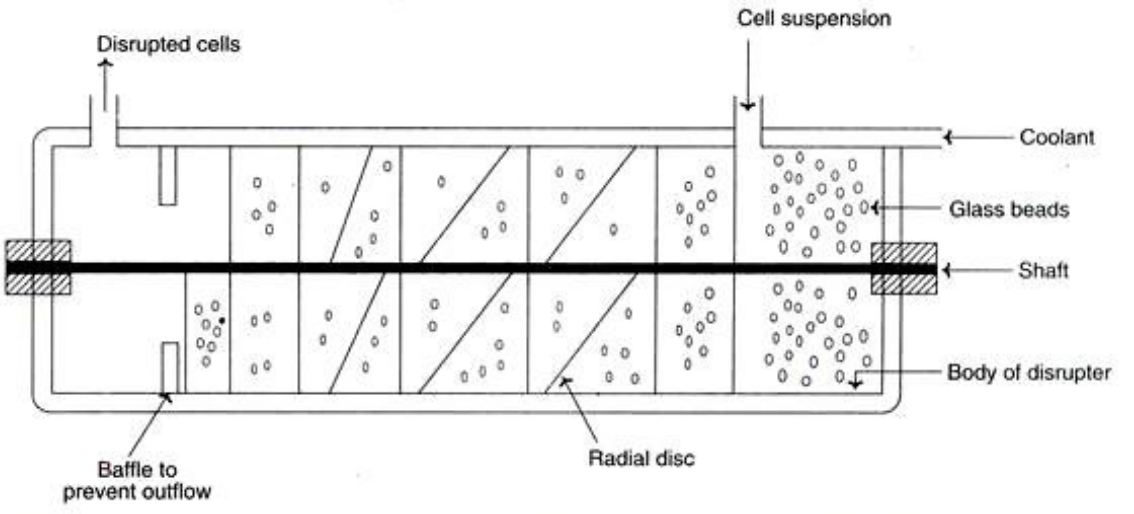


Fig. 20.6 : Diagrammatic representation of a cell disrupter.

Mechanical and non-mechanical methods:

Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods (described below) are non- mechanical in nature.

Chemical methods of cell disruption:

Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

Alkalies:

Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.

Organic solvents:

Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

Detergents:

Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt

precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromato-graphy for purification.

Enzymatic methods of cell disruption:

Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram-positive bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme. For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

Concentration:

The filtrate that is free from suspended particles (cells, cell debris etc.) usually contains 80-98% of water. The desired product is a very minor constituent. The water has to be removed to achieve the product concentration. The commonly used techniques for concentrating biological products are evaporation, liquid-liquid extraction, membrane filtration, precipitation and adsorption. The actual procedure adopted depends on the nature of the desired product (quality and quantity to be retained as far as possible) and the cost factor.

Evaporation:

Water in the broth filtrate can be removed by a simple evaporation process. The evaporators, in general, have a heating device for supply of steam, and unit for the separation of concentrated product and vapour, a condenser for condensing vapour, accessories and control equipment. The capacity of the equipment is variable that may range from small laboratory scale to industrial scale. Some of the important types of evaporators in common use are briefly described.

Plate evaporators:

The liquid to be concentrated flows over plates. As the steam is supplied, the liquid gets concentrated and becomes viscous.

Falling film evaporators:

In this case, the liquid flows down long tubes which gets distributed as a thin film over the heating surface. Falling film evaporators are suitable for removing water from viscous products of fermentation.

Forced film evaporators:

The liquid films are mechanically driven and these devices are suitable for producing dry product concentrates.

Centrifugal forced film evaporators:

These equipment evaporate the liquid very quickly (in seconds), hence suitable for concentrating even heat-labile substances. In these evaporators, a centrifugal force is used to pass on the liquid over heated plates or conical surfaces for instantaneous evaporation.

Liquid-Liquid Extraction:

The concentration of biological products can be achieved by transferring the desired product (solute) from one liquid phase to another liquid phase, a phenomenon referred to as liquid-liquid extraction. Besides concentration, this technique is also useful for partial purification of a product. The efficiency of extraction is dependent on the partition coefficient i.e. the relative distribution of a substance between the two liquid phases. The process of liquid-liquid extraction may be broadly categorized as extraction of low molecular weight products and extraction of high molecular weight products.

Extraction of low molecular weight products:

By using organic solvents, the lipophilic compounds can be conveniently extracted. However, it is quite difficult to extract hydrophilic compounds. Extraction of lipophilic products can be done by the following techniques.

Physical extraction:

The compound gets itself distributed between two liquid phases based on the physical properties. This technique is used for extraction of non-ionising compounds.

Dissociation extraction:

This technique is suitable for the extraction of ionisable compounds. Certain antibiotics can be extracted by this procedure.

Reactive extraction:

In this case, the desired product is made to react with a carrier molecule (e.g., phosphorus compound, aliphatic amine) and extracted into organic solvent. Reactive extraction procedure is quite useful for the extraction of certain compounds that are highly soluble in water (aqueous phase) e.g., organic acids.

Supercritical fluid (SCF) extraction:

This technique differs from the above procedures, since the materials used for extraction are supercritical fluids (SCFs). SCFs are intermediates between gases and liquids and exist as fluids above their critical temperature and pressure. Supercritical CO2, with a low critical temperature and pressure is commonly used in the extraction. Supercritical fluid extraction is rather expensive, hence not widely used (SCF has been used for the extraction of caffeine from coffee beans, and pigments and flavor ingredients from biological materials).

Extraction of high molecular weight compounds:

Proteins are the most predominant high molecular weight products produced in fermentation industries. Organic solvents cannot be used for protein extraction, as they lose their biological

activities. They are extracted by using an aqueous two-phase systems or reverse micelles formation.

Aqueous two-phase systems (ATPS):

They can be prepared by mixing a polymer (e.g., polyethylene glycol) and a salt solution (ammonium sulfate) or two different polymers. Water is the main component in ATPS, but the two phases are not miscible. Cells and other solids remain in one phase while the proteins are transferred to other phase. The distribution of the desired product is based on its surface and ionic character and the nature of phases. The separation takes much longer time by ATPS.

Reverse miceller systems:

Reverse micelles are stable aggregates of surfactant molecules and water in organic solvents. The proteins can be extracted from the aqueous medium by forming reverse micelles. In fact, the enzymes can be extracted by this procedure without loss of biological activity.

