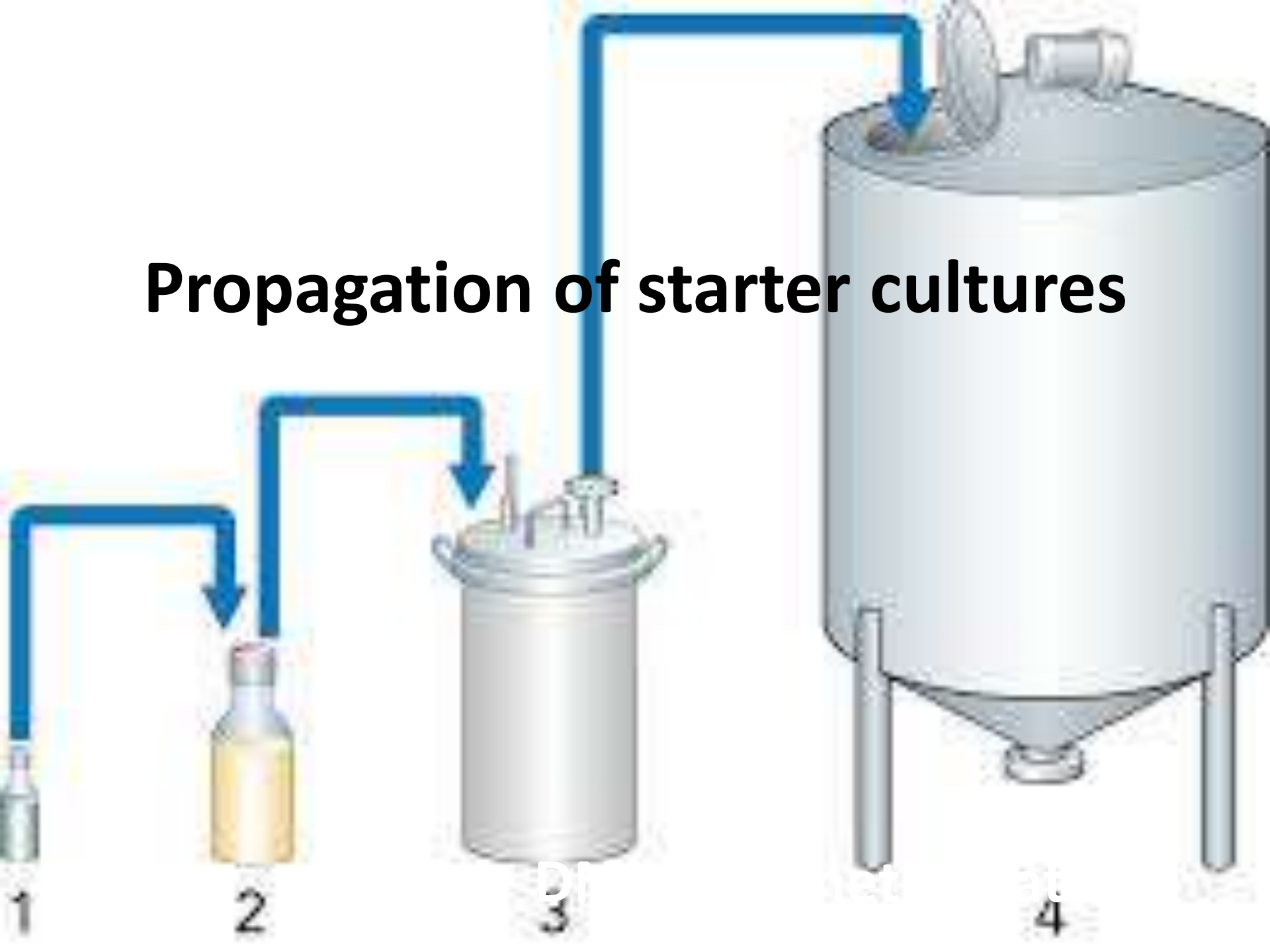


# Propagation of starter cultures



## **What is Starter Propagation????**

- **Starter propagation is the most important operation of the quality control unit of dairy plant.**
- **Main aim of propagation is to maintain pure cultures and activate cultures without any loss of viability .**
- **Fermentation process of any cultured dairy product relies on 'purity' and 'activity' of starter culture.**
- **Starter must be active, contain maximum number of viable organisms and free from contaminants.**

## Traditional Methods

- In traditional method, starter are propagated in the laboratory itself taking the stock cultures which are in inactive 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.
- Dairy product manufacturers inoculate culture into raw/pasteurized milk or other suitable substrate.



