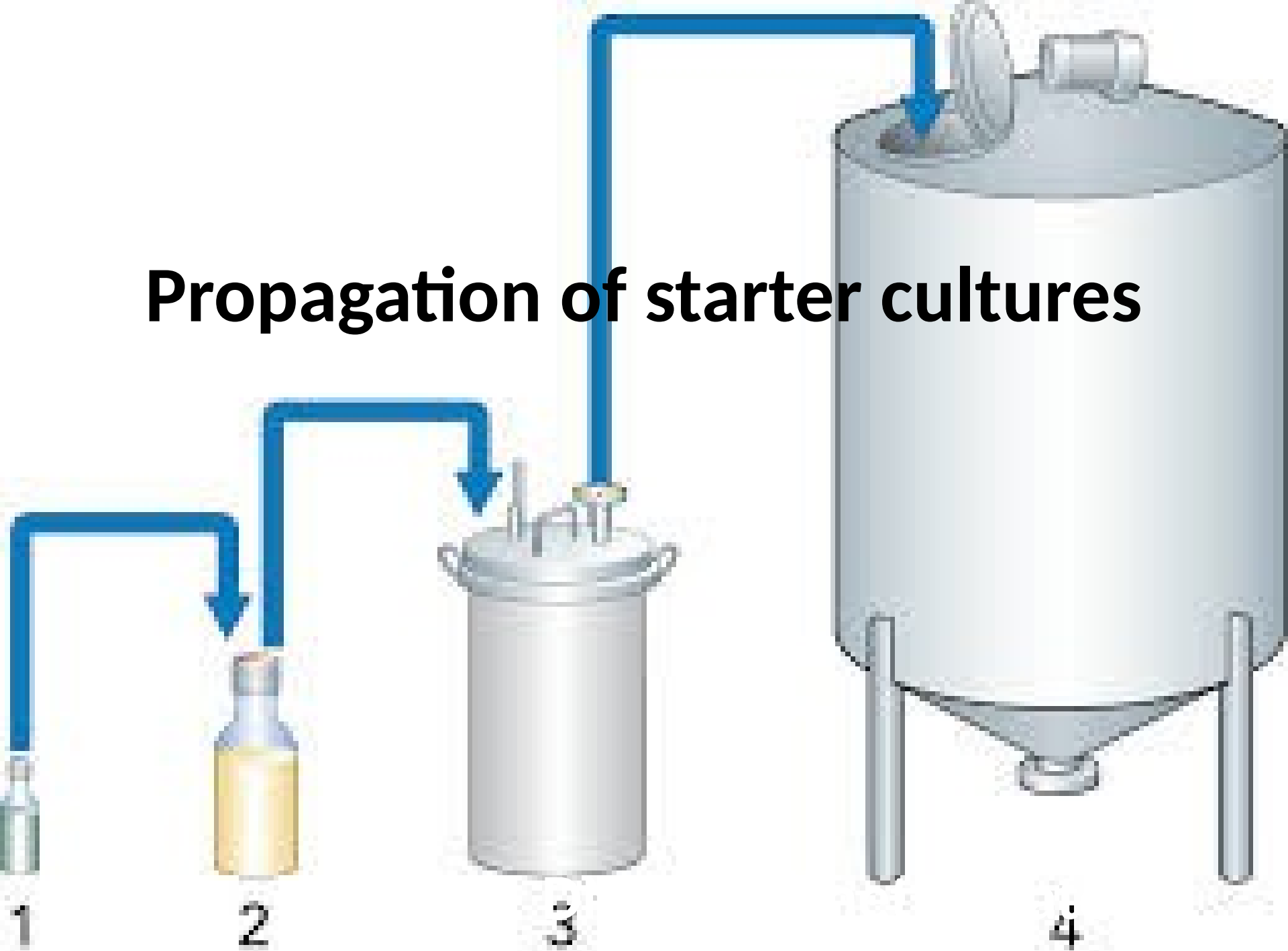


Propagation of starter cultures



What is starter propagation????

- Starter propagation is the most important operation of the quality control unit of dairy plant as the quality of starter is having a direct bearing on the quality of finished product.
- The main aim of propagation is to maintain pure cultures and activate cultures without any loss of viability .
- The culture organisms are preserved in small quantities known as stock cultures'.
- Fermentation process of any cultured dairy product relies on the 'purity' and 'activity' of the starter culture.
- A starter must be active, contain maximum number of viable organisms, free from contaminants.
- To obtain the above qualities of culture, the inoculation is carried out under aseptic conditions and growth is initiated in a sterile medium.

