Batch Cultures

Clip slide

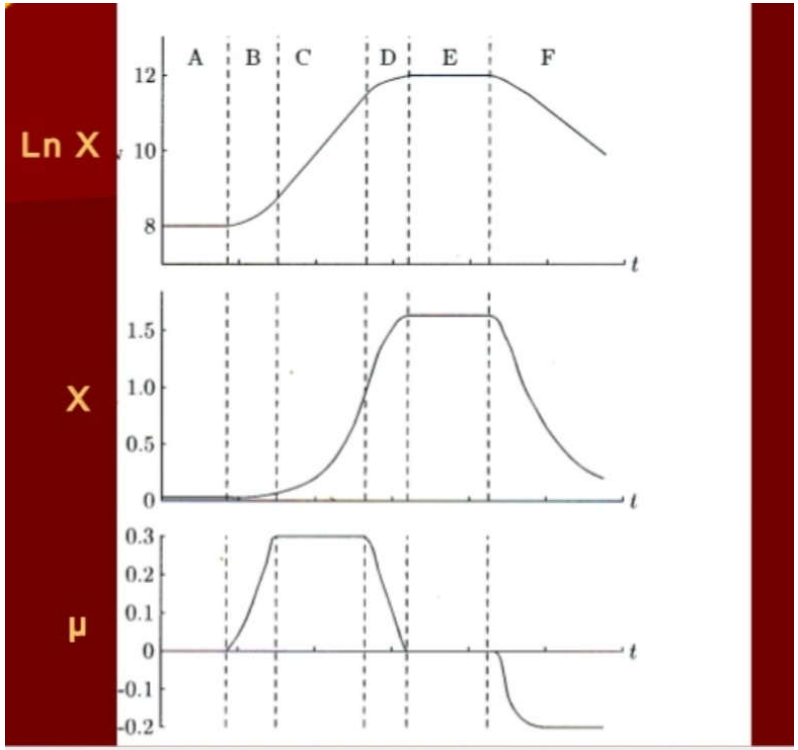
- In a batch operation, all necessary medium components and the inoculum are added at the beginning and not during period of fermentation.
- Therefore, their concentrations are not controlled but are allowed to vary as the living cells take them up.
- The products, be they intra- or extracellular, are harvested only at the end of the run.
- Basic controls for pH, temperature, dissolved oxygen, and foam are applied during the course of batch culture.
- The pH, dissolved oxygen, and temperature are normally held constant during the course of batch reactor operation.
- The only optimization parameters are the initial medium composition.
- However, profile optimizations of temperature and pH may lead to improved performance over the operations carried out at constant temperature and constant pH.

- Batch cultivation is closed system where there is no interaction between the system and the surrounding during the process. Except air during the aerobic cultivation.
- In Batch cultivation we prepare medium, sterilize it and inoculate the culture into the bioreactor.
- Allow the cells to grow and produce the product.
- Once the product formation reaches maximum harvest the fermentation broth.

How cells grow during Batch cultivation

After inoculating the medium and start measuring the biomass at different time intervals, you may find six different phases. They are

1. Lag phase
2. Accelerated growth phase
3. Exponential growth phase
4. Decelerated growth phase
5. Stationary phase
6. Death phase



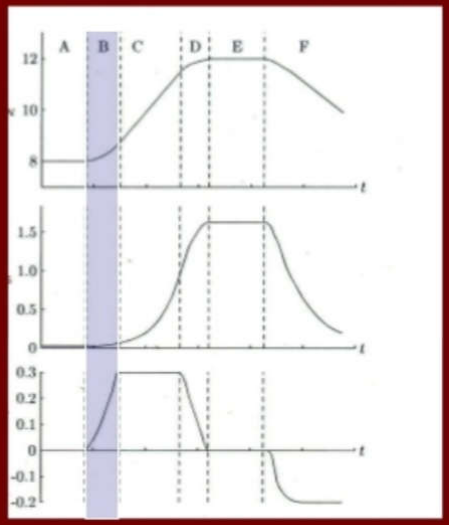
How to avoid lag phase

Lag phase is non productive period in the industrial fermentations. Hence minimizing it is essential.

- **The stage of culture from where the inoculum is drawn is important. Exponentially growing cells will have adequate concentrations of intermediates and intracellular pool of compounds. Hence if the inoculum is drawn from this stage they will not suffer dilution effect.**
- Size of the inoculum – If the size of the inoculum is large then the lag phase can be minimized. Generally 10% are used for yeast and mold and 5% for bacteria.
- Medium of inoculum should be same of that production medium.
- In certain cases such as recombinant *E.coli* cultivation to minimize plasmid loss higher percentage of inoculum will be used.

Accelerated growth phase

- At the end of Lag phase, when growth begins the division rate increase gradually and reaches a maximum value.
- The sp growth rate increases to maximum during this phase.



Exponential growth phase

- Cell division occurs in this phase.
- Often cell dry weight is used for cell concentration. During exponential phase we write as

$$\frac{dX}{dt} = \mu X$$

Where μ - Specific growth rate

X- cell dry weight

Rearranging and integrating the eqn

$$\int_{X_o}^{X} \frac{dX}{X} = \mu \int_{0}^{t} dt$$

$$\ln X \Big|_{X_o}^{X} = \mu t \Big|_{0}^{t}$$

$$\ln\left(\frac{X}{X_o}\right) = \mu t$$

$$X = X_o e^{\mu t}$$

Other phases of growth

- The end of the exponential phase occur when any of the essential nutrients is depleted or toxic metabolite accumulated in the system. During this phase the growth rate declines.
- Stationary phase will follow this phase. The length of stationary phase may vary with cell type, previous growth conditions etc., In certain cases the product formation will occur during this phase
- Following this is the death phase where the cells will start to lyse and the cell density decreases.

5.7. GROWTH YIELDS

When microbial growth is limited by low concentration of required nutrient, the final net growth or yield of cells increases with the initial amount of the limiting nutrient present. The rate of growth also increases with nutrient concentration but in a hyperbolic manner. The shape of the curve reflects the rate of nutrient uptake by microbial transport proteins.

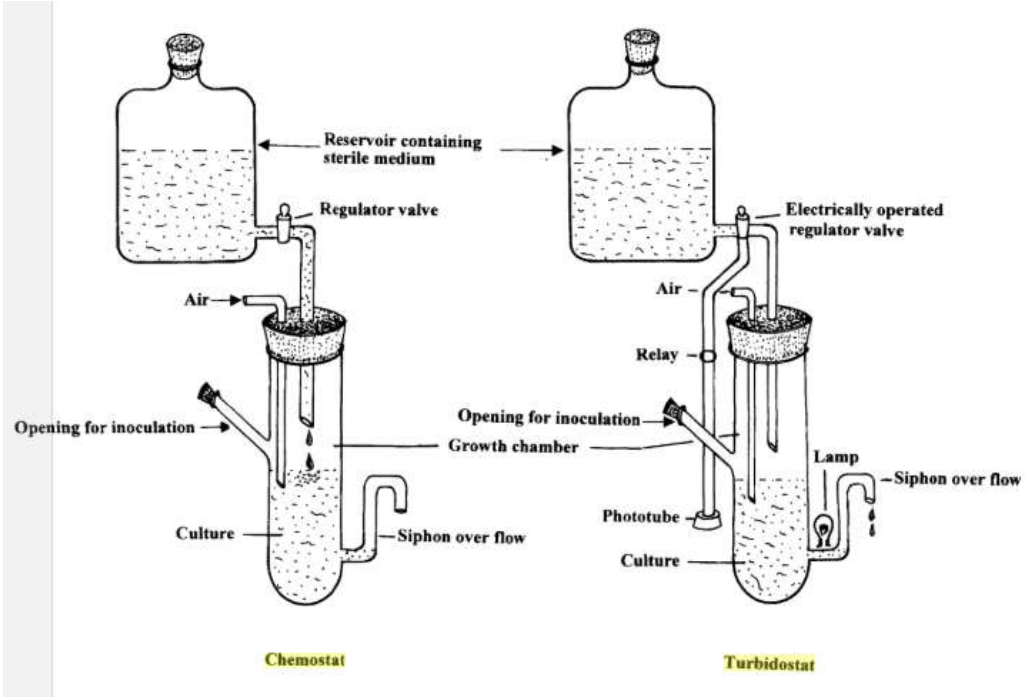
When the nutrient quantity limits the production of bacteria, it is possible to define a growth yield constant (Y). The growth yield constant is the amount of dry weight of cells produced per weight of nutrient used

$$Y = \frac{\text{mass of microorganism formed}}{\text{mass of substrate consumed}}$$