Membrane Filtration:

Membrane filtration has become a common separation technique in industrial biotechnology. It can be conveniently used for the separation of biomolecules and particles, and for the concentration of fluids. The membrane filtration technique basically involves the use of a semi-permeable membrane that selectively retains the particles/molecules that are bigger than the pore size while the smaller molecules pass through the membrane pores.

Membranes used in filtration are made up of polymeric materials such as polyethersulfone and polyvinyl di-fluoride. It is rather difficult to sterilize membrane filters. In recent years, micro-filters and ultrafiIters composed of ceramics and steel are available. Cleaning and sterilization of such filters are easy. The other types of membrane filtration techniques are described briefly.

Membrane adsorbers:

They are micro- or macro porous membranes with ion exchange groups and/or affinity ligands. Membrane adsorbers can bind to proteins and retain them. Such proteins can be eluted by employing solutions in chromatography.

Pervaporation:

This is a technique in which volatile products can be separated by a process of permeation through a membrane coupled with evaporation. Pervaporation is quite useful for the extraction, recovery and concentration of volatile products. However, this procedure has a limitation since it cannot be used for large scale separation of volatile products due to cost factor.

Perstraction:

This is an advanced technique working on the principle of membrane filtration coupled with solvent extraction. The hydrophobic compounds can be recovered/ concentrated by this method.

Precipitation:

Precipitation is the most commonly used technique in industry for the concentration of macromolecules such as proteins and polysaccharides. Further, precipitation technique can also be employed for the removal of certain unwanted byproducts e.g. nucleic acids, pigments.

Neutral salts, organic solvents, high molecular weight polymers (ionic or non-ionic), besides alteration in temperature and pH are used in precipitation. In addition to these non-specific protein precipitation reactions (i.e. the nature of the protein is unimportant), there are some protein specific precipitations e.g., affinity precipitation, ligand precipitation.

Neutral salts:

The most commonly used salt is ammonium sulfate, since it is highly soluble, non-toxic to proteins and low-priced. Ammonium sulfate increases hydrophobic interactions between protein molecules that result in their precipitation. The precipitation of proteins is dependent on several factors such as protein concentration, pH and temperature.

Organic solvents:

Ethanol, acetone and propanol are the commonly used organic solvents for protein precipitation. They reduce the dielectric constant of the medium and enhance electrostatic interaction between protein molecules that lead to precipitation. Since proteins are denatured by organic solvents, the precipitation process has to be carried out below 0°C.

Non-ionic polymers:

Polyethylene glycol (PEG) is a high molecular weight non-ionic polymer that can precipitate proteins. It reduces the quantity of water available for protein solvation and precipitates protein. PEG does not denature proteins, besides being non-toxic.

Ionic polymers:

The charged polymers such as polyacrylic acid and polyethyleneimine are used. They form complexes with oppositely charged protein molecules that causes charge neutralisation and precepitation.

Increase in temperature: The heat sensitive proteins can be precipitated by increasing the temperature.

Change in pH: Alterations in pH can also lead to protein precipitation.

Affinity precipitation:The affinity interaction (e.g., between antigen and antibody) is exploited for precipitation of proteins.

Precipitation by ligands :Ligands with specific binding sites for proteins have been successfully used for selective precipitation.

Adsorption:

The biological products of fermentation can be concentrated by using solid adsorbent particles. In the early days, activated charcoal was used as the adsorbent material. In recent years, cellulose-based adsorbents are employed for protein concentration.And for concentration of low molecular weight compounds (vitamins, antibiotics, peptides) polystyrene, methacrylate and acrylate based matrices are used. The process of adsorption can be carried out by making a bed of adsorbent column and passing the culture broth through it. The desired product, held by the adsorbent, can be eluted.

Purification by Chromatography:

The biological products of fermentation (proteins, pharmaceuticals, diagnostic compounds and research materials) are very effectively purified by chromatography. It is basically an analytical technique dealing with the separation of closely related compounds from a mixture. Chromato-graphy usually consists of a stationary phase and mobile phase. The stationary phase is the porous solid matrix packed in a column (equilibrated with a suitable solvent) on to which the mixture of compounds to be separated is loaded. The compounds are eluted by a mobile phase.

A single mobile phase may be used continuously or it may be changed appropriately to facilitate the release of desired compounds. The eluate from the column can be monitored continuously (e.g. protein elution can be monitored by ultraviolet adsorption at 280 nm), and collected in fractions of definite volumes. The different types of chromatography techniques used for separation (mainly proteins) along with the principles are given in Table 20.2. A large number of matrices are commercially available for purification of proteins e.g., agarose, cellulose, polyacrylamide, porous silica, cross- linked dextran, polystyrene.

TABLE 20.2 Chromatographic techniques along with the principles for separation of proteins

Chromatography	*Principle*
Gel-filtration (size exclusion)	Size and shape
Ion-exchange	Net charge
Chromatofocussing	Net charge
Affinity	Biological affinity and molecular recognition
Hydrophobic interaction	Polarity (hydrophobicity of molecules)
Immobilized metal-ion affinity	Metal ion binding

Formulation:

Formulation broadly refers to the maintenance of activity and stability of a biotechnological products during storage and distribution. The formulation of low molecular weight products (solvents, organic acids) can be achieved by concentrating them with removal of most of the

water. For certain small molecules, (antibiotics, citric acid), formulation can be done by crystallization by adding salts. Proteins are highly susceptible for loss of biological activity; hence their formulation requires special care. Certain stabilizing additives are added to prolong the shelf life of protein. The stabilizers of protein formulation include sugars (sucrose, lactose), salts (sodium chloride, ammonium sulfate), polymers (polyethylene glycol) and polyhydric alcohols (glycerol). Proteins may be formulated in the form of solutions, suspensions or dry powders.

Drying:

Drying is an essential component of product formulation. It basically involves the transfer of heat to a wet product for removal of moisture. Most of the biological products of fermentation are sensitive to heat, and therefore require gentle drying methods. Based on the method of heat transfer, drying devices may be categorized as contact, convection, radiation dryers. These three types of dryers are commercially available.

Spray drying:

Spray drying is used for drying large volumes of liquids. In spray drying, small droplets of liquid containing the product are passed through a nozzle directing it over a stream of hot gas. The water evaporates and the solid particles are left behind.