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Traditional Methods

- **In traditional method (simple microbiological technique), the starter are propagated in the laboratory itself taking the stock cultures which are in inactive state or dormant state.**
- **Commercial manufacturers provide starter cultures in lyophilized (freeze-dried), frozen or spray-dried forms. These cultures cannot be added directly in the milk to initiate fermentation process because they are in an inactive state.**
- **They need to be brought in to active form and also scale up to prepare bulk quantities.**
- **The dairy product manufacturers need to inoculate the culture into raw/pasteurized milk or other suitable substrate.**

Number of steps are to be followed during propagation of starter culture:

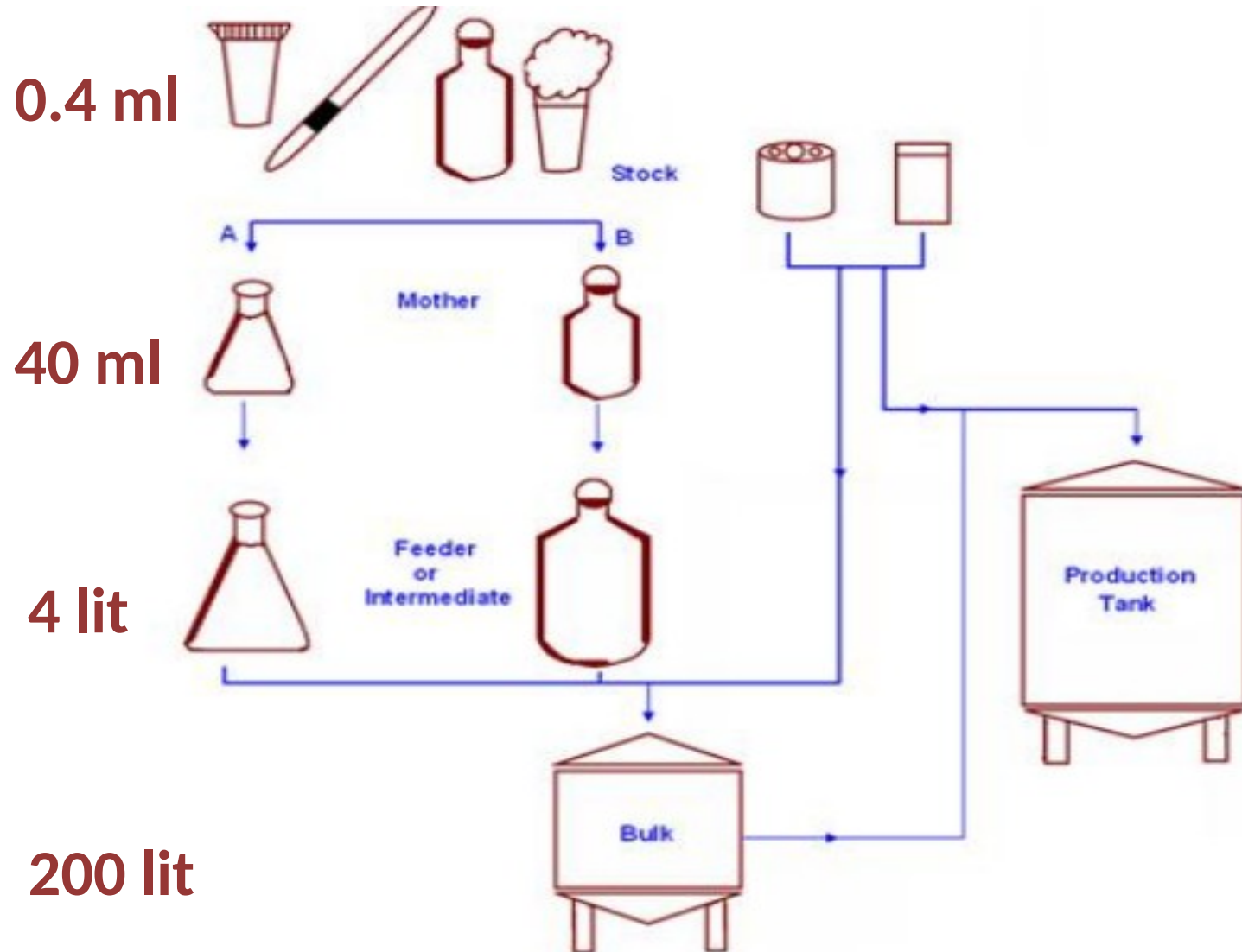
1. Selection of pure culture

2. Inoculation of starter culture

3. Incubation of starter culture

4. Sterilization of starter culture

Scaling up system of propagation: Stock and mother cultures are propagated in laboratory and feeder and bulk cultures in the starter room of the dairy plant.