The molar growth yield constant is Y_m, which is the dry weight of the cells produced (in grams) per mole of substrate used. For example, the $Y_{glucose}$ for aerobically growing cells is about 0.5, which means about 50% of the sugar is converted to cell material and 50% oxidised to CO_2. For certain sugars and bacteria, the efficiency of conversion of cell material can be much lower (e.g, 20%). In dilute media, some bacteria are able to increase their efficiency and assimilate upto 80% of the sugar acquired.

Continuous Cultures

- In a continuous operation, one or more feed streams containing the necessary nutrients are fed continuously, while the effluent stream containing the cells, products, and residuals is continuously removed.
- A steady state is established by maintaining an equal volumetric flow rate for the feed and effluent streams.
- In so doing, the culture volume is kept constant, and all nutrient concentrations remain at constant steady state values.
- Continuous reactor operations are common in chemical industries. With the exception of single-cell protein production, certain beer production, and municipal waste treatment processes, continuous cultures have not been adopted widely by industry.
- It is not a dominant mode of industrial operation primarily because of the difficulty in maintaining sterility (contamination by other organisms) and protecting against phage attacks or mutations and because often, steady state operations are found to yield poorer results than dynamic operations, for reasons not yet fully understood.



CONTINUOUS GROWTH KINETICS

- The actual growth rate depends not only on the volumetric flow rate of the medium into the reactor, but also on the dilution rate(D)

$$D = F/V$$

The net change in the cell concentration over a period of time may be expressed as:

dX/dt = rate of growth in reactor – rate o loss from reactor(μX-Dx)

Under steady state conditions, the rate of growth = rate of loss

$$dX/dt = 0$$

Therefore, $\mu X = DX$ & $\mu = D$

- For any given dilution rate, under steady state conditions, the residual substrate concentration in the reactor can be predicted by substituting D for μ in the Monod equation

$$D = \mu_{max} S_r / K_s + S_r$$

where S_r = steady state residual substrate concentration in the reactor at the fixed dilution rate. Rearrangement gives,

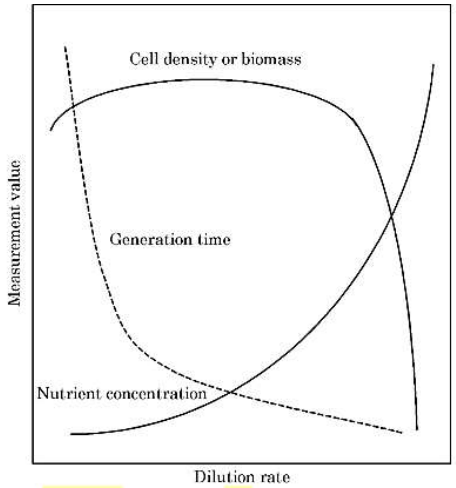
$$D(K_s + S_r) = \mu_{max} S_r \text{ or } DK_s + DS_r = \mu_{max} S_r$$

Dividing by S gives,

$$DK_s / S_r + D = \mu_{max}$$

hence,

$$S_r = DK_s / \mu_{max} - D$$

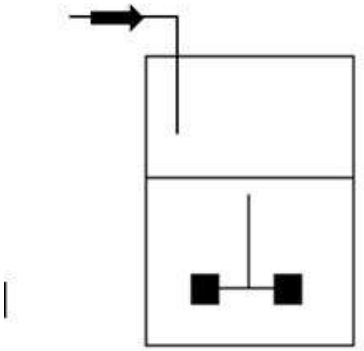


BATCH CULTIVATION	CONTINUOUS CULTIVATION
1. The bacteria are inoculated into the bioreactor (always stirred tank bioreactor). 2. Then, under certain conditions (temperature, pH, aeration, etc.) the bacteria go through all the growth phases (lag, exponential, stationary).	1. The fresh medium flows into the fermentor continuously, and part of the medium in the reactor is withdrawn from the fermenter at the same flow rate of the inlet flow. 2. The bacteria is grown under certain conditions (temperature, pH, aeration)
Advantages: • can be used for diff reactions every day. • Safe: can be properly sterilized. • Little risk of infection or strain mutation • Complete conversion of substrate is possible	Advantages: • Works all the time: low labor cost, good utilization of reactor • Often efficient: due to the autocatalytic nature of microbial reactions,. • the productivity can be high. • Automation may be very appealing. • Constant product quality
Dis-advantages: •High labor cost •Much idle time – Sterilization, growth, cleaning •Safety – filling emptying, cleaning.	Dis-advantages: •promised continuous production for months fails due to a. infection. b. spontaneous mutation of microorganisms to non producing strain

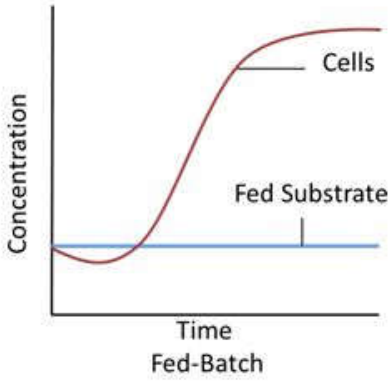
Fed-Batch Cultures

Clip slide

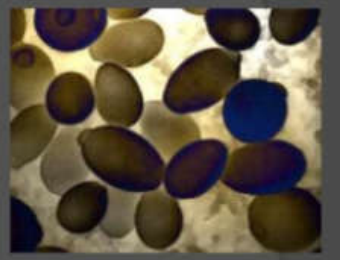
- A fed-batch culture is a semi-batch operation in which the nutrients necessary for cell growth and product formation are fed either intermittently or continuously via one or more feed streams during the course of an otherwise batch operation.
- The culture broth is harvested usually only at the end of the operational period, either fully or partially (the remainder serving as the inoculum for the next repeated run).
- This process may be repeated (repeated fed-batch) a number of times if the cells are fully viable and productive.
- Thus, there are one or more feed streams but no effluent during the course of operation.
- Sources of carbon, nitrogen, phosphates, nutrients, precursors, or inducers are fed either intermittently or continuously into the culture by manipulating the feed rates during the run. The products are harvested only at the end of the run.
- Therefore, the culture volume increases during the course of operation until the volume is full.
- Thereafter, a batch mode of operation is used to attain the final results. Thus, the fed-batch culture is a dynamic operation.
- By manipulating the feed rates, the concentrations of limiting nutrients in the culture can be manipulated either to remain at a constant level or to follow a predetermined optimal profile until the culture volume reaches the maximum, and then a batch mode is used to provide a final touch.
- In so doing, the concentration of the desired product or the yield of product at the end of the run is maximized. This type of operation was first called a *fed-batch culture* or *fed-batch fermentation*.