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

**Stock culture**

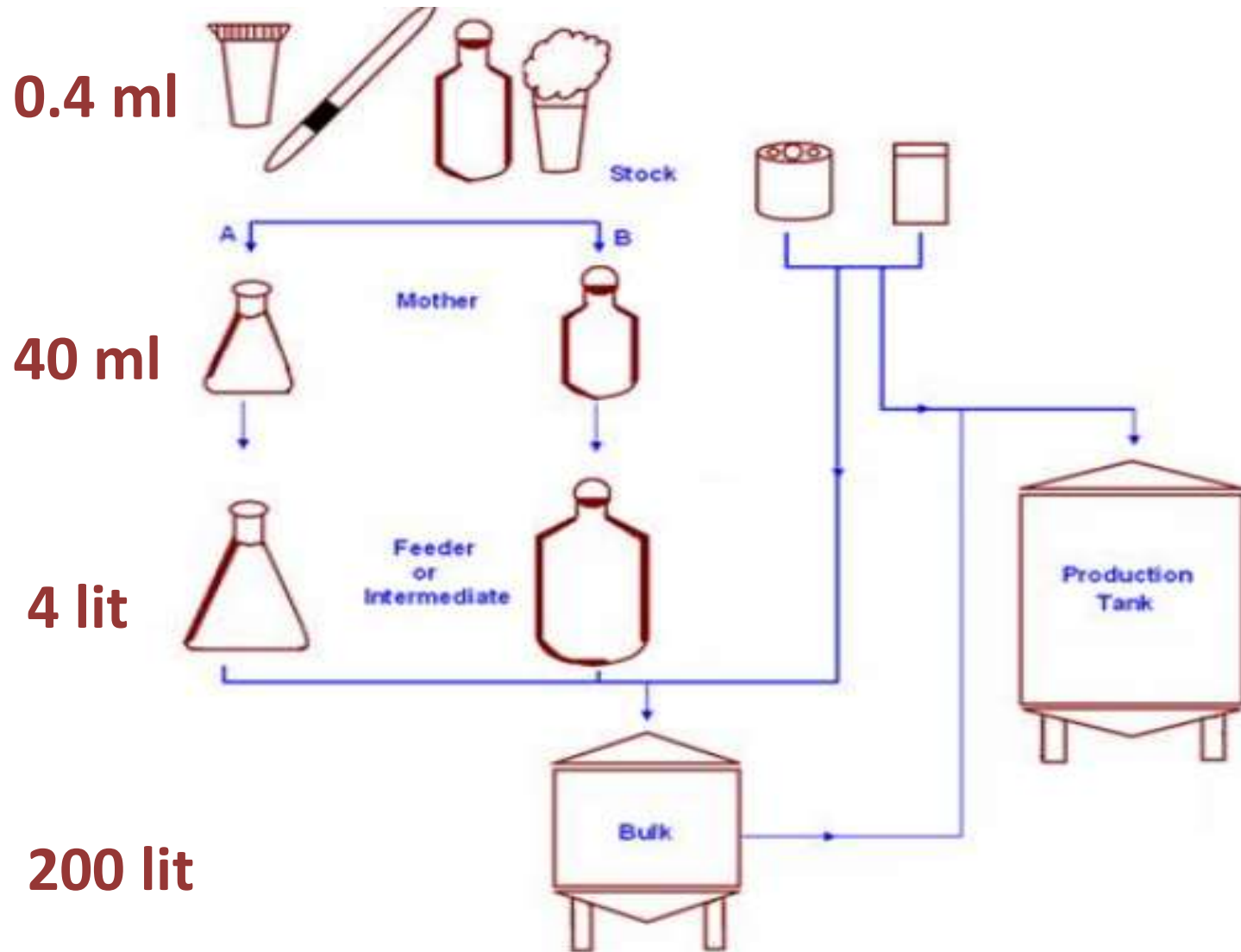
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graph TD; A[Stock culture] --> B[Mother culture (first inoculation): all cultures will originate from this preparation]; B --> C[Intermediate culture (feeder) is used in preparation of larger volumes of prepared starter]; C --> D[Bulk starter culture - this stage is used in dairy product production];
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**Mother culture (first inoculation): all cultures will originate from this preparation**