Freeze-drying:

Freeze-drying or lyophilization is the most preferred method for drying and formulation of a wide-range of products—pharmaceuticals, foodstuffs, diagnostics, bacteria, viruses. This is mainly because freeze-drying usually does not cause loss of biological activity of the desired product.

Lyophilization is based on the principle of sublimation of a liquid from a frozen state. In the actual technique, the liquid containing the product is frozen and then dried in a freeze-dryer under vacuum. The vacuum can now be released and the product containing vials can be sealed e.g., penicillin can be freeze dried directly in ampules.

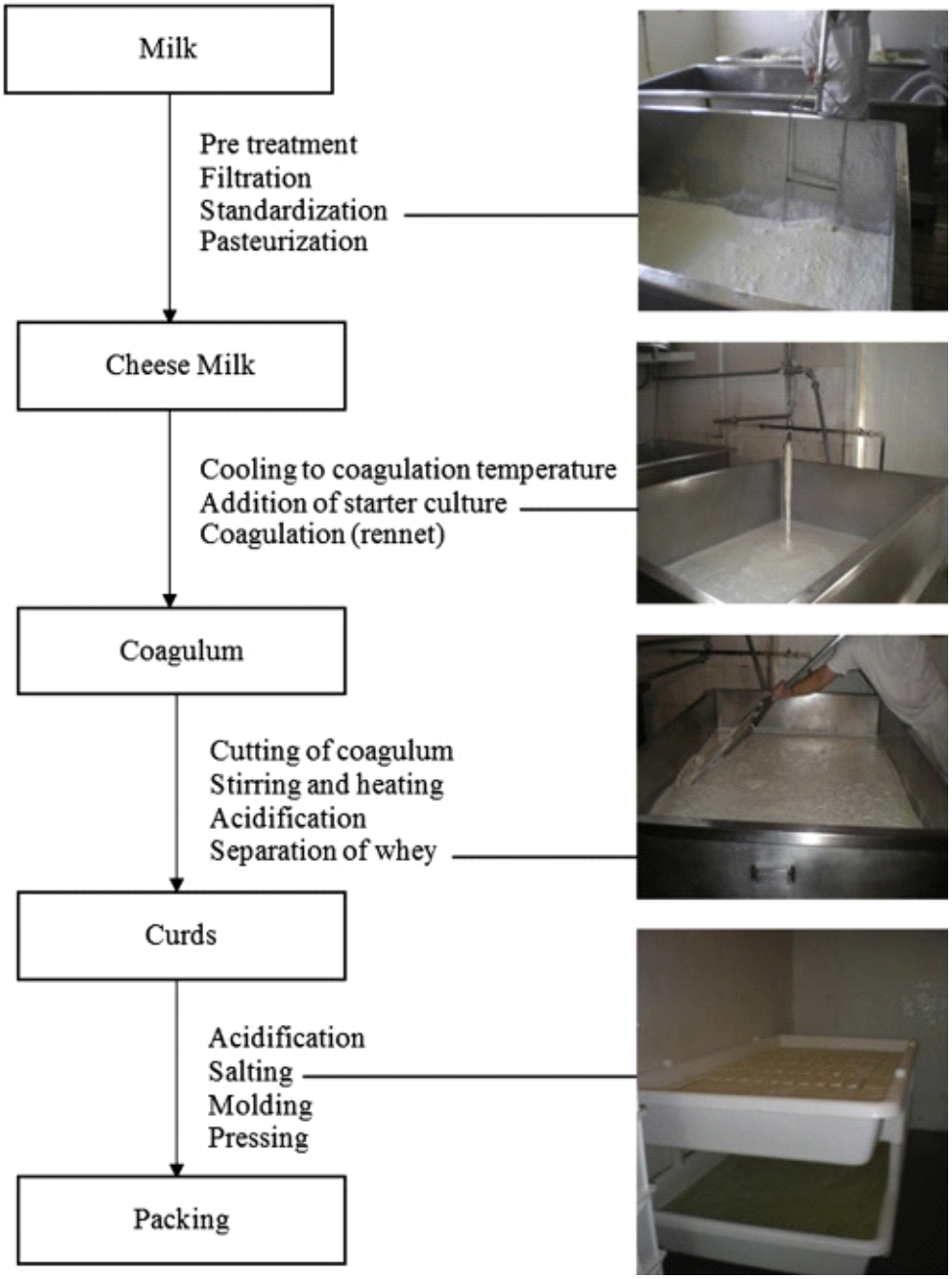
UNIT V-APPLICATION OF MICROBES IN FOOD PROCESSING

Milk processing

Milk is a valuable nutritious food that has a short shelf-life and requires careful handling. Milk is highly perishable because it is an excellent medium for the growth of microorganisms – particularly bacterial pathogens – that can cause spoilage and diseases in consumers. Milk processing allows the preservation of milk for days, weeks or months and helps to reduce food-borne illness.

The usable life of milk can be extended for several days through techniques such as cooling (which is the factor most likely to influence the quality of raw milk) or fermentation. Pasteurization is a heat treatment process that extends the usable life of milk and reduces the numbers of possible pathogenic microorganisms to levels at which they do not represent a significant health hazard. Milk can be processed further to convert it into high-value, concentrated and easily transportable dairy products with long shelf-lives, such as butter, cheese and ghee.

Processing of dairy products gives small-scale dairy producers higher cash incomes than selling raw milk and offers better opportunities to reach regional and urban markets. Milk processing can also help to deal with seasonal fluctuations in milk supply. The transformation of raw milk into processed milk and products can benefit entire communities by generating off-farm jobs in milk collection, transportation, processing and marketing.



Cheese Processing and Critical Points of Contamination

Although there are more than 600 different types of cheeses, the manufacturing process of all cheeses can be arranged in five general, essential steps including pretreatment of raw milk, milk coagulation, treating of curd, ripening, and packaging. Technological processing of all cheeses has as their basic principle milk coagulation, which transforms milk into a solid mass that is then separated from the whey. Milk coagulates when casein micelles become unstable, which may be achieved by the addition of enzymes or acids. Some cheeses may be produced by mixed

coagulation process, when rennet (the substance responsible for enzymatic coagulation) and specific lactic acid bacteria or lactic acid (responsible for acid coagulation) are added.