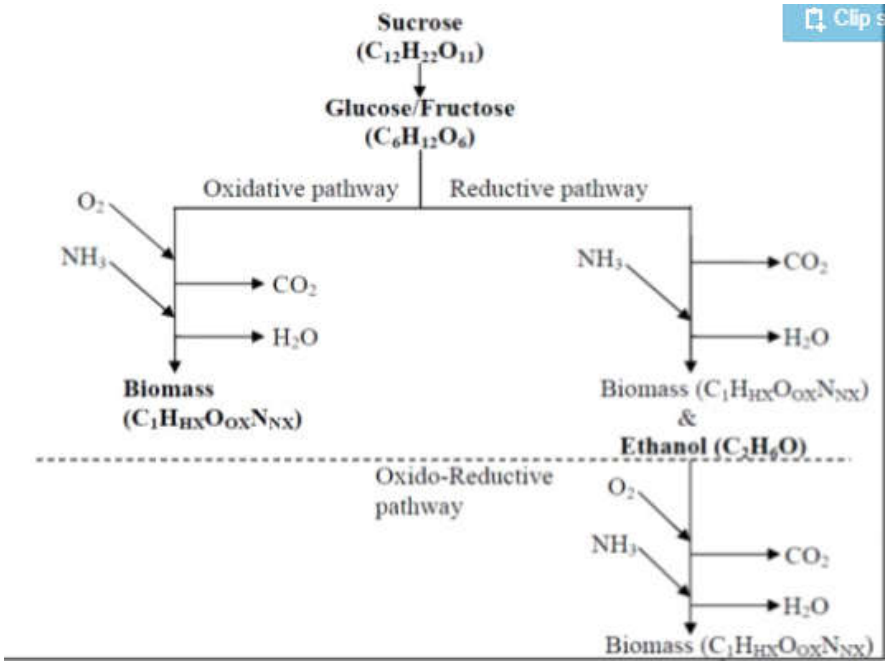
Batch



- *Marketed in the form of cake, powder or cream*
- *By-products are not required so --- directed towards max. biomass production*
- *Saccharomyces cerevisiae*
  - *Most commonly used organism*
  - *Unicellular*
  - *Rich in protein & vitamin B*
  - *Budding*
  - *Enzymes*
    - *Maltase; converts maltose to glucose*
    - *Invertase; sucrose to glucose & fructose*
    - *Zymase complex; sugars to CO_2 & ethanol*



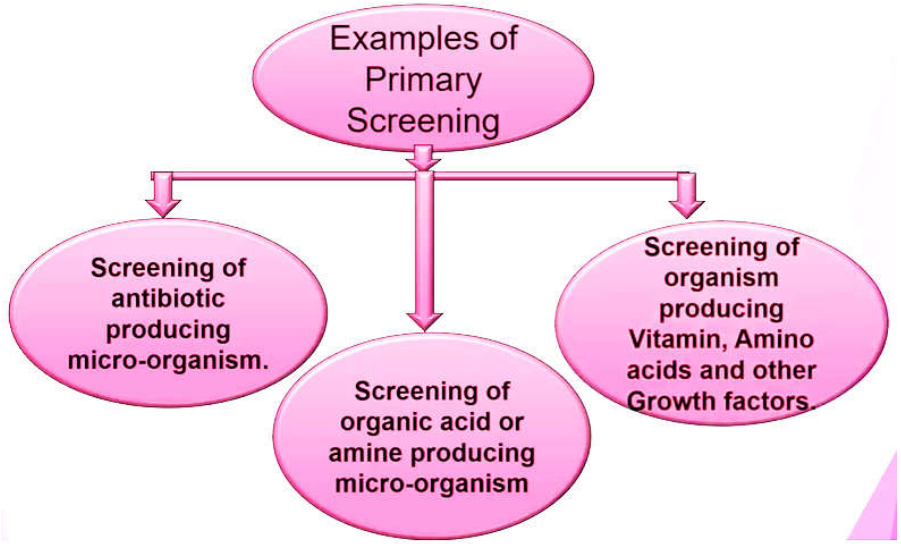
- Process Biochemistry
- Grow either in the absence or presence of O2
- Grows efficiently… O2 present
- Grows inefficiently… O2 not present
  - Produces ethanol in large quantity
- Fed-batch is best method
  - Incremntal feeding & high aeration



Screening allows the discarding of many valueless microorganisms, at the same time it allows the easy detection of the useful microorganisms that are present in the population in very less number

PRIMARY SCREENING TECHNIQUE

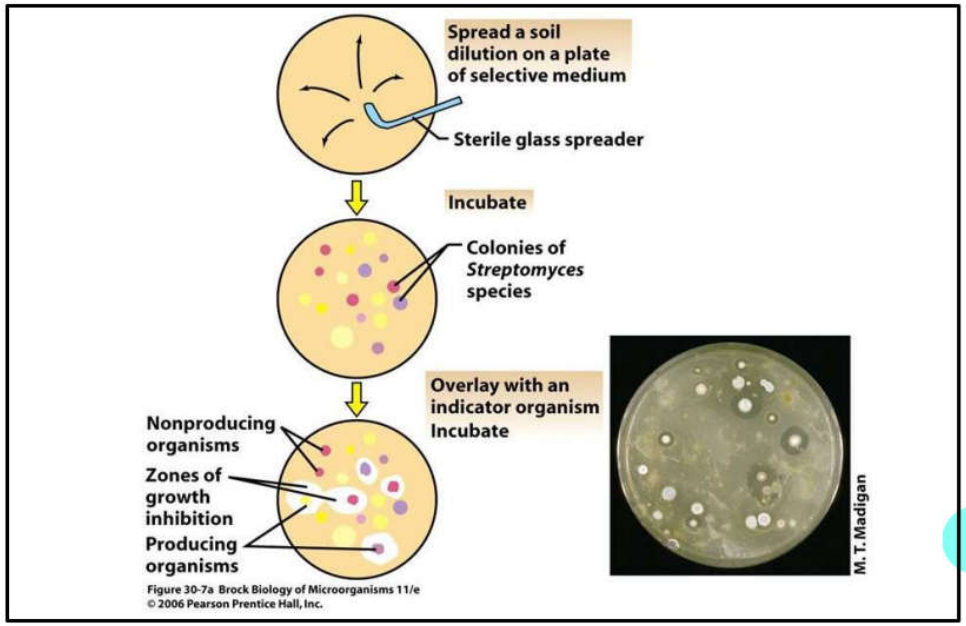
- Detection and isolation of industrially important micro-organism from mixed population using simple techniques is called as primary screening techniques.
- In primary screening technique simple methods are used to detect valuable micro-organism based on certain biochemical characters of microbes.
- Generally the sample is collected, serially diluted and isolated on suitable agar medium by specific technique.



2) PRIMARY SCREENING OF ANTIBIOTIC PRODUCING MICROORGANISMS

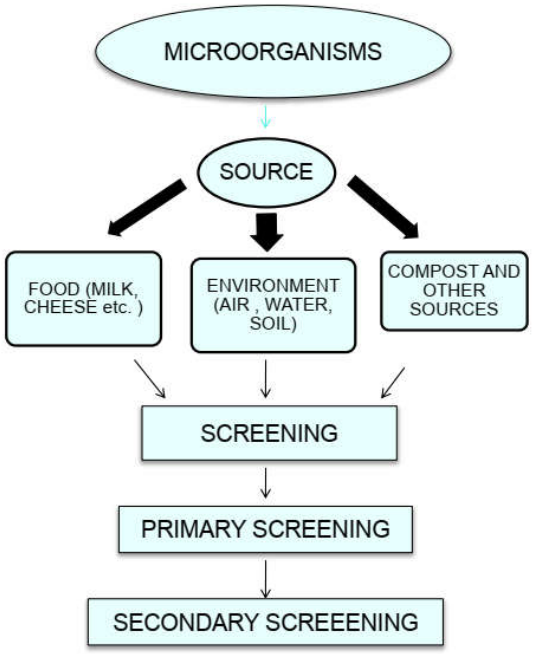
- ❖ Crowded plate technique is used for screening of antibiotic producing microorganisms.
- ❖ Does not give information about the sensitivity of antibiotics towards other microorganisms.
- ❖ Dilutions are made and then pouring and spreading of soil samples that give 300 to 400 or more colonies per plate.
- ❖ Colonies showing antibiotic activity are indicated by zone of inhibition around the colony .
- ❖ Such colonies are sub cultured and purified by streak before making stock cultures.