1. The mother culture is traditionally prepared in 40 ml bottles provided with a cap containing a membrane.
2. The bottle is filled with skim milk, autoclaved, and then cooled to the appropriate inoculation temperature.
3. The master culture is injected into the bottle for mother culture with a sterilized syringe inserted through the membrane.
4. Following a suitable period of incubation and subsequent cooling, the mother culture is inoculated into the skim milk, used for the intermediate culture; the milk is first heated to a minimum of 95°C for 30-45 mins and cooled to adequate incubation temperature.
5. Heating and cooling are done in a specially designed incubator.
6. After an appropriate incubation period and cooling to about 10 – 12°C, the intermediate culture is transferred by displacement with filtered air through a tube to the bulk starter tank.
7. Before being inoculated the skim milk, is heated by circulation of heating medium and coolant through the tank jacket.
8. Air supplied to and evacuated from the tank passes through a sterile HEPA.

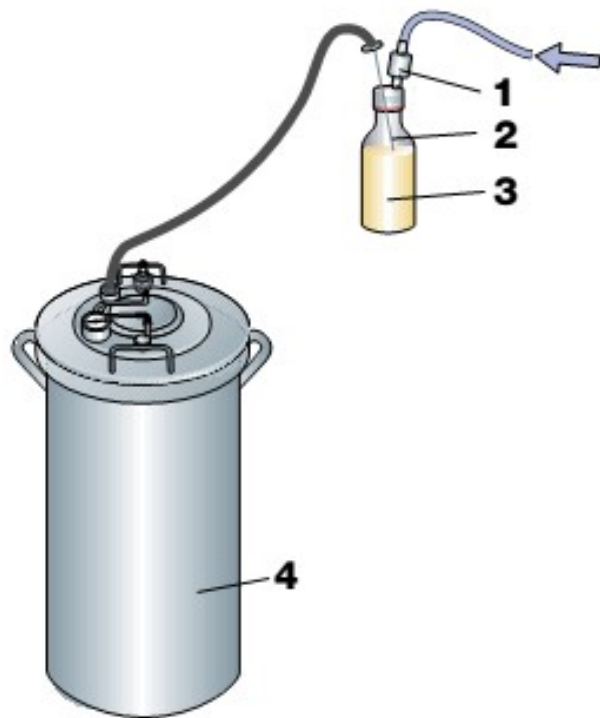
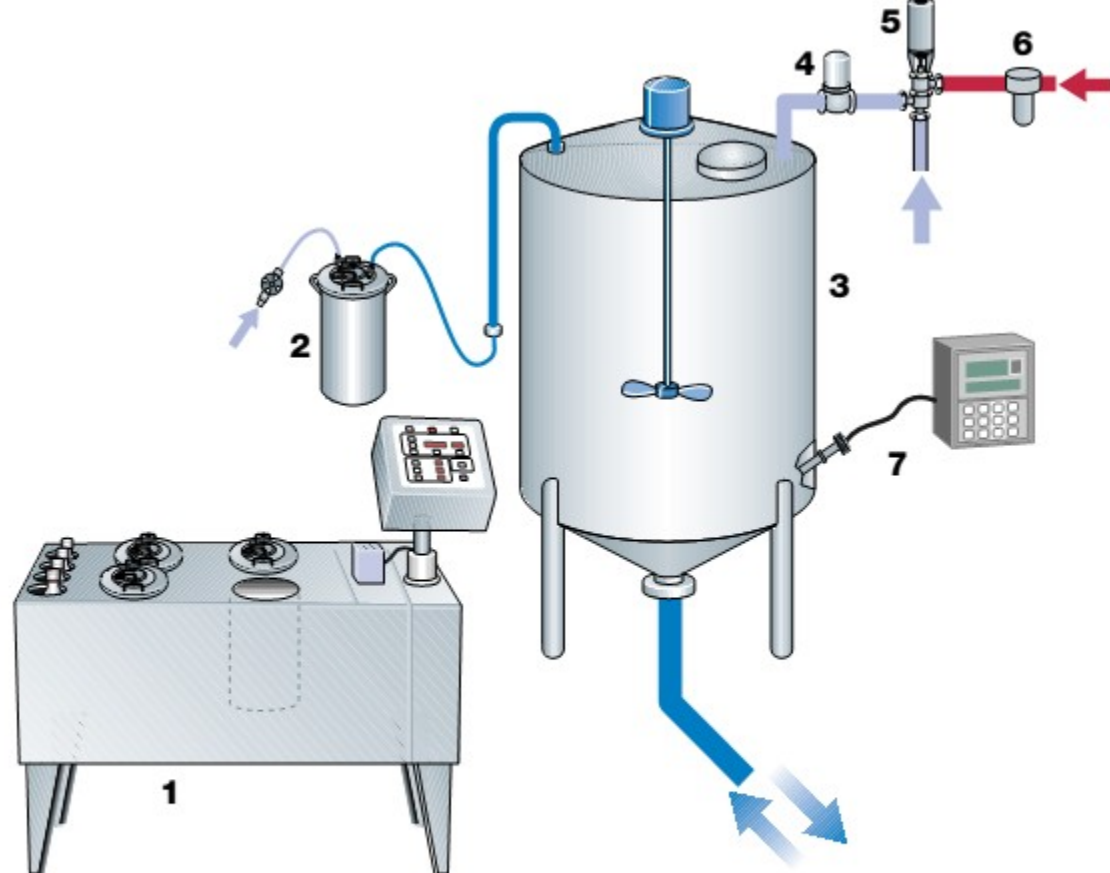