Butter Manufacture

Butter is essentially the fat of the milk. It is usually made from sweet cream and is salted. However, it can also be made from acidulated or bacteriologically soured cream and saltless (sweet) butters are also available. Well into the 19th century butter was still made from cream that had been allowed to stand and sour naturally. The cream was then skimmed from the top of the milk and poured into a wooden tub. Buttermaking was done by hand in butter churns. The natural souring process is, however, a very sensitive one and infection by foreign micro-organisms often spoiled the result. Today's commercial buttermaking is a product of the knowledge and experience gained over the years in such matters as hygiene, bacterial acidifying and heat treatment, as well as the rapid technical development that has led to the advanced machinery now used. The commercial cream separator was introduced at the end of the 19th century, the continuous churn had been commercialized by the middle of the 20th century.

Definitions and Standards

Milkfat

The lipid components of milk, as produced by the cow, and found in commercial milk and milk-derived products, mostly comprised of triglyceride.

Butterfat

Almost synonymous with milkfat; all of the fat components in milk that are separable by churning.

Anhydrous Milkfat (AMF)

The commercially- prepared extraction of cow's milkfat, found in bulk or concentrated form (comprised of 100% fat, but not necessarily all of the lipid components of milk).

Butter oil

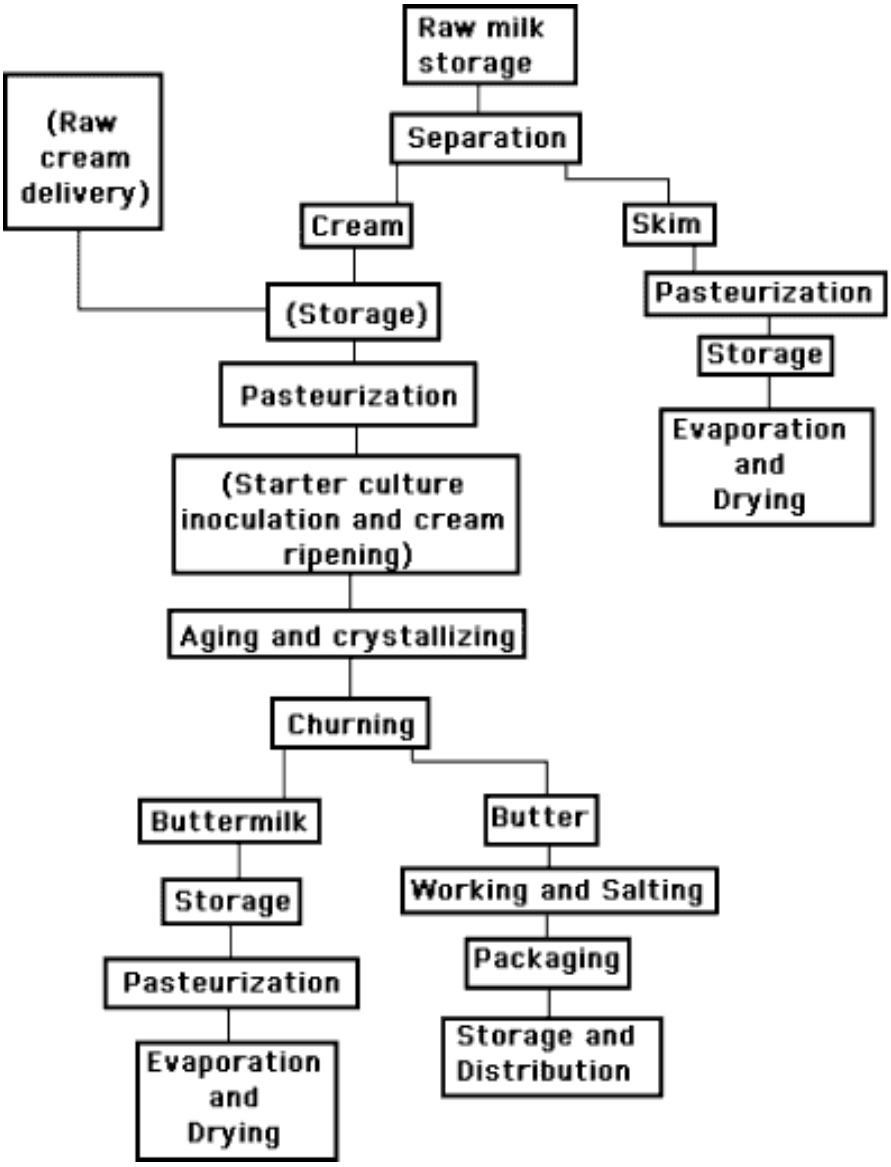
Synonymous with anhydrous milkfat; (conventional terminology in the fats and oils field differentiates an oil from a fat based on whether it is liquid at room temp. or solid, but very arbitrary).

Butter

A water-in-oil emulsion, comprised of >80% milkfat, but also containing water in the form of tiny droplets, perhaps some milk solids-not-fat, with or without salt (sweet butter); texture is a result of working/kneading during processing at appropriate temperatures, to establish fat crystalline network that results in desired smoothness (compare butter with melted and recrystallized butter); used as a spread, a cooking fat, or a baking ingredient.

The principal constituents of a normal salted butter are fat (80 - 82%), water (15.6 - 17.6%), salt (about 1.2%) as well as protein, calcium and phosphorous (about 1.2%). Butter also contains fat-soluble vitamins A, D and E.

Butter should have a uniform color, be dense and taste clean. The water content should be dispersed in fine droplets so that the butter looks dry. The consistency should be smooth so that the butter is easy to spread and melts readily on the tongue.



The buttermaking process involves quite a number of stages. The continuous HYPERLINK "https://www.uoguelph.ca/foodscience/node/1687/"buttermaker has become the most common type of equipment used.

The cream can be either supplied by a fluid milk dairy or separated from whole milk by the butter manufacturer. The cream should be sweet (pH >6.6, TA = 0.10 - 0.12%), not rancid and not oxidized.

If the cream is separated by the butter manufacturer, the whole milk is preheated to the required temperature in a milk pasteurizer before being passed through a separator. The cream is cooled and led to a storage tank where the fat content is analyzed and adjusted to the desired value, if

necessary. The skim milk from the separator is pasteurized and cooled before being pumped to storage. It is usually destined for concentration and drying.