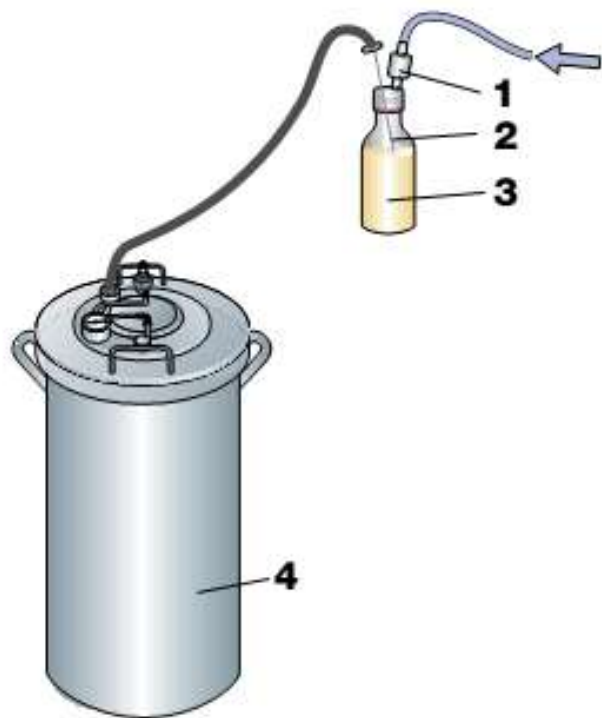
**Intermediate culture (feeder) is used in preparation of larger volumes of prepared starter**