Fig 10.10 Aseptic transfer of mother culture to intermediate culture container.

- 1 Sterile filter
- 2 Aseptic needle
- 3 Mother culture flask
- 4 Intermediate culture container



- Milk/culture
- Air
- Steam

Fig. 10.11 Aseptic transfer of intermediate culture to bulk starter tank.

- 1. Incubator
- 2. Intermediate culture container
- 3. Bulk starter tank
- 4. HEPA filter
- 5. Air valve
- 6. Steam filter
- 7. pH measurement unit

Factors Affecting Starter Propagation

- 1. Selection of milk:** Milk is a good medium for the propagation of starters because it gives better and milder flavour, better viscosity. The milk should be of high quality and first grade, milk of abnormal quality (mastitic, colostrums or late lactation milk) should not be used, free from antibiotic residues, free from bacteriophage, should not contain residues of detergents and sanitizers, low bacterial load, high SNF content, have clean flavour and odour. Since milk of above characteristics is difficult to obtain, NFDM (non fat dry milk) is used for the propagation of starters as it avoids daily variations. Flavour and odour defects are easy to detect if skim milk is used for the starter propagation. Skim milk with a total solids content of 10-11% is desirable for the propagation of starters.

- **Lactic acid bacteria cannot synthesize various vitamins and amino acids. Lactococci require niacin, pantothenic acid, pyridoxine, and biotin for growth. Although milk contains many of the essential amino acids for starter culture microorganisms, these are not present in sufficient quantity to sustain maximal growth rates.**
- **Ingredients commonly used to formulate any starter culture media are: Lactose is always used as the major carbohydrate, although low concentrations of maltose, sucrose, or glucose are sometimes added to stimulate growth. Yeast extract is a source of nitrogen as well as a supplier of vitamins, minerals, and other growth stimulants. Casein hydrolysates are added to provide readily available amino acids. Neutralizers, such as ammonium or potassium hydroxide, help prevent excessive acidity. Phosphates are commonly used in culture media, because they act both as acid-neutralizing and phage-inhibitory agents.**

2. Treatment of milk: Milk is usually subjected to heat treatment before using for the inoculation of starters.

The purpose of heat treatment is to:

- inactivate harmful organisms and bacteriophage in milk.
- inactivate the natural germicidal properties of milk (immunoglobulin, LP system, lactoferrin and lysozyme).
- reduce the oxidation reduction potential of milk by driving out the dissolved oxygen.
- denature the proteins to make available the nitrogenous compounds for the bacteria and also formation of -SH compounds which in small concentration act as stimulants for the growth of starters.
- improve the viscosity of the finished culture due to denaturation of whey proteins by improving their water retention properties to minimize the wheying off.

A heat treatment of milk to 90C for 1 h or boiling/steaming for 30 min is usually preferred. Autoclaving at 121C for 15 min is also followed but this may result in the formation of curd with soft and sloppy body and texture. It may also give rise to scorched flavour making the curd difficult to judge.

pH control during culture propagation is necessary as lactobacilli grow best under slightly acidic conditions, other starter culture microorganisms prefer conditions near neutrality. Eg., As the pH decreases below the optimal range, growth slows, and pH continues to decrease, cells become susceptible to sub-lethal acid injury and gradually lose their activity. The greater the loss of activity, the longer the ripening time required before rennet addition when making Cheddar cheese.

3. Inoculation: Inoculation is preferred with sterilized equipment. Inoculation has to be carried out in separate rooms with UV lamp designated for this purpose or by using laminar flow system. Freeze dried ampoule is sterilized with alcohol and liquid culture tubes are shown to flames. The amounts of inoculums depend on the activity of starter, temperature of incubation and time of incubation. Normally 0.5%-2.0% is used subject to variations depending upon the situation. Possibilities of external contamination are minimized as far as possible by working quickly.