From the intermediate storage tanks, the cream goes to pasteurization at a temperature of 95oC or more. The high temperature is needed to destroy enzymes and micro-organisms that would impair the keeping quality of the butter.

If ripening is desired for the production of cultured butter, mixed cultures of S. cremoris, S. lactis diacetyl lactis, Leuconostocs, are used and the cream is ripened to pH 5.5 at 21oC and then pH 4.6 at 13oC. Most flavour development occurs between pH 5.5 - 4.6. The colder the temperature during ripening the more the flavour development relative to acid production. Ripened butter is usually not washed or salted.

In the aging tank, the cream is subjected to a program of controlled cooling designed to give the fat the required crystalline structure. The program is chosen to accord with factors such as the composition of the butterfat, expressed, for example, in terms of the iodine value which is a measure of the unsaturated fat content. The treatment can even be modified to obtain butter with good consistency despite a low iodine value, i.e. when the unsaturated proportion of the fat is low.

As a rule, aging takes 12 - 15 hours. From the aging tank, the cream is pumped to the churn or continuous HYPERLINK "https://www.uoguelph.ca/foodscience/node/1687/"buttermaker via a plate heat exchanger which brings it to the requisite temperature. In the churning process the cream is violently agitated to break down the fat globules, causing the fat to coagulate into butter grains, while the fat content of the remaining liquid, the buttermilk, decreases.

Thus the cream is split into two fractions: butter grains and buttermilk. In traditional churning, the machine stops when the grains have reached a certain size, whereupon the buttermilk is drained off. With the continuous buttermaker the draining of the buttermilk is also continuous.

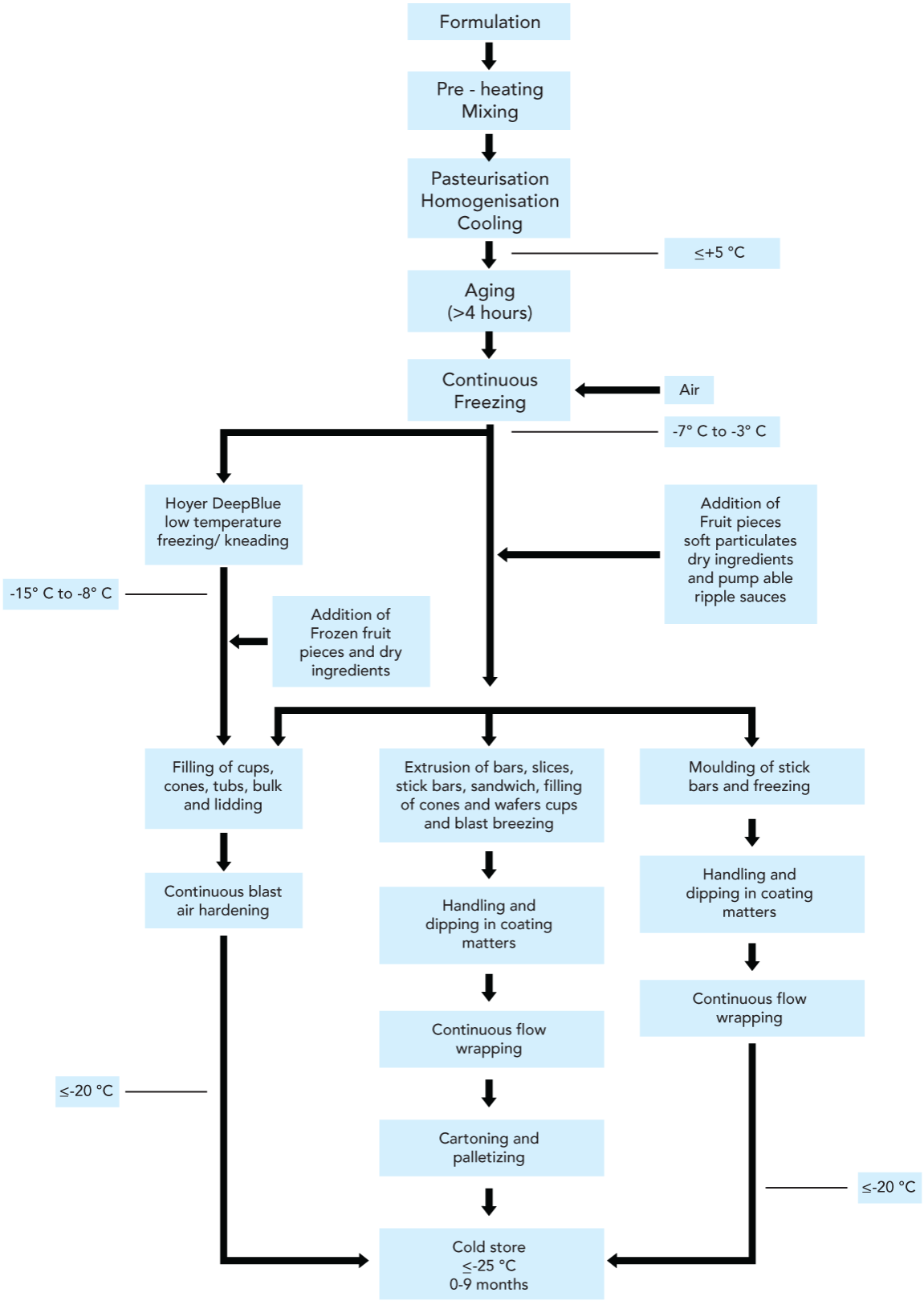
After draining, the butter is worked to a continuous fat phase containing a finely dispersed water phase. It used to be common practice to wash the butter after churning to remove any residual buttermilk and milk solids but this is rarely done today.

Salt is used to improve the flavour and the shelf-life, as it acts as a preservative. If the butter is to be salted, salt (1-3%) is spread over its surface, in the case of batch production. In the continuous buttermaker, a salt slurry is added to the butter. The salt is all dissolved in the aqueous phase, so the effective salt concentration is approximately 10% in the water.

After salting, the butter must be worked vigorously to ensure even distribution of the salt. The working of the butter also influences the characteristics by which the product is judged - aroma, taste, keeping quality, appearance and colour. Working is required to obtain a homogenous blend of butter granules, water and salt. During working, fat moves from globular to free fat. Water droplets decrease in size during working and should not be visible in properly worked butter. Overworked butter will be too brittle or greasy depending on whether the fat is hard or soft. Some water may be added to standardize the moisture content. Precise control of composition is essential for maximum yield.