**Bulk starter culture - this stage is used in dairy product production**

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

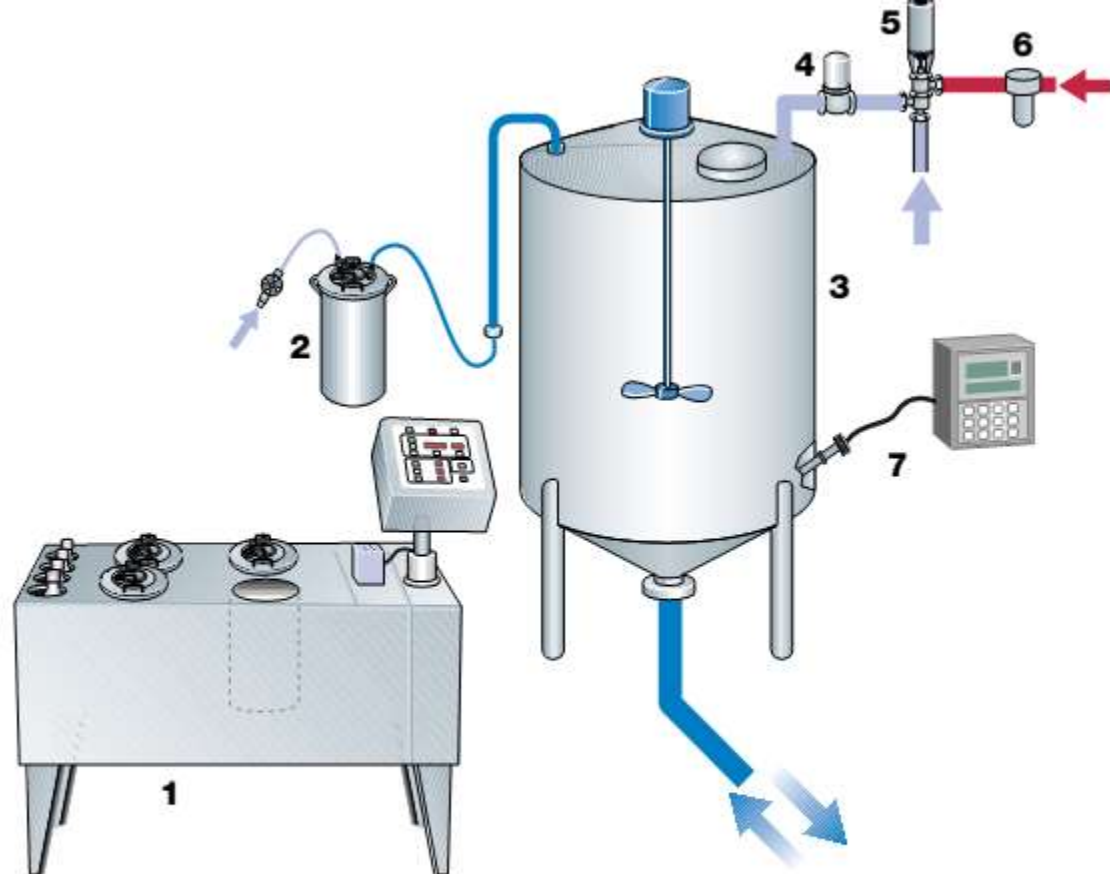


- 1. Mother culture is traditionally prepared in 40 ml bottles provided with a cap containing membrane.**
- 2. Bottle is filled with skim milk, autoclaved and then cooled to appropriate inoculation temperature.**
- 3. Master culture is injected into bottle of mother culture with a sterilized syringe inserted through the membrane.**
- 4. After suitable period of incubation and subsequent cooling, mother culture is inoculated into skim milk, used for intermediate culture; milk is first heated to 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, intermediate culture is transferred by displacement with filtered air through a tube to the bulk starter tank.**
- 7. Before inoculation, skim milk is heated by circulation of heating medium and coolant through the tank jacket.**



**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 propagation of starters because it gives better and milder flavour, better viscosity.
- Milk should be of high quality and first grade, abnormal quality (mastitic, colostrums or late lactation milk) should be avoided, free from antibiotic residues and bacteriophage, absence of residues of detergents and sanitizers, low bacterial load, have clean flavour and odour.
- NFDM (non fat dry milk) is used for the propagation of starters as it avoids daily variations.
- Skim milk (Total solids content of 10-11%) is desirable for the propagation of starters.