❖ The purified cultures are then tested to find the Microbial Inhibition Spectrum.



SCREENING

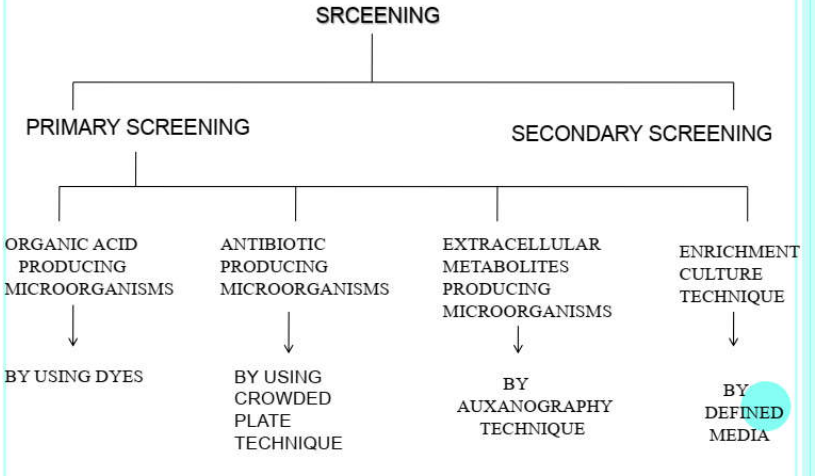
The procedure of isolation, detection , and separation of microorganisms of our interest from a mixed population by using highly selective procedures is called SCREENING



IMPORTANT THINGS TO BE CONSIDERED WHILE SCREENING :-

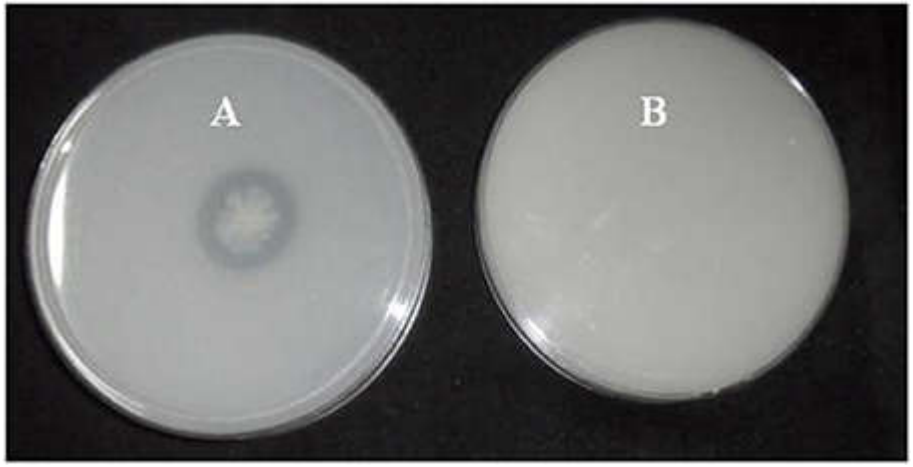
- 1.) CHOICE OF SOURCE - Samples from screening is taken from soil, water, air, milk, compost etc.
- 2.) CHOICE OF SUBSTRATE -Nutrients and growth factors should be supplied for growth of desired microorganism.
- 3.) CHOICE OF DETECTION - Proper isolation and detection of desired microorganisms is important

TYPES OF SCREENING



PRIMARY SCREENING

- It's a process for detection and isolation of microorganisms of our interest.
- Determines which microorganisms are able to produce a compounds.
- Does not provide much idea about the production or yield potential of microorganisms.
- It separate out only a few microorganisms, only few have commercial value while discards the valueless microorganisms .



1) PRIMARY SCREENING OF ORGANIC ACID PRODUCING MICROORGANISMS

❖The ph indicating **dyes** may be used for detecting microorganism that are capable of producing organic acids.

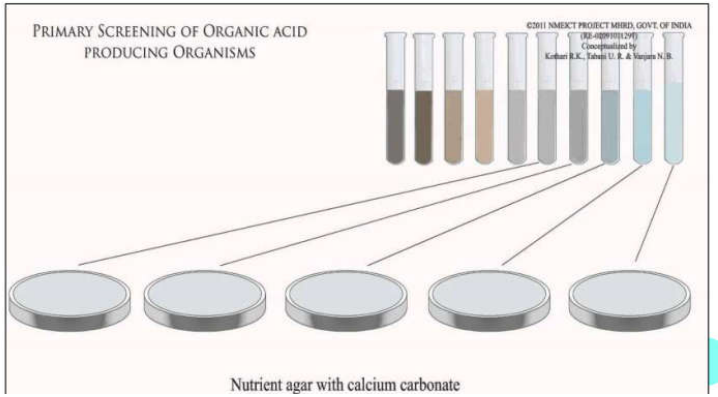
❖These dyes undergo color changes according to its ph.

❖Dyes such as **Neutral red, Bromothymol blue** are added to the poorly buffered nutrient agar media .

❖Colonies are subcultured to make stock culture.

❖Further testing is needed since inorganic acids, bases are also metabolic products of microbial growth.

❖ Incorporation of $CaCO_3$ in medium is also used to screen organic acid producing microbes on basis of formation of clear zone of dissolved $CaCO_3$ around the colony.



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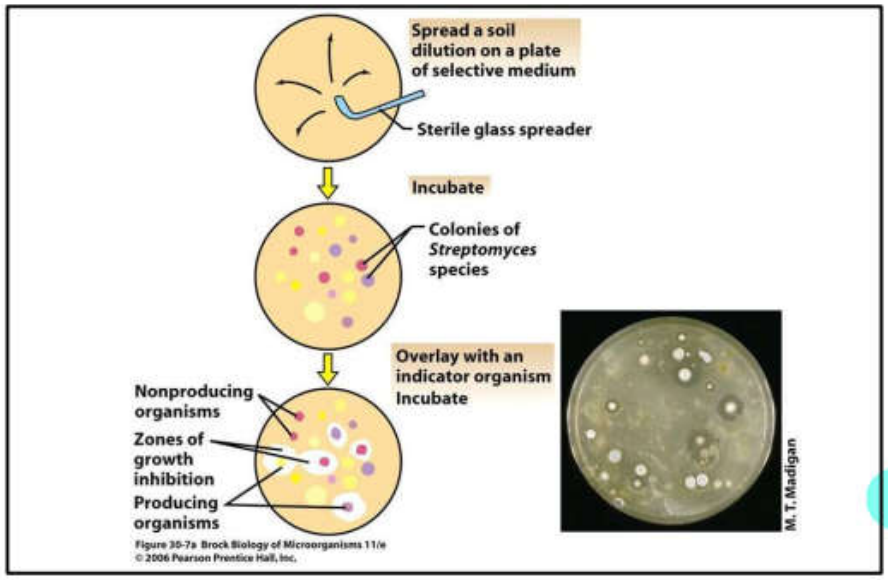


Auxanography technique

- **Auxanography technique:** This technique is employed for the detection and isolation of microorganisms capable of producing certain extracellular substances such as growth stimulating factors like amino acids, vitamins etc.

This technique has two major steps are:-

A) Preparation of first plate:

- A filter paper is put across the bottom of petri dish.
- The nutrient agar is prepared and poured on the paper disc.
- Allowed to solidify
- Soil sample is diluted and proper dilutions are inoculated

Auxanography technique

B)Preparation of second plate:

- A minimal media lacking the growth factors is prepared and seeded with the test organism.
- The seeded medium is poured onto fresh petri plate and the plate allowed to set.
- The agar in first plate is then lifted and placed on the second plate without inverting.
- The growth factor produced on agar can diffuse into the lower layer containing test organism
- The zones of stimulated growth of test organism around colonies is an indication that organism produce growth factor extracellularly.