The finished butter is discharged into the packaging unit, and from there to cold storage.

Ice cream production



he basic steps in the manufacturing of ice cream are generally as follows:

- blending of the mix ingredients

- pasteurization
- homogenization
- aging the mix
- freezing
- packaging
- hardening

Processing of Fruits and Vegetables

In previous blog post we wrote about storage of fresh fruits and vegetables. But fruits and vegetables can be also stored in many other forms, such as canned, frozen, dried or juced. Modern lifestyle and diet, which prompted the human to adequately storage a variety of fruits and other plant organs, influenced on the development and implementation of the many methods and procedures for preservation of fruits and vegetables. Technological procedures of processing of fruit and vegetables can be classified into few processing methods:

- Traditional processing methods - drying, concentrating, heating (cooking, baking, frying) cooling, use of additives - preservatives, acidification, fermentation
- Improved traditional methods of processing - the application of increased temperatures (sterilization, pasteurization), the application of low temperature (cooling, freezing), aseptic packaging, controlled atmosphere -CA, freeze-drying, microfiltration and membrane processes, packaging (MA and vacuum)
- Procedures that are investigating - high voltage pulse techniques, photodynamic inactivation, microwave processing - heating, high pressure treatment, ionizing radiation, heating of electrical resistance effect and induction

Heat treatment and sterilization

One of the traditional methods of preserving process of fruits and vegetables is a thermal treatment, which involves the use of heat, ie. increased temperatures. Heat treatment is carried out by methods of sterilization, pasteurization and blanching, thus hermetically sealed packaging is used (usually made of metal, glass or plastic). Fruits, vegetables and their products represent a significant segment of the human diet, as they create the preconditions of proper nutrition. From a global point of view fruits and vegetables are present in the human diet all over the world, but it is also interesting that the relatively large producers of fruits and vegetables are developing countries.

Fruit and vegetable products

It is a large number of products that can be produced from fruits and vegetables. In the table below you can see the list of products.

Fruit products	Vegetable products
frozen fruit	frozen vegetables

jam	vegetable juice
fruit jellies	concentrated vegetable juice
pasteurized fruit	pasteurized vegetables
fruit cheese	sterilized vegetables
frozen pulp of the fruit	frozen pulp of the fruit dried vegetables
pasteurised mash	marinated vegetables
candied fruits	biological canned vegetables
dried fruit	vegetable sauce
fruit juice	other vegetable products
fruit juice powder	
fruit syrup	
low-calorie products	
compote	
marmalade	

Frozen, canned or dried fruits and vegetables have a lot of benefits, such as longer shelf life, convenience, year round availability, most vitamins are retained as they are generally processed and packaged within hours of being picked, easy storage, easy preparation etc. To learn more about processing of fruit, vegetables and also cereals, check out the knowledge base in Agrivi farm management system. Start using Agrivi now.

Meat, Poultry and Fish

Meat, poultry and fish are foods that may naturally contain bacteria that cause diseases.
The prevention of diseases whose source is in these foods is dependent on keeping to and insisting on the regulations that are detailed here, starting with the stage of buying, through storage and treatment at home.

Proper purchasing of meat, poultry and fish

- Purchase only those products having clear markings of the last use-by-date. For products that are pre-packaged, check that the last use-by-date has not passed;

- When purchasing raw (uncooked) food, that is not pre-packaged at the butcher shop, ask the butcher to show the original label of the food, including clear marking of the last use-by-date;
- Buy only in places that are air-conditioned, well-lit and clean;
- Buy only in places where the employees are cleanly and properly dressed;
- Buy raw (uncooked) meat and poultry that was ground in your presence or only if it is packaged and labeled.
- Buy only products that have characteristic appearances, colors and odors. Fish have special signs of freshness, and it is recommended that you pay attention to them (see below);
- Buy only products that are kept in the store according to the manufacturer's instructions. Fresh meat, poultry and fish/raw (uncooked) must be refrigerated (0o-4oC) or frozen (-18o C) during all stages of marketing, and they should not be purchased if they are not in the refrigerator or the freezer.
  The manufacturer's recommended temperature appears on the product, and you can check that the product is properly kept in the store in the refrigerator or the freezer according to the manufacturer's recommendations (the manufacturer decides how the product is to be stored, and products marked as "chilled" are not to be frozen in the store or frozen products kept in the refrigerator).
  You are able to recognize if a frozen product in the store has been thawed and refrozen: if there are lumps of ice in the package, this is a sign that the product has thawed and been re-frozen, and it is not recommended to purchase this product.
- Purchase pre-packaged products only if the packaging is whole and proper;
- Place raw (uncooked) meat, poultry and fish in a clean bag, separate from other products you have bought.
  A separate, clean package will prevent dripping and contamination of other products when you are shopping.
- In the store, remove the meat, poultry and fish from the refrigerator as close as possible to the check-out time.
- Purchase chicken and turkey (frozen or chilled) only if it has the symbol of the veterinary supervision.
  The symbol appears on the product's label and contains the words "checked and approved" and the unique veterinary supervision number of the slaughterhouse.

Highlights for purchasing fish

- Buy fish that are not pre-packaged only if they are displayed in a refrigerator and are covered with ice chips;
- It is completely forbidden to touch the fish by hand before they are cleaned;
- Buy fish that are not pre-packaged only after they have been cleaned by the seller;
- Buy fish that are not pre-packaged only if they have the normal appearance, odor and color;
- The fish's eyes are black, clear and bulging;
- The gills are red and not white or gray, and without a rotten smell.

Be sure to take these five simple actions:

1. Separation – separate meat according to its type, poultry and fish separate from each other and from other foods

- Use special utensils (knives and boards) for dealing with raw (uncooked) meat, poultry and fish and which are kept separate from others used to prepare food that is ready to eat without cooking (like salad). It is recommended to deal separately with meat, poultry and fish.