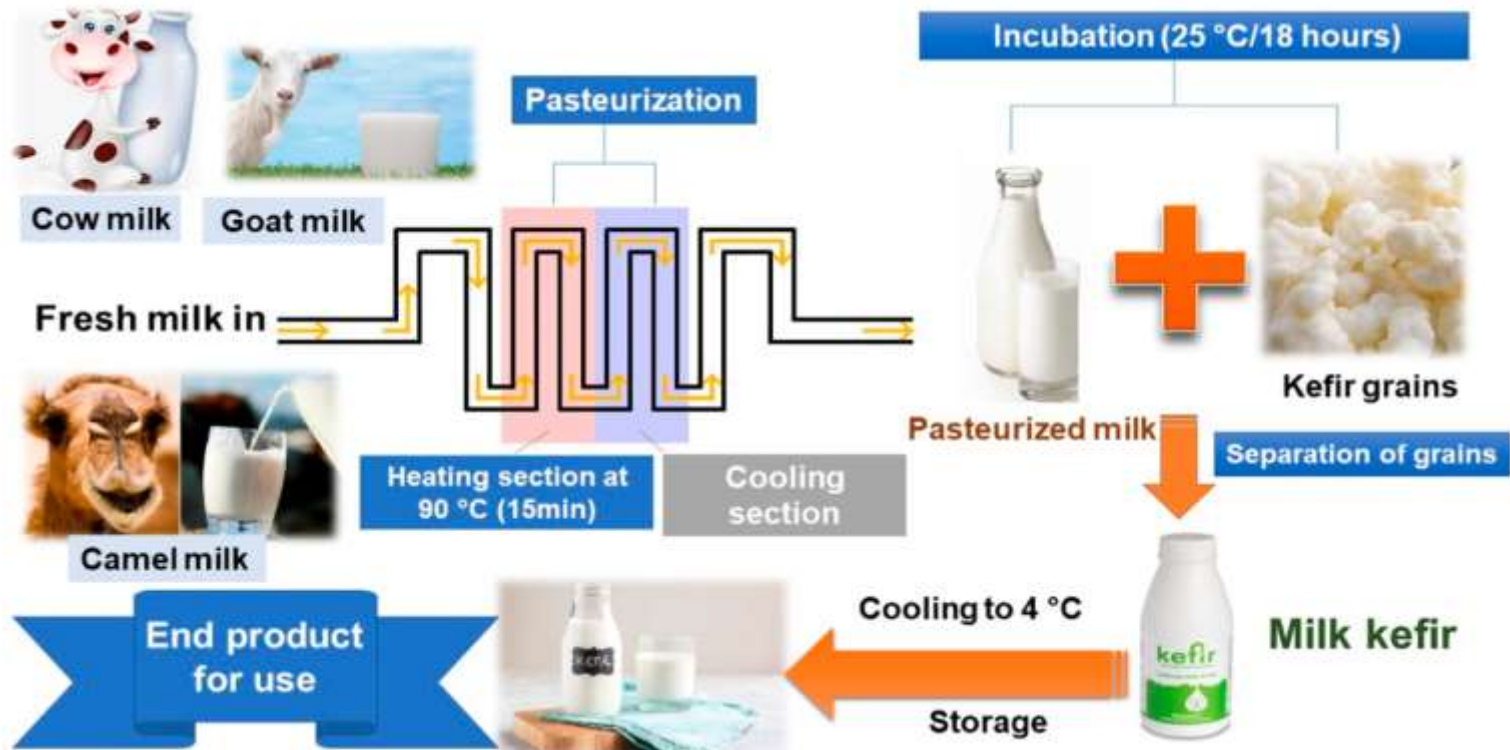


- **Lactococci require niacin, pantothenic acid, pyridoxine, and biotin for growth. Milk do not contain sufficient quantity of essential amino acids to sustain maximal growth rates.**
- **To formulate any starter culture media are: **Lactose** is major carbohydrate, **Yeast extract** is a source of nitrogen and supplier of vitamins and minerals. **Casein hydrolysates** provide readily available amino acids. **Neutralizers** (ammonium or potassium hydroxide) help prevent excessive acidity. **Phosphates** act both as acid-neutralizing and phage-inhibitory agents.**

## **2. Treatment of milk: Purpose of heat treatment is to:**

- inactivate harmful organisms and bacteriophage in milk.**
- inactivate natural germicidal properties of milk (immunoglobulin, LP system, lactoferrin and lysozyme).**
- reduce oxidation reduction potential of milk by driving out the dissolved oxygen.**
- denature proteins to make available the nitrogenous compounds for the starter bacteria.**

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



### **3. Inoculation:**

- Inoculation is preferred with sterilized equipment.**
- Inoculation to be carried out in separate rooms with UV lamp or by using laminar flow system.**
- Freeze dried ampoule is sterilized with alcohol and liquid culture tubes are shown to flames.**
- Amount of inoculums (normally 0.5-2.0%) depend on activity of starter, temperature and time of incubation.**

### **4. Container/Utensils:**

- Stainless steel, aluminium enameled wares and glass are best material for starter propagation.**
- Copper and copper alloys should be avoided.**
- Starter vessel should be jar or cylindrical container free from crevices.**

## **5. Incubation:**

- **Incubation temperature depends on amount of inoculum and on the type of starter culture.**
- **For mesophiles, temperature is 21-22 C for 14-16 h and for thermophiles, 41-43 C for 3-4 h.**