3) PRIMARY SCREENING EXTRACELLULAR METABOLITE PRODUCING MICROORGANISM

❖Auxanography technique is employed for detecting microorganisms able to produce growth factors , vitamins , amino acids etc. extracellularly.

❖ The 2 major steps are:-

A.)Preparation of first plate

- A filter paper strip is put across the bottom of petri dish.
- The nutrient agar is prepared and poured on the paper disc
- and allowed to solidify.
- Soil sample is diluted and proper dilutions are inoculated.

B.) Preparation of second plate

- A minimal media lacking the growth factors is prepared and seeded with the test organism.
- The seeded medium is poured onto fresh petri plate and the plate is allowed to set.

❖The agar in first plate is then lifted and placed on the second plate without inverting.

❖The growth factors produced on agar can diffuse into the lower layer containing test organism.

❖The zones of stimulated growth of test organism around colonies is an indication that organism produce growth factor extracellularly.

4) ENRICHMENT CULTURE TECHNIQUE

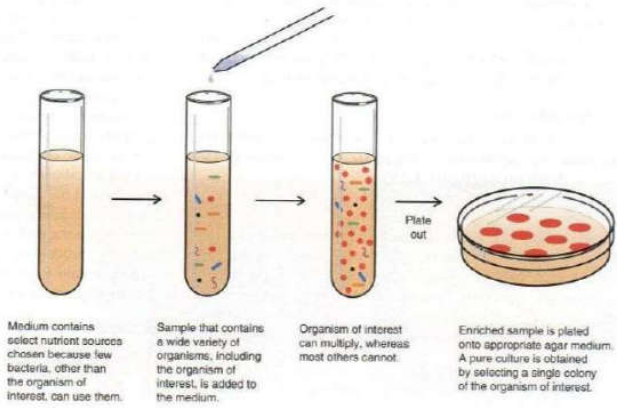
❖This was designed by Beijerinck to isolate the desired microorganism from heterogeneous microbial population.

❖ It consists of following steps :

a.) Nutrient broth is inoculated with microbial source material and incubated.

b.) A small portion of all inoculums is plated onto the solid medium and well isolated colonies are obtained.

c.) Suspected colonies from the plate are sub cultured on fresh media and subjected for further testing.



SECONDARY SCREENING

It's a systematic screening programme intended to isolate industrially important or useful microorganisms .

SOME IMPORTANT POINTS ASSOCIATED WITH SECONDARY SCREENING ARE:-

- It is useful in sorting of microorganisms that have real commercial value. The microorganisms having poor applicability in fermentation process are discarded.
- Provides the information whether the product formed by microorganisms is new or not. This may be accomplished by paper , thin layer, chromatographic technique.
- It should show whether the product possess physical properties such as UV light absorption or fluorescence or chemical properties that can be employed to detect the compound during use of paper chromatography.
- It is conducted on agar plates, in flasks or in small fermentor containing liquid media.
- It gives an idea about the economic position of the fermentation process involving the use of a newly discovered culture.
- It helps in providing information regarding the product yield potentials of different isolates.
- It determines the optimum conditions for growth or accumulation of a product associated with a particular culture.
- Chemical, physical and biological properties of a product are also determined during secondary screening. Moreover, it reveals whether a product produced in the culture broth occurs in more than one chemical form.
- It detects gross genetic instability in microbial cultures. This type of information is very important, since microorganisms tending to undergo mutation or alteration is some way may lose their capability for maximum accumulation of the fermentation products.
- It tells about the chemical stability of the fermentation product.
- It can be qualitative or quantitative in its approach.

DIFFERENCE BETWEEN PRIMARY SCREENING AND SECONDARY SCREENING

PRIMARY SCREENING

- Desired micro-organisms are isolated and detected.
- Isolated micro-organisms are used for secondary screening.
- Basic information about the isolated colony is obtained.

SECONDARY SCREENING

- Industrially Important organism are characterize by using highly selective procedures.
- The organisms that are capable to give high yield of product by using cheap raw material are Screened.
- Detail information of isolated colony is obtained and determined whether it can be used on industrial scale.
- Here Capabilities and yield potential of microbes is determined
- Here valueless organism are discarded and valuable organism that are capable to produce product on industrial scale are used further

EXAMPLE OF SECONDARY SCREENING – ANTIBIOTIC PRODUCNING STREPTOMYCES SPECIES

1. Streptomyces isolates are streaked as a narrow band on nutrient agar plates are incubated .
2. Test organisms are then streaked from the edge of plates without touching streptomyceal isolate and then the plates are then incubated .
3. At the end of incubation, growth inhibitory zones for each organism are measured in millimeters .
4. Such organisms are again subjected for further testing by growing the culture in sterilized liquid media and incubated at constant temperature in a mechanical shaker.

Introduction

- To maintain pure culture for extended periods in a viable conditions, without any genetic change is referred as Preservation.
- During preservation most important factor is to stop microbial growth or at least lower the growth rate.
- Due to this toxic chemicals are not accumulated and hence viability of microorganism is not affected.

Objectives of Preservation

- To maintain isolated pure cultures for extended periods in a viable conditions.
- To avoid the contamination
- To restrict genetic change(Mutation)

Application of Preservation

- **Academic Use:**
- **Research Purpose:**
- **Fermentation Industry:**
- **Biotechnological Field:**

Once a microorganism has been isolated and grown in pure culture, it becomes necessary to maintain the viability and purity of the microorganism by keeping the pure culture free from contamination. Normally in laboratories, the pure cultures are transferred periodically onto or into a fresh medium (subculturing) to allow continuous growth and viability of microorganisms. The transfer is always subject to aseptic conditions to avoid contamination.

- Since repeated subculturing is time consuming, it becomes difficult to maintain a large number of pure cultures successfully for a long time. In addition, there is a risk of genetic changes as well as contamination. Therefore, it is now being replaced by some modern methods that do not need frequent subculturing. These methods include refrigeration, paraffin method, cryopreservation, and lyophilization (freeze drying).

- **Methods of Preservation**

- 1. Periodic transfer to fresh media (Subculturing)
- 2. Storage in sterile soil
- 3. Saline suspension
- 4.Oil overlay

- **5. Suspended Metabolism**
- **6. Storage at low temperature**
- **7. Freez dying**
- **8. Storage in silica gel**

Periodic transfer to fresh media

- Strains can be maintained by periodically preparing a fresh culture from the previous stock culture. The culture medium, the storage temperature, and the time interval at which the transfers are made vary with the species and must be ascertained beforehand. The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible.

- Many of the more common heterotrophs remain viable for several weeks or months on a medium like **Nutrient Agar**. The transfer method has the disadvantage of failing to prevent changes in the characteristics of a strain due to the development of variants and mutants.

Advantages

- 1. It is a simple method, any special apparatus are not required.
- Easy to recover the culture
- Disadvantage
- Risk of contamination is more
- It may be possible to change in genetic and biochemical characteristics

Lyophilization (Freeze-Drying)

Freeze-drying is a process where water and other solvents are removed from a frozen product **via sublimation.** Sublimation occurs when a frozen liquid goes directly to a gaseous state without entering a liquid phase. It is recommended using slow rates of cooling, as this will result in the formation of vertical ice crystal structures, thus allowing for more efficient water sublimation from the frozen product.

Freeze-dried products are hygroscopic and must be protected from moisture during storage. Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years. Lyophilized or freeze-dried pure cultures and then sealed and stored in the dark at 4°C in refrigerators.