2**. Cleanliness** – wash your hands, dishes and work spaces often

- Adherence to the cleanliness of the equipment and your hands before and after dealing with meat, poultry or fish is designed to prevent the transfer of bacteria that cause diseases from the meat, poultry or fish to other foods and to cooked products ("cross-contamination").
- After cleaning, koshering, dismembering and all other treatments before cooking, all the remnants that are not to be used should be immediately disposed of, and dishes and hands should be thoroughly and well washed (with soap and water);
- It is especially important to scrub the cutting board with dishwashing liquid, rinse it thoroughly with hot water and dry it in the air – in order to remove the layer of bacteria that is not visible to the naked eye. Washing meat, poultry and fish before cooking may cause environmental contamination by bacteria. If you do wash the product, everything that has come into contact with the rinsing water (sink, countertop, etc.) should be disinfected.

3. **Cooking –** avoid eating meat, poultry and fish that are not cooked

- If you have a cooking thermometer, you can check that temperature at the center of the product has reached a minimum of 70oC. After every check, the thermometer should be thoroughly washed to prevent it reinfecting the cooked product;
- If you cook the meat, poultry or fish when it is frozen, the cooking time needs to be extended compared with the thawed product, so that the entire product is properly cooked and will reach the recommended temperature;
- If you roast or fry the products on a grill (barbeque) or in a frying pan – one should use items that are as thin as possible in order to ensure thorough cooking;
- Use of a microwave oven is only meant for heating products that are ready to eat or to thaw frozen products. It is not recommend to use a microwave oven for cooking products;
- It is not recommended to taste the products during the cooking process, before they have reached the recommended temperature.

4**. Refrigeration** – keep products in the refrigerator or the freezer according to the instructions of the manufacturer or the butcher shop / butcher

- It is recommend to eat the meat immediately after cooking or to place it in the refrigerator (meat that has been cooked and left out of the refrigerator for more than two hours should not be used).

5**. Thawing** – meat, poultry and fish should only be thawed in the refrigerator or in a microwave oven

- The product should be thawed in a closed dish in order to avoid contamination of other products and especially food that is ready to eat;
- When thawing in a microwave oven, make sure to cook immediately after thawing.
- It is not recommended to thaw meat, poultry or fish and refreeze them – not even parts of them.
- When buying frozen products, it is recommended to purchase them in a size that is suitable for your immediate needs, in order to avoid thawing quantities larger than are needed.

Storage of meat, poultry and fish at home

- Store fresh meat, poultry and fish only in the refrigerator at a temperature of 0o – 4oC. They should be cooked as soon as possible (according to the instructions of the manufacturer or the butcher). If you wish to store them for a longer period of time, it is recommend to buy in advance frozen meat, poultry and fish;
- Store frozen meat, poultry and fish only in a freezer at a temperature that does not exceed -18oC;
- Use the stored products no later than the last use-by-date that appears on the package;
- Avoid contact between raw (uncooked) foods of various types, such as meat, eggs and vegetables;
- Avoid contact between raw (uncooked) foods used as raw materials for cooking and foods that are already ready to be eaten.

Special highlights concerning the purchase, treatment and storage of ground meat

- Ground meat is especially sensitive to bacterial growth and is therefore likely to be dangerous if not treated properly;
- Buy only products that are packaged and labeled or demand that the meat be ground in front of you;
- Buy only frozen ground meat that is produced in a factory with a manufacturer's license; Butcher shops are forbidden to freeze ground meat or any other type of meat. Therefore, check that the frozen ground meat is marked with the name and address of the manufacturing plant.
- Fresh ground meat should be stored in the refrigerator and frozen ground meat in the freezer, until it is used.
- Fresh ground meat should be cooked as soon as possible
  If you wish to store ground meat for a lengthy period of time, it is recommended to buy frozen ground meat and to make sure to use it no later than the last use-by-date that the manufacturer has set;
- It is recommended not to mix ground meat from various types of animals, except during thorough cooking.
  The various types of ground meat (chicken, turkey, beef, mutton, goat, pork) have different types of characteristic bacteria that cause illnesses;
- Be sure not to consume ground meat that has not been thoroughly cooked or fried. It is recommended to eat the meat immediately after cooking or to refrigerate it (meat that has been cooked and left out for more than two hours should not be used).

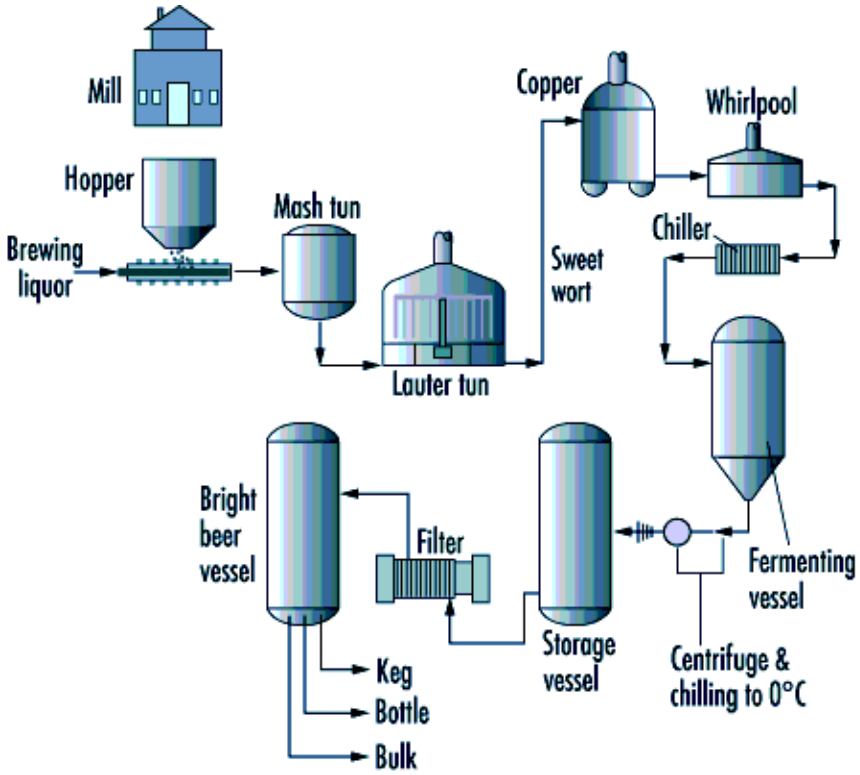
BREWING INDUSTRY

Brewing is one of the oldest industries: beer in different varieties was drunk in the ancient world, and the Romans introduced it to all their colonies. Today it is brewed and consumed in almost every country, particularly in Europe and areas of European settlement.