## **6. Cooling of starters:**

- **After incubation the culture is cooled to stop further development through refrigeration.**

## **Drawbacks in traditional method of propagation:**

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

# Preservation of Starter Cultures

1. 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.
2. 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. Mother culture is used to inoculate successively larger amounts of medium (using 1% inoculum) until sufficient inoculum volume is obtained to prepare the bulk culture.
3. Preparing bulk culture inoculum at 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:**

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

**Freeze-Dried Cultures:** 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 (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**

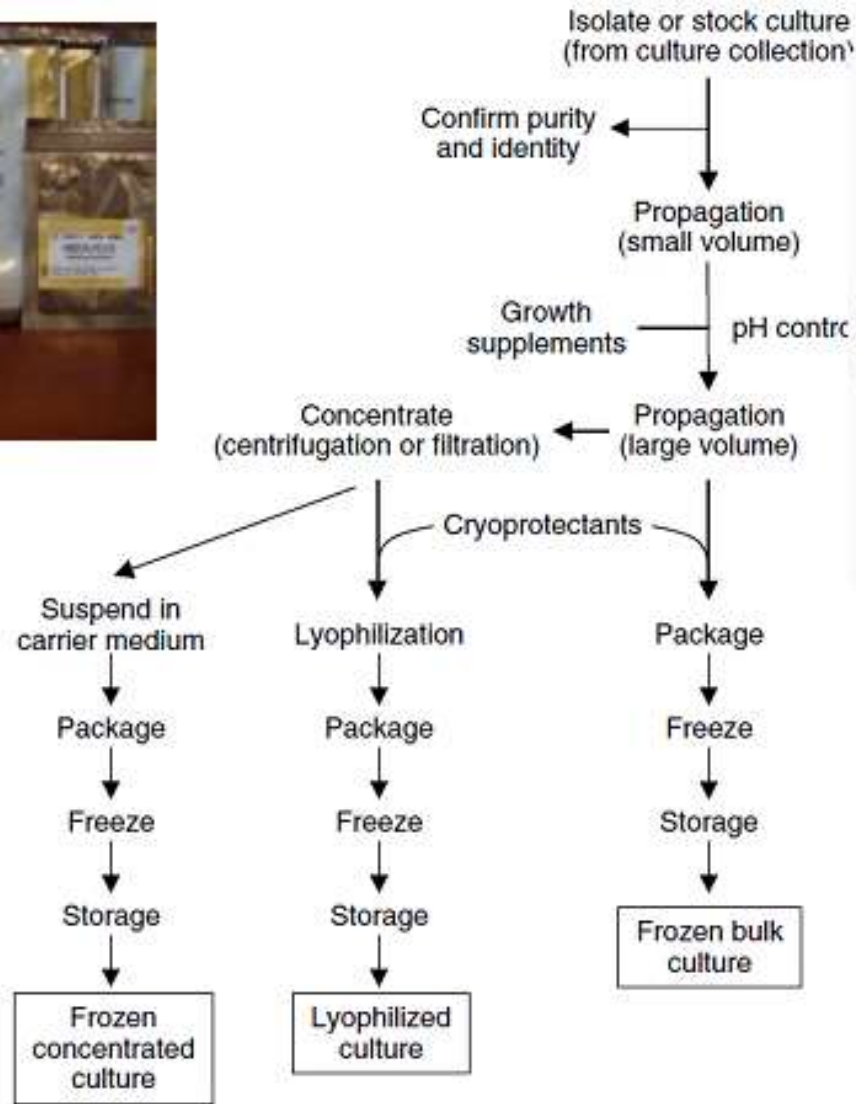
- **Evaporation at 27 C to 22% dry matter after cell concentration**

- **Spray drying at 70 C to 9% moisture**

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

## **Starter concentrates:**

- 1. 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).**
- 2. Cells retain all enzymes and biochemical activities required for bringing desirable fermentations in product.**
- 3. Higher number of bacterial cells is achieved in the starter concentrate, efficiency of starter is of very high order.**
  - a. Uniform product can be prepared.**
  - b. No incubation or sub culturing stages are required.**