Freeze-drying method is the most frequently used technique by culture collection centers. Many species of bacteria preserved by this method have remained viable and unchanged in their characteristics for more than 30 years.

Advantage of Lyophilization

Only minimal storage space is required; hundreds of lyophilized cultures can be stored in a small area.

Small vials can be sent conveniently through the mail to other microbiology laboratories when packaged in a special sealed mailing containers.

Lyophilized cultures can be revived by opening the vials, adding liquid medium, and transferring the rehydrated culture to a suitable growth medium.

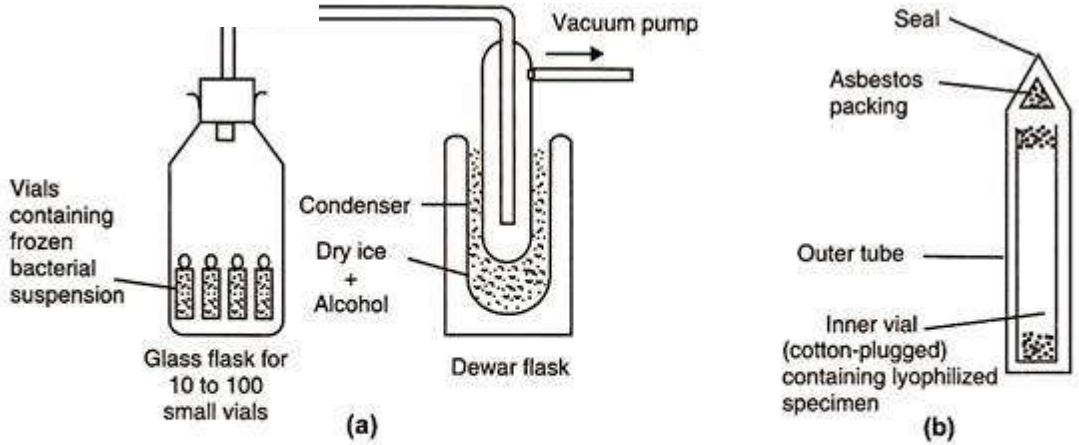


Fig. 2.16: (a) Small cotton-plugged vials containing frozen suspension of the microorganisms are placed in the glass-flask, which is attached to a condenser. The condensor is connected with a high-vacuum pump and this system brings about desiccaton of the cultures.

(b) After desiccation of the cultures as in (a) the vials are removed, placed individually in a large tube covered with asbestos packing and under vacuum.

Cryopreservation

- Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times.
- In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at -196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival.

- This liquid nitrogen method has been successful with many species that cannot be preserved by lyophilization and most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics, however this method is expensive.

Agar Slant Cultures:

- All microbiology laboratories preserve micro-organisms on agar slant. The slants are incubated for 24hr or more and are then stored in a refrigerator. These cultures are periodically transferred to fresh media. Time intervals at which the transfers are made which varies with the origin and condition of growth.

Agar Slant Culture Covered with Oil (Parafin Method):

- The agar slants are inoculated and incubated until good growth appears. They are then covered with sterile mineral oil to a depth of 1 cm above the tip of slant surface. Many bacterial species can preserved by covering the growth on the agar slants with steril mineral oil. Oil must cover the slants completely.Oil reduces the loss of water and also slow down the exchange of gas within organism and sorrounding
- This is a simple and most economical method of preserving bacteria and fungi where they remain viable for several years at room temperature. The layer of paraffin prevents dehydration of the medium and by ensuring an aerobic condition, the microorganism remain in dormant state.

Saline Suspension:

- Sodium chloride in high concentration is frequently an inhibitor of bacterial growth. Bacteria are suspended in 1% salt solution (sublethal concentration in screw cap tubes to prevent evaporation). The tubes are stored at room temperature. Whenever needed the transfer is made on agar slant.

Preservation by Drying in Vacuum:

- The organisms are dried over calcium chloride in vacuum and are stored in the refrigerator.

Media Used in Industrial Microbiology

- Use of appropriate growth medium is important to
  - Allow to harness the organism's full industrial potentials.
  - Prevent formation of toxic products.
- The basic nutrient requirements of Industrial media
  - All microbiological media must satisfy the needs of the organism in terms of
    - carbon, nitrogen, minerals, growth factors, and Water
  - In addition they must not contain materials which are inhibitory to growth.

Media Used in Industrial Microbiology

- **Media formulation**
  - Under laboratory conditions
    - Purified chemicals are used since volume is limited to a few litters
  - At industrial scale
    - Made with unpurified raw materials.

Criteria for the choice of raw materials used in industrial media

- In deciding the raw materials to be used in the production of a given products using designated microorganism the following factors should be taken into account.
  - Media cost must not be above the selling price.
  - The raw material must be readily available in order not to halt production.
  - Proximity of the user-industry to the site of production of the raw materials is important
- Ease of disposal of wastes resulting from the raw materials.
- The quality of the raw material in terms of its composition must be reasonably constant.
- Media must have Adequate chemical composition.
- The raw material must contain the precursors necessary for the synthesis of the finished product.

Fermentation media

- Most fermentations require
- 1-liquid media, often referred to as broth,
- 2-solid-substrate fermentations.
- In most industrial fermentation processes there are several stages where media are required.

1- several inoculum (starter culture) propagation steps,

2- pilot-scale fermentations

3-the main production fermentation.

- Where biomass or primary metabolites are the target product, the objective is to provide a production medium that allows optimal growth of the microorganism.
- For secondary metabolite production, such as antibiotics, their biosynthesis is not growth related. For this purpose, media are designed to provide an initial period of cell growth, followed by conditions optimized for secondary metabolite production. At this point the supply of one or more nutrients (carbon, phosphorus or nitrogen source) may be limited and rapid growth stopped

The main factors that affect the final choice of raw materials

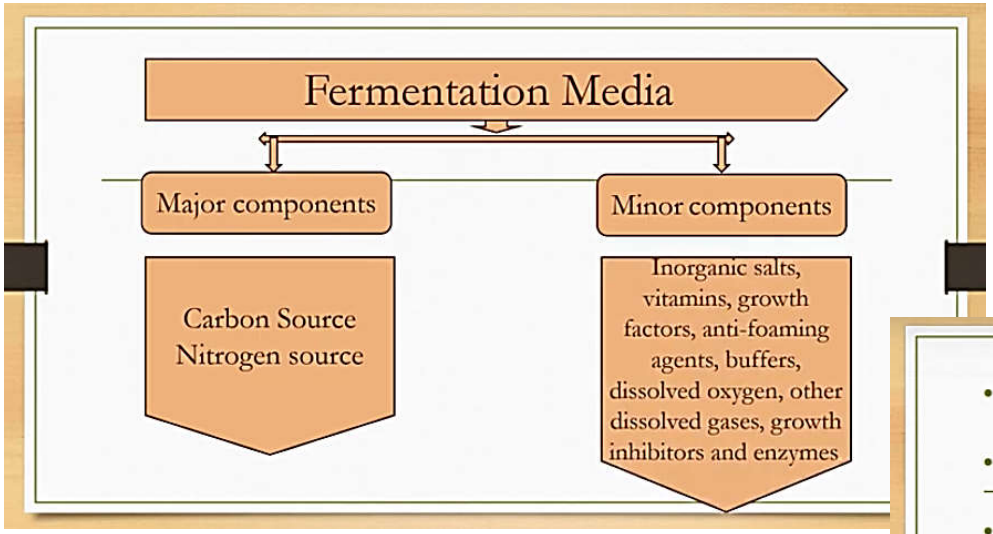
- **1** Cost and availability
- **2** Ease of handling in solid or liquid forms, along with associated transport and storage costs, e.g. requirements for temperature control.
- **3** Sterilization requirements and any potential denaturation problems.
- **4** Formulation, mixing, complexing and viscosity characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages.
- **5** The concentration of target product , its rate of formation and yield per gram of substrate utilized.
- **6** The levels and range of impurities, and the potential for generating further undesired products during the process.
- **7** Overall health and safety implications.