4. Container/Utensils: Stainless steel, aluminium enameled wares and glass are the best material for starter propagation. Copper and copper alloys should be avoided. The form of starter vessel should be jar or cylindrical container free from crevices.

- 5. Incubation:** Incubation temperature depends on the amount of inoculum and on the type of starter culture. For the mesophilic organisms the temperature is 21-22C for 14-16 h and for the thermophilic organisms it is 41-43C for 3-4 h. Change in temperature may affect the composition of starters in mixed population.
- 6. Cooling of starters:** After incubation the culture is cooled to stop further development. Refrigeration appears to give appropriate cooling effect.

The drawbacks in traditional method of propagation:

1. Time consuming
2. Requires skilled operators
3. May lead to contamination by bacteriophage

Preservation of Starter Cultures

- Frozen concentrated cultures that can be used to inoculate directly milk from which product will be manufactured. Using these “direct-to-vat” or “direct-vat-set” cultures reduces cost as well as avoids the possibility that starter culture will become contaminated with phage during preparation within the plant.
- Culture can be prepared at the plant called as bulk culture, can be prepared from commercially available frozen concentrated or freeze-dried cultures, or the inoculum can be prepared at the plant. Preparing inoculum at the plant involves starting with a “mother” culture maintained in small amounts (approx. 100 mL) of medium. The mother culture is used to inoculate successively larger amounts of medium (using a 1% inoculum) until sufficient inoculum volume is obtained to prepare the bulk culture.
- Preparing bulk culture inoculum at the plant carries an increased risk of phage contamination, so most plants purchase an inoculum either as a frozen concentrated or freeze-dried preparation.

Frozen Concentrated Cultures: Contain 10^{10} – 10^{11} cfu/g, a sufficient concentration to allow 70 mL to inoculate 1000 L of medium for bulk culture preparation. Preparation of frozen concentrated cultures involves (1) growing cultures under optimal conditions using pH control, (2) harvesting the cells via centrifugation or ultrafiltration, (3) standardizing the cell suspension to a specific activity, (4) adding a cryoprotectant, (5) packaging (6) rapid freezing using liquid nitrogen. The pH of the cell concentrate should be 6.6 for lactococci and 5.4–5.8 for lactobacilli. Cryoprotective agents that can be used, including glycerol, monosodium glutamate, sucrose, and lactose. The frozen concentrate should be stored at -196°C (liquid nitrogen) for best retention of activity.

Freeze-Dried Cultures: Active freeze-dried cultures can be added directly to milk in the cheese vat. The major disadvantage is the longer lag phase they exhibit, adding an additional 30–60 min to the time required to make Cheddar cheese. Preparation of freeze-dried cultures is initially similar to that of frozen concentrates. After freezing, the culture concentrate is placed under high vacuum to dehydrate by sublimation. The dry cells are then packaged under aseptic conditions, preferably in the absence of oxygen.

Spray-Dried Cultures: Survival of cultures after spray drying is usually much lower than after freeze drying, because cells are simultaneously exposed to both thermal and dehydration stresses. The viability of spray-dried cultures depends on many factors including, growth conditions, age of culture, processing, rehydration conditions, and cryoprotectant used (Contd.)



•Milk

•Hydrolysis with trypsin for 4 h at 37 C

•Steaming of milk

•Cooling of milk

•Inoculation with starter culture

•Incubation at 20 C

•Evaporation at 27 C to 22% dry matter after cell concentration

•Spray drying at 70 C to 9% moisture

•Vacuum drying at 27 C to 5-6% moisture

Starter concentrates: The process consists of concentrating the starter culture bacteria grown under controlled conditions in a liquid medium into a smaller volume and then placing in frozen state (-196 C). The resulting cells retain all enzymes and biochemical activities required for bringing desirable fermentations in the product. Since higher number of bacterial cells is achieved in the starter concentrate, the efficiency of the starter is of very high order.

- a. Uniform product can be prepared.
- b. No incubation or sub culturing stages are required.
- c. Concentrates can eliminate the need for bulk starter facilities including tanks, laboratories etc.

Propagation of starter cultures by traditional method is a cumbersome process which involves a technical person to propagate the active and pure culture without any contamination.

Production systems for bulk starter cultures

The aim of production systems used for bulk starter cultures is to produce a pure active culture free from contamination, mainly from bacteriophage.