## Production of industrial starter cultures

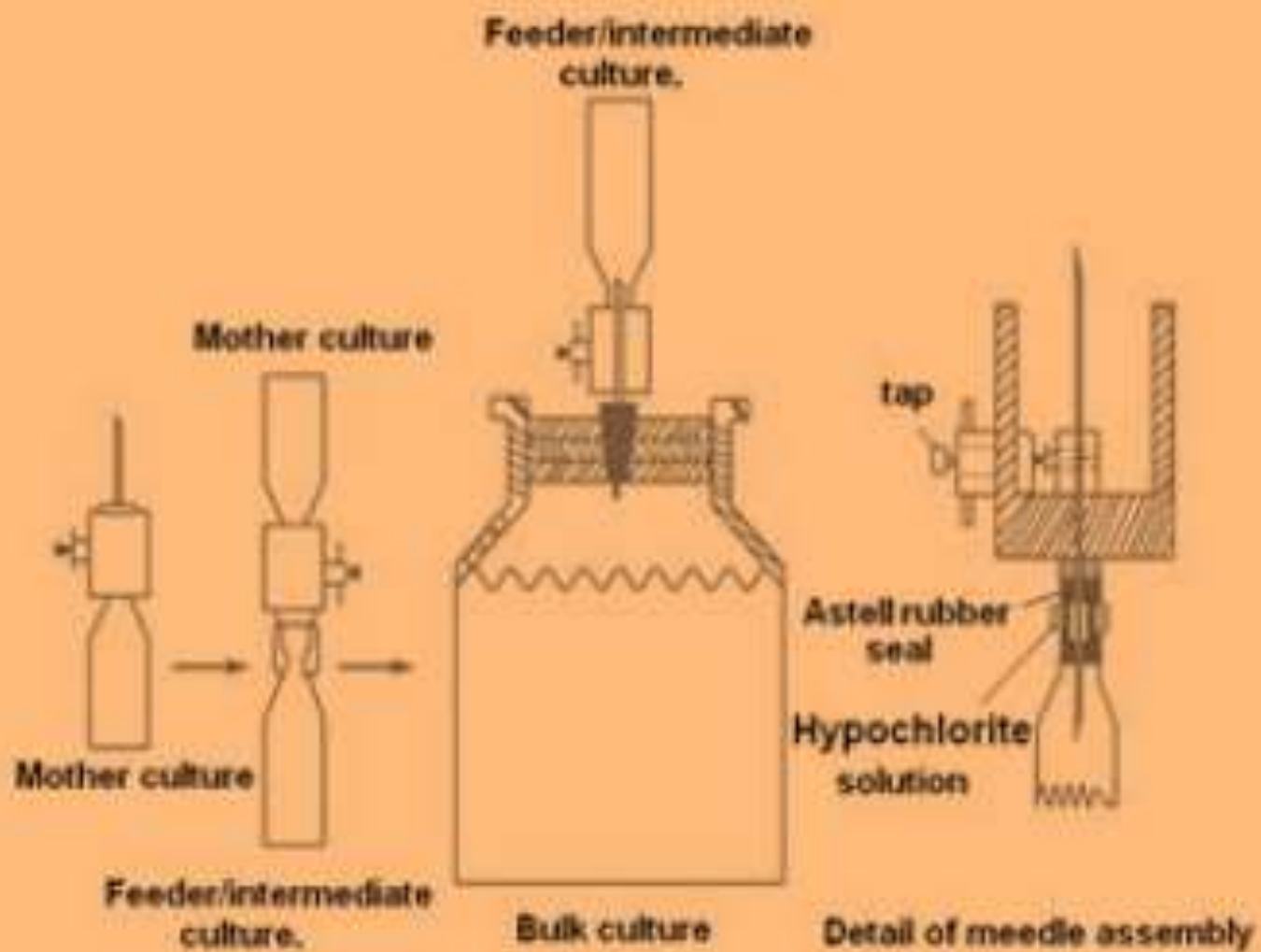
## **Employment of mechanically protected equipment**