Process Overview

The grain used as the raw material is usually barley, but rye, maize, rice and oatmeal are also employed. In the first stage the grain is malted, either by causing it to germinate or by artificial means. This converts the carbohydrates to dextrin and maltose, and these sugars are then extracted from the grain by soaking in a mash tun (vat or cask) and then agitating in a lauter tun. The resulting liquor, known as sweet wort, is then boiled in a copper vessel with hops, which give a bitter flavour and helps to preserve the beer. The hops are then separated from the wort

and it is passed through chillers into fermenting vessels where the yeast is added—a process known as pitching—and the main process of converting sugar into alcohol is carried out. (For discussion of fermentation see the chapter Pharmaceutical industry.) The beer is then chilled to 0 °C, centrifuged and filtered to clarify it; it is then ready for dispatch by keg, bottle, aluminium can or bulk transport. is a flow chart of the brewing process.



Hazards and Their Prevention

Manual handling accounts for most of the injuries in breweries: hands are bruised, cut or punctured by jagged hoops, splinters of wood and broken glass. Feet are bruised and crushed by falling or rolling barrels. Much can be done to prevent these injuries by suitable hand and foot protection. Increase in automation and standardization of barrel size (say at 50 l) can reduce the lifting risks. The back pain caused by lifting and carrying of barrels and so on can be dramatically reduced by training in sound lifting techniques. Mechanical handling on pallets can also reduce ergonomic problems. Falls on wet and slippery floors are common. Non-slip surfaces and footwear, and a regular system of cleaning, are the best precaution.

Handling of grain can produce barley itch, caused by a mite infesting the grain. Mill-worker's asthma, sometimes called malt fever, has been recorded in grain handlers and has been shown to be an allergic response to the grain weevil (Sitophilus granarius). Manual handling of hops can produce a dermatitis due to the absorption of the resinous essences through broken or chapped skin. Preventive measures include good washing and sanitary facilities, efficient ventilation of the workrooms, and medical supervision of the workers.

When barley is malted by the traditional method of steeping it and then spreading it on floors to produce germination, it may become contaminated by Aspergillus clavatus, which can produce growth and spore formation. When the barley is turned to prevent root matting of the shoots, or when it is loaded into kilns, the spores may be inhaled by the workers. This may produce

extrinsic allergic alveolitis, which in symptomatology is indistinguishable from farmer's lung; exposure in a sensitized subject is followed by a rise in body temperature and shortness of breath. There is also a fall in normal lung functions and a decrease in the carbon monoxide transfer factor.

A study of organic dusts containing high levels of endotoxin in two breweries in Portugal found the prevalence of symptoms of organic dust toxic syndrome, which is distinct from alveolitis or hypersensitivity pneumonia, to be 18% among brewery workers. Mucous membrane irritation was found among 39% of workers (Carveilheiro et al. 1994).

In an exposed population, the incidence of the disease is about 5%, and continued exposure produces severe respiratory incapacity. With the introduction of automated malting, where workers are not exposed, this disease has largely been eliminated.

Machinery

Where malt is stored in silos, the opening should be protected and strict rules enforced regarding entry of personnel, as described in the box on confined spaces in this chapter. Conveyors are much used in bottling plants; traps in the gearing between belts and drums can be avoided by efficient machinery guarding. There should be an effective lockout/tagout programme for maintenance and repair. Where there are walkways across or above conveyors, frequent stop buttons should also be provided. In the filling process, very serious lesions can be caused by bursting bottles; adequate guards on the machinery and face guards, rubber gloves, rubberized aprons and non-slip boots for the workers can prevent injury.

Electricity

Owing to the prevailing damp conditions, electrical installations and equipment need special protection, and this applies particularly to portable apparatus. Ground fault circuit interrupters should be installed where necessary. Wherever possible, low voltages should be used, especially for portable inspection lamps. Steam is used extensively, and burns and scalds occur; lagging and protection of pipes should be provided, and safety locks on steam valves will prevent accidental release of scalding steam.

Carbon dioxide

Carbon dioxide (CO2) is formed during fermentation and is present in fermenting tuns, as well as vats and vessels that have contained beer. Concentrations of 10%, even if breathed only for a short time, produce unconsciousness, asphyxia and eventual death. Carbon dioxide is heavier than air, and efficient ventilation with extraction at a low height is essential in all fermentation chambers where open vats are used. As the gas is imperceptible to the senses, there should be an acoustic warning system which will operate immediately if the ventilation system breaks down. Cleaning of confined spaces presents serious hazards: the gas should be dispelled by mobile ventilators before workers are permitted to enter, safety belts and lifelines and respiratory protective equipment of the self-contained or supplied-air type should be available, and another worker should be posted outside for supervision and rescue, if necessary.

Gassing

Gassing has occurred during relining of vats with protective coatings containing toxic substances such as trichloroethylene. Precautions should be taken similar to those listed above against carbon dioxide.

Refrigerant gases

Chilling is used to cool the hot wort before fermentation and for storage purposes. Accidental discharge of refrigerants can produce serious toxic and irritant effects. In the past, chloromethane, bromomethane, sulphur dioxide and ammonia were mainly used, but today ammonia is most common. Adequate ventilation and careful maintenance will prevent most risks, but leak detectors and self-contained breathing apparatus should be provided for emergencies frequently tested. Precautions against explosive risks may also be necessary (e.g., flameproof electrical fittings, elimination of naked flames).

Hot work

In some processes, such as cleaning out mash tuns, workers are exposed to hot, humid conditions while performing heavy work; cases of heat stroke and heat cramps can occur, especially in those new to the work. These conditions can be prevented by increased salt intake, adequate rest periods and the provision and use of shower baths. Medical supervision is necessary to prevent mycoses of the feet (e.g., athlete's foot), which spread rapidly in hot, humid conditions.

Throughout the industry, temperature and ventilation control, with special attention to the elimination of steam vapour, and the provision of PPE are important precautions, not only against accident and injury but also against more general hazards of damp, heat and cold (e.g., warm working clothes for workers in cold rooms).

Control should be exercised to prevent excessive consumption of the product by the persons employed, and alternative hot beverages should be available at meal breaks.

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