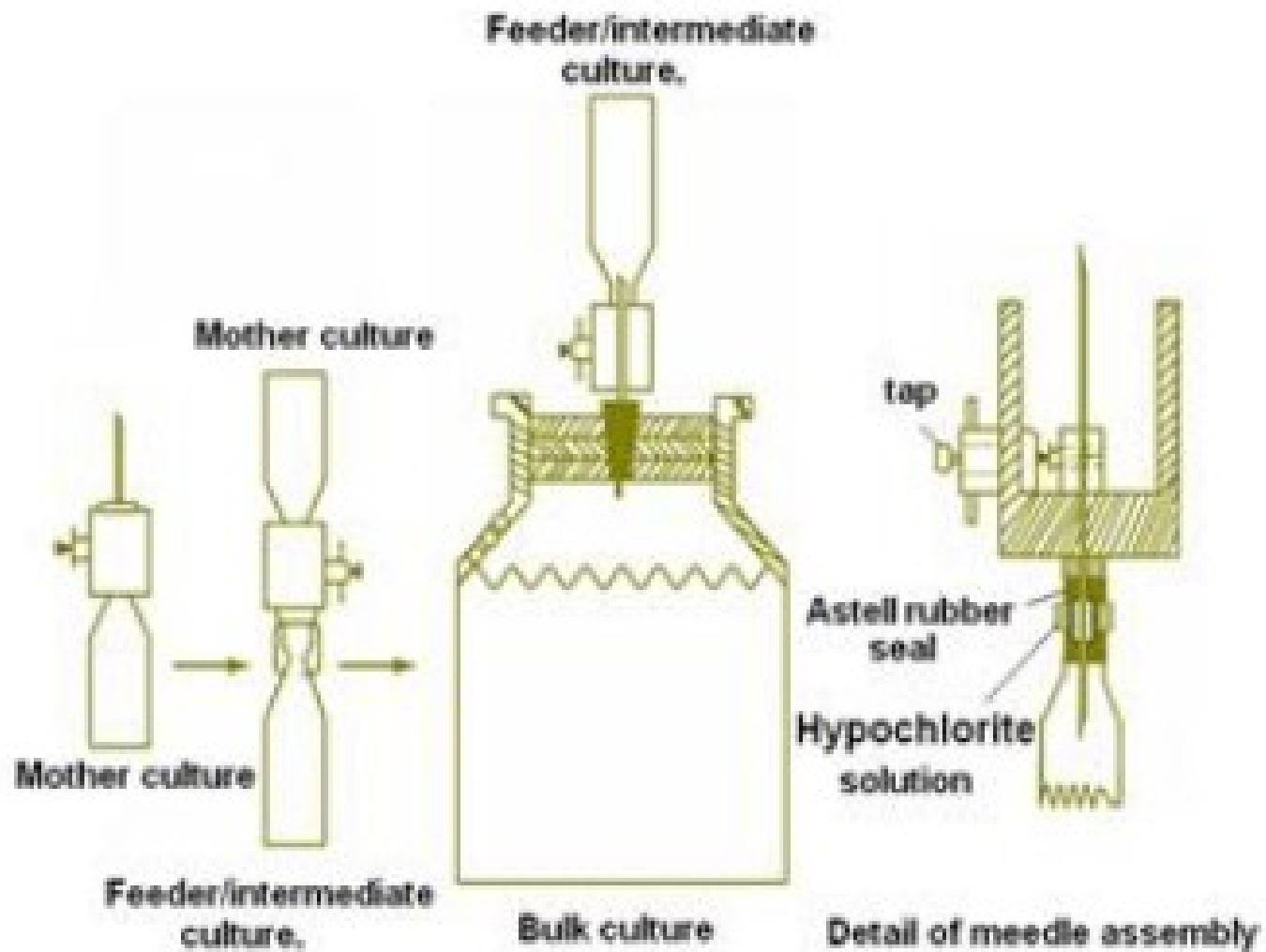
- 1. Simple microbiological technique**
- 2. Employment of mechanically protected equipment**
- 3. Propagation in Phage Resistant Medium /Phage Inhibitory Medium (PRM/PIM)**

Employment of mechanically protected equipment

Mechanically protected equipment has two aspects:

- a) Growth medium is heated and cooled to incubation temperature in a completely closed vat.
- b) Inoculation of the starter takes place through a barrier which prevents the entry of contaminated external air.

Lewis system: The technique consists of using reusable polythene bottles (115 and 850 g capacity) for mother and feeder cultures respectively. These bottles are fitted with Astell rubber seals, and the growth medium (i.e., 10-12g 100g⁻¹ reconstituted, antibiotic-free, skimmed milk powder) is sterilized in the same bottles. The starter culture transfers are carried out by means of two-way hypodermic needles. The Lewis system requires a pasteurized bulk starter tank so that the growth medium can be heat-treated inside the sealed vessel. During the heating or cooling of the milk, no air escapes from or enters the tank. The top of the tank is flooded with sodium hypochlorite solution (100mg/liter-I), so that the transfer of the starter inoculum (feeder) to the bulk starter medium is through a sterile barrier.