Carbon sources

- A carbon source is required for all biosynthesis leading to reproduction, product formation and cell maintenance.
- In most fermentations it also serves as the energy source.
- Carbon requirements may be determined from the biomass yield coefficient (*Y*), an index of the efficiency of conversion of a substrate into cellular material.

- $Y_{carbon(g/g)}$ = Biomass produced(g) / Carbon substrate utilized (g)
- For commercial fermentations the determination of yield coefficients for all other nutrients is usually essential.
- Various organisms may exhibit different yield coefficients for the same substrate due to the pathway by which the compound is metabolized.
- For example, *Saccharomyces cerevisiae* grown on glucose
- has biomass yield coefficients of 0.56 under aerobic condition and 0.12 g/g under anaerobic conditions

- Carbohydrates are traditional carbon and energy sources for microbial fermentations, although other sources may be used, such as alcohols, alkanes and organic acids. Animal fats and plant oils may also be incorporated into some media, often as supplements to the main carbon source.

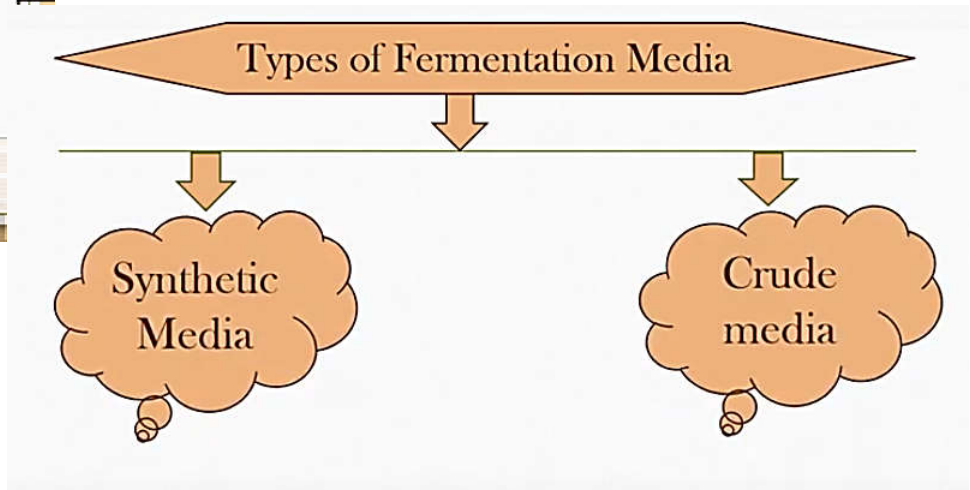


- Requirement of Nutrients depend upon type of organism used and type of fermentation that is carried out.
- Types of nutrients present in the fermentation media determine the yield of the product.
- There are two uses of fermentation media

1. Growth Media – It contains less nutrients and used for growth of micro-organism and preparation of Innoculum for fermentation.
2. Fermentation media – It contains large amount of nutrients this media is used in fermentation industries for production economically important products.

Role of fermentation media

- During fermentation process organisms enjoy luxurious metabolism with high amount of nutrients, optimum conditions.
- Due to presence of large amount of nutrients the micro-organism become hyperactive and consume excess nutrients and results in partial degradation of fermentation media.
- The waste product execrated by microbes may be the desired product.
- The substrate given to microbes should not reach inhibitory concentration levels as it may result death of cells.
- Water present in cell cytoplasm is also a important factor.
- Excess substrate may increase osmotic pressure and effect enzyme activities in a cell.
- Microbes excrete this excess substrate in the form of partially digested fermentation media.



Synthetic media

- The exact composition of nutrients is predetermined.
- Here level and concentration of nutrients can be controlled. Media can be re-designed
- Used in experiment related to effect of nutrients on growth of microbes and yield of product.
- Metabolic pathways and main ingredient that is involved in product formation can be determined.
- Synthetic media can be optimized as per our requirement.
- Product recovery is easy as all pure ingredients are used.
- It is expensive media and cannot be used on industrial scale as the process is not economical and profitable.

Crude Media

1)Crude media contains a rough composition of media.
2) It gives high yield of product and contains undefined sources of ingredients.
3) It contains high level of nutrients, vitamins, proteins, growth factors, anti-foaming agents and precursors.
4) It is important to ensure that crude media should not contain toxic substance.

Ingredients of Crude media

Inorganic nutrients | Carbon source | Nitrogen source | Growth factors | Precursors | Buffers

1) **Inorganic nutrients** – It contains salts containing anion and cations along with carbon source. Specific ions like magnesium ions, phosphates and sulphate are added to balance crude media.

2) **Carbon source** - Simple to complex carbohydrates are used. Different sugars, organic acids, fatty acids, proteins, peptides are added as a carbon source. In fermentation media crude source of carbon is added.
Examples -Black strap molasses, Corn molasses, Beet molasses, Starch, Corn, Rice, Rye, Milo, wheat, straws, wood waste, saw meal etc.

3) **Nitrogen source** - Non-proteolytic micro-organism need pure form of urea, ammonia and nitrate as a source of nitrogen. When fermentation organism is proteolytic in nature animal and plant raw material is used as a nitrogen source.
Examples - Distillery dried solubles, Casein, Cereal grains, peptones, yeast extract, hydrolysate, and soybean meal etc

4) **Growth factor** - Crude media constituents provides enough amount of growth factors so no extra addition of growth factor is required. If there is a lack of any kind if vitamins or nutrients, growth factors can be added to media. Examples are yeast extract, and beef extract.

5) **Precursors** – It is a crude constitute, Precursor in cooperate in the molecules of product without changing the final product. It improves yield and quality of the product. Example, Cobalt chloride is added fermentation of vitamin B12.

6) **Buffers** -Buffers are used to control drastic changes of pH. Sometimes, media components may act as buffers. For example, protein, peptides, amino-acids act as good buffers at neutral pH. Sometimes inorganic buffers like K_2HPO_4, KH_2PO_4, and $CaCO_3$ can be added as required. Generally, during the fermentation process, pH changes to acidic or alkaline pH. The cheapest and easily available buffer is $CaCO_3$.

Media Used in Industrial Microbiology

- Some raw materials used in compounding industrial media
  a) Corn steep liquor
  b) Pharmamedia
  c) Distillers soluble
  d) Soya bean meal
  e) Molasses
  f) Sulfite liquor
  g) Other Substrates (alcohol, acetic acid, methanol, methane, and fractions of crude petroleum)

Corn Steep Liquor Corn steep liquor is a by-product of starch extraction from maize and its first use in fermentations was for penicillin production in the 1940s. The extract composition of the liquor varies depending on the quality of the maize and the processing conditions. Concentrated extracts generally contain about 4% (w/v) nitrogen, including a wide range of amino acids, along with vitamins and minerals.

Molasses

The byproducts of sugar industry include the beet and cane molasses. They are the concentrated syrups or mother liquors recovered at any of several steps in the sugar-refining process, and they are named depending on the particular step from which it is recovered. Blackstrap molasses prepared from sugarcane is the cheapest and most used sugar source for industrial fermentations. Blackstrap molasses is the mother liquor left after the crystallization of sugar and it contains approximately 52 per cent total sugars of sucrose in which about 30 per cent approximately is sucrose. When molasses is used as a fermentation component it should contain at least 50 per cent fermentable sugars.

The high-test or invert molasses is produced by partially inverting the whole cane juice to prevent sugar crystallization of sugars.

Sugar is partially hydrolysed to monosaccharide with heat and acid then neutralized and further concentrated without removal of any sugar. It contains approximately about 75-80 per cent sugar which is mostly the original sugar of the cane juice and some of which is partially hydrolyzed to D-glucose and D-fructose. It is preferable to blackstrap molasses because of the lower levels of non-fermentable solids including salts and un-fermentable sugars.