**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:**

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



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

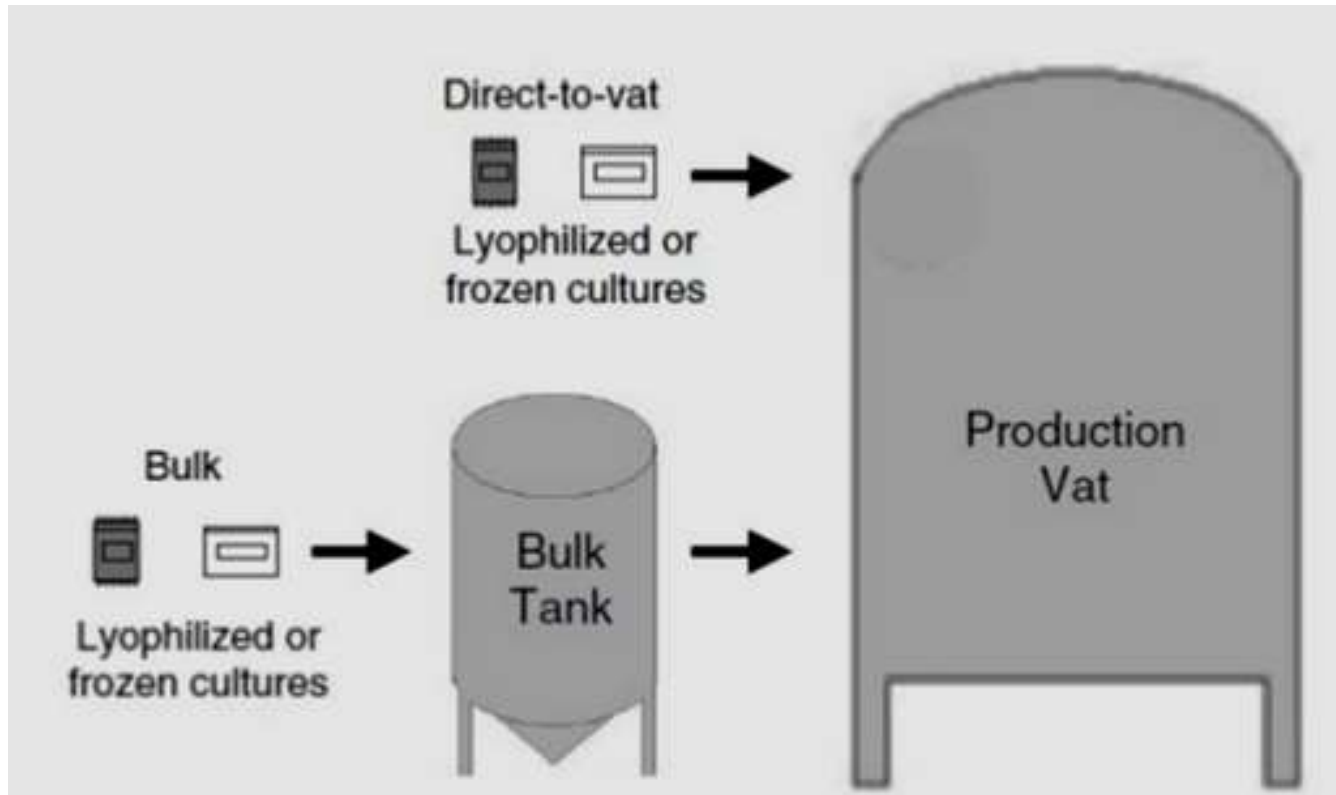
- ✓ Phage resistant medium is used mostly for mesophilic starter cultures by using milk solids, sugar, stimulatory compounds (yeast extract, pancreatic extract), phosphate citrate buffer, chelating agents (ammonium or sodium phosphate).
- ✓ Chelating agents bind with  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  as these ions are required by bacteriophage during proliferation and replication as well as for 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	$\text{FeSO}_4$	Phosphates
Sucrose	Hydrolyzed casein				Hydroxides
Glucose					Oxides

# Starter Systems

**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**