Tetra-Pak system:

- 1. In the Lewis system, the starter culture transfer from one container to another relies on squeezing the polythene bottle to eject the culture. In Tetra Pak system uses filter-sterilized air, under pressure, for transferring the culture.**
- 2. The mother and feeder intermediate cultures are prepared in a special unit called a “Viscubator,” and the capacity of these containers is 0.5 and 20 liters, respectively. The former unit is a glass bottle that is sealed with a rubber stopper and a metal screw-cap with an annular space, and the feeder container is a stainless steel canister which has two fittings: one is for compressed air, and the other is in the form of a length of stainless steel pipe that connects to the bulk starter tank during culture transfer.**
- 3. During culture transfer (i.e., from mother + feeder) two disposable sterile syringes are used: One is short and the other is long enough to reach the bottom of the bottle. Through the short syringe, compressed, sterile air causes a buildup of pressure in the head space of the bottle which displaces and forces the culture through the long needle to the feeder vessel.**

- 1. For the manufacture of starter cultures under aseptic conditions, Tetra Pak has developed a bulk starter tank of aseptic design.**
- 2. The tank is constructed using stainless steel, and it is capable of withstanding negative and positive pressures up to 30 and 100 kPa, respectively.**
- 3. It has an aseptic design (i.e., hermetically sealed and triple jacketed).**
- 4. The growth medium is heated in the tank, and the tank can be fitted with a stationary integrated pH meter that is designed to withstand the great temperature differences that occur between the heat treatment of the medium and growth or storage of the starter culture.**
- 5. The tank is fitted with high-efficiency particulate air (HEPA) filters that can be sterilized by steam at 140°C.**
- 6. A two-speed motor is used to operate the agitator of the tank.**

Propagation in Phage Resistant Medium /Phage Inhibitory Medium (PRM/PIM)

Phage resistant medium is used mostly for mesophilic starter cultures by using the ingredients like milk solids, sugar, stimulatory compounds (yeast extract, pancreatic extract), phosphate citrate buffer, chelating agents (ammonium or sodium phosphate). These chelating agents bind with Ca^{++} and Mg^{++} as these ions are required by the bacteriophage during the proliferation and replication. The bacteriophage also requires these ions for the phage adsorption onto the bacterial cell.