In addition to the sucrose, blackstrap molasses contains small amounts of complex polysaccharides invert sugars (due to the action of enzyme invertase), calcium organic acids (malic, citric, lactic, humic and acetic and propionic) nitrogen containing compounds like (aspartic and succinic acid) and alkali stable vitamins (niacin, riboflavin). The dark coloured nitrogen containing polymeric substances result from "browning" a reaction of the sugar with amino acids, heat and alkali are used in this processing. The overall composition of the various molasses differs according to the specific geographic area of production.

Pharmamedia

- Yellow fine powder made from cotton-seed embryo.
- It is used in the manufacture of tetracycline and some semi-synthetic penicillins.
- rich in protein, (56% w/v) and contains 24% carbohydrate, 5% oil, and 4% ash
- rich in calcium, iron, chloride, phosphorous, and sulfate.

Sulfite Liquor

- Sulfite liquor (also called waste sulfite liquor, sulfite waste liquor or spent sulfite liquor) is the aqueous effluent resulting from the sulfite process for manufacturing cellulose or pulp from wood.
- During the sulfite process, hemicelluloses hydrolyze and dissolve to yield the hexose sugars, glucose, mannose, galactose, fructose and the pentose sugars, xylose, and arabinsoe.
- Used as a medium for the growth of microorganisms after being suitably neutralized with $CaCO_3$ and enriched with ammonium salts or urea, and other nutrients.

Antifoam agents

High degree of aeration and agitation will result in foam formation.

Foaming reduces oxygen transfer. Air bubbles entrapped in the foam and again and again they recirculate in the medium. This will result in oxygen depleted bubbles residing in the system

To control foam antifoam agents are added.

- Most of the antifoams are surface tension lowering substances
- This will result in rigid bubble formation and resistance to oxygen transfer.
- Also antifoams in the liquid may favour coalescence of bubbles in freely moving areas which again will decrease oxygen transfer.
- OTR can be reduced dramatically even by factor of 10.

Disadvantages of Foaming:

1. Reduce process productivity since bursting bubbles can damage proteins,
2. Can result in loss of sterility if the foam escapes the bioreactor.
3. Leads to over-pressure if a foam-out blocks an exit filter.

Antifoam agent : soya bean oil , corn oil, lard oil and silicones (sterilized before adding)

AIR AND MEDIA STERILIZATION

PRINCIPLE

- Sterilization removes infecting micro-organisms it can also remove pathogenic micro-organisms or spoiling agents.
- Sterilization is accomplished either by chemical or physical means.
- Moist heat is a most common physical agent.
- It allows for satisfactory industrial sterilization.
- The other method of sterilization is the removal of infecting micro-organisms.
- This is done by filtration. Numerous type of filter papers are available for this purpose.
- It depends on the-
  (i)- The size of micro-organisms and
  (ii)-The retention efficiency of the filter.
- Usually sterilization of gases and biostatic fluids is done by filtration.

STERILIZATION OF MEDIA

AIR AND MEDIA STERILIZATION

- Usually media are sterilized before they are inoculated.
- Sterilization of media is decided by the chemical composition.
- Sterilization of media may be done by one of the following three methods-
  (i)-by boiling
  (ii)-by passing live steam
  (iii)-by subjecting the medium to steam under pressure(i.e. autoclaving)
- The classical technique of making the medium sterile by the use of steam may be carried out in two ways-

  (i)-batch wise in fermentor and
  (ii)-continuous sterilization

BATCH WISE IN FERMENTOR

- This is the simplest method of sterilizing production media.

- The vessel is equipped with a coil or jacket for heating and cooling.

- Also the agitator may be fitted to aid heat-exchange.

- It is needed to raise the temperature of the medium to 120^0C with steam to maintain this for a period of 20 minutes before cooling the system.
- There is an interconnecting pipeline between the batch the batch cooker and the fermentor for transferring the sterile medium from the cooker to steam sterilized fermentor.

ADVANTAGE

- The batch cooker method saves the production time, since the fermentor is unoccupied between two fermentor runs.

LIMITATION

- It occupies increased plant space.

- It involve higher cost of the additional equipment required, and

- It involves increased steam usages.

CONTINUOUS STERILIZATION

- This methods involves passing of production medium through a heat exchanger, a holding coil and a cooler.

- The temperature of medium undergoing sterilization is raised to the desired level in the heat exchanger.

- The medium is then passes on to a holding coil.

➢Where it is maintained at the sterilizing temperature for a predetermined time period.

➢Finally the medium is rapidly cooled by counter circulating it in the exchanger against the cool input medium, and then against cold water.

➢In continuous sterilization the temperature is higher than 120^0C.

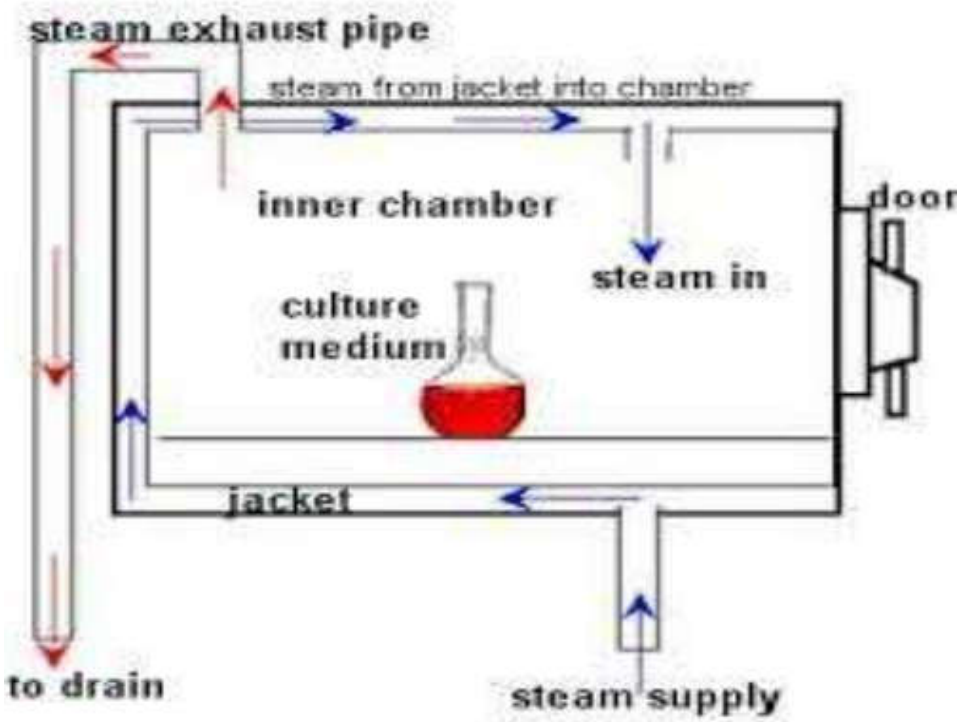
ADVANTAGES

➢It saves both production time and plant space.

➢It gives improved quality of the medium.

➢It involves some economy in steam cost.

➢It allows the use of lower sterilizing temperature or shorter holding period.



➢With aerobic fermentation continuous supply of sterile air is vital for successful fermentation.

➢Air can be sterilized by many methods namely-

(i)-filtration

(ii)-heat

(iii)-electrostatic repulsion

(iv)-U.V. light

(iv)-chemical agents

AIR STERILIZATION

- The sterilization of air in fermentation industries is widely carried out by the filtration method.
- For sterilizing large volumes of air was studied by Terjesen and cherry.
- They used a performed slab wool 3 inches thick.
- The air velocity through the slab was kept below 1ft./sec. to avoid channeling through the slag wool material.

AIR STERILIZATION