Ingredients Used in Formulating Bulk Starter Media for Lactic Acid Bacteria

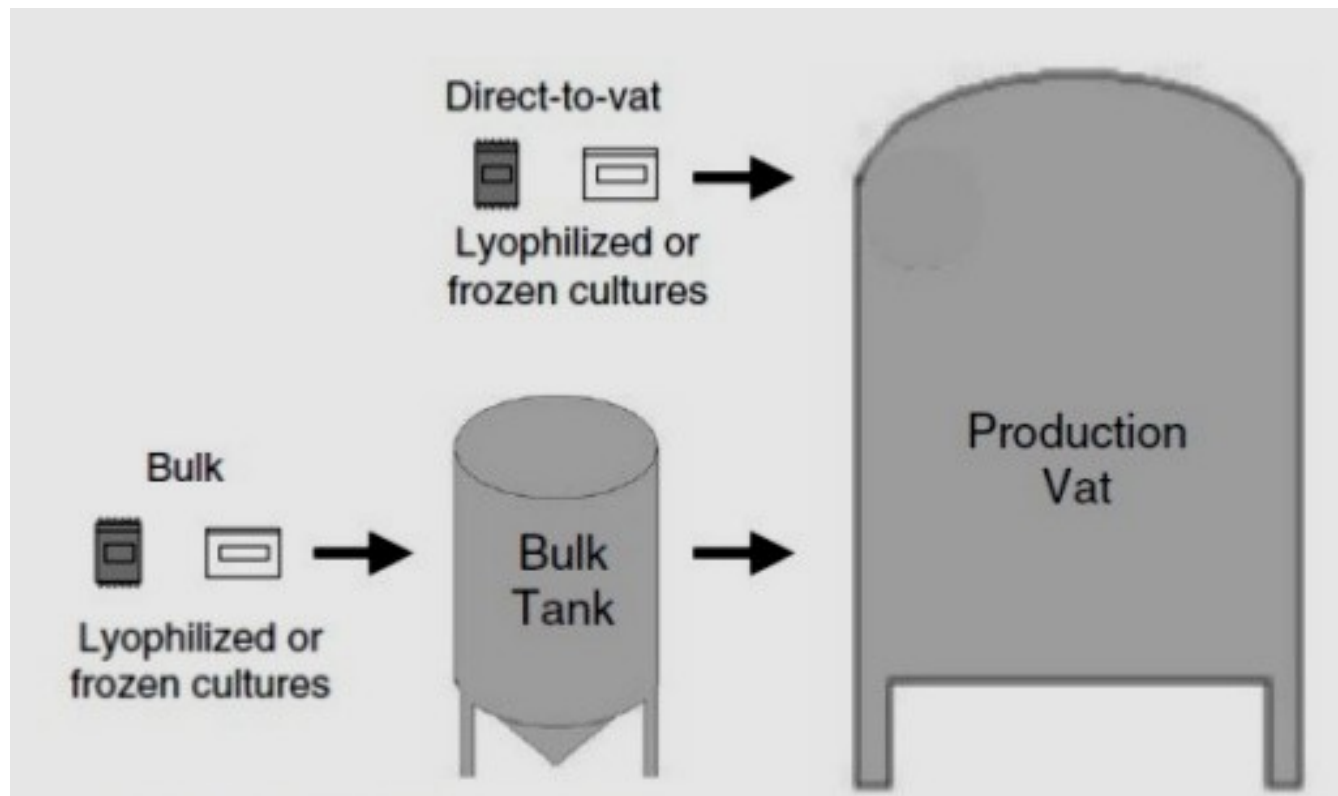
Carbohydrate	Nitrogen source	Vitamins and minerals	Phage-inhibitory agents	Antioxidants	Neutralizers
Lactose	Milk protein	Yeast extract	Phosphates	Ascorbic acid	Carbonates
Maltose	Whey protein	Corn steep liquor	Citrates	FeSO_4	Phosphates
Sucrose	Hydrolyzed casein				Hydroxides
Glucose					Oxides

Starter Systems

Bulk set: The supplier provides the user with a small amount of culture which is then fermented by the user through one or two stages to produce a volume of bulk starter. Typically fermentation takes place at 22-26 C for 14-16 h for mesophilic and at 43 C for 4.5-5 h for thermophilic starter. A significant portion of this time is taken up by the lag phase as cells repair themselves from their storage mode. This starter then may be used immediately by inoculating into production milk (1% for cheddar, 3% for yoghurt).

Direct vat inoculation: This is made available in more concentrated form in large volumes such that the product can be inoculated directly into the production milk. In both cases inoculation into pasteurized medium ensures an active starter dominating the fermentation.

DVS cultures (Direct Vat Set cultures) are freeze dried bacterial cultures that can be directly added to the milk. Such cultures are not reusable. One used, they are no longer useful for the processing. DVS cultures are also called Direct Vat Inoculate (DVI). In order to produce cultured dairy product, DVS cultures need to be added directly to the milk for production in vat.



Bulk vs Direct-to-vat culture preparation

Main starter operating systems for controlling the bacteriophages

Rotational: Culture rotations control bacteriophagic infection by limiting the length of time that a specific strain or mixture of strains is used. Cultures following each other in the series are susceptible to different phage types and are therefore unaffected by phages that may have infected the previous culture. Cultures can be rotated on a daily basis or after each vat of milk is inoculated. Short rotations over 2–3 days using 6–12 strains and long (5–10 days) rotations of up to 30 strains are used. However, the use of a limited number of cultures at any one time is recommended to reduce exposure to phages and maintain product uniformity.

Non-rotational: This system uses genetic derivatives of a single strain, each with a different phage-resistance mechanism. When used in rotation or as mixtures, resistant phages fail to develop, because they cannot overcome the multiple resistance mechanisms. This avoids the lack of product uniformity associated with conventional culture rotations and allows continuous use of strains with special properties. When the phage levels are likely to affect acid production (10^5 or above detected), the strain is removed and replaced by a suitable alternative.

Dutch P/L system: It is based on the proposition that if mixed cultures are transferred in an environment which deliberately exposes them to bacteriophage and the resulting mix of cultures may include new phage resistant mutants and this will help operate satisfactorily in the production environment.

For Effective Starter Systems...

1. Recognize the physical as well as human constraints.
2. Ensure all staff involved understands the bacteriophage, its seriousness and the part they can play in limiting its effects.
3. Ensure that the movements of air, water, people and product are known and their role as potential channels for phage attack.
4. Consider the role of HACCP.
5. Be obsessive in hygiene and production disciplines associated with starter production and usage.
6. Institute prospective phage monitoring by daily whey testing.