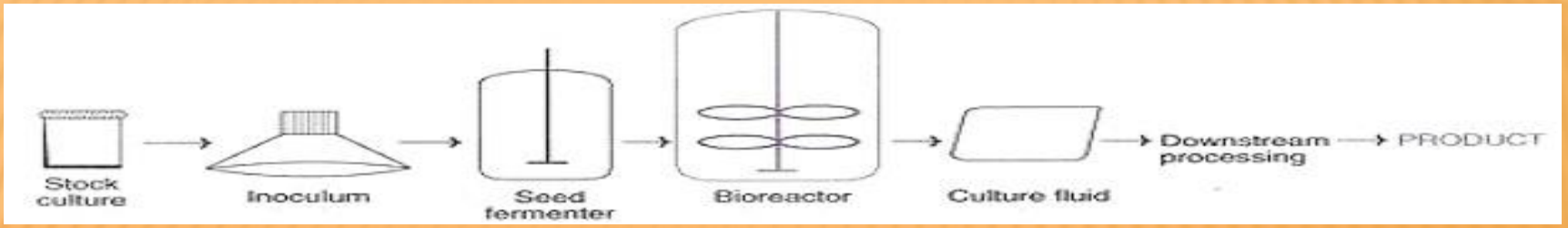
UNIT I
FERMENTATION TECHNOLOGY



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- The biochemical activity of organisms, during their growth, development, reproduction, even senescence and death.
- Chemical change induced in a complex organic compound by the action of an enzyme, whereby the substance is split into simpler compounds.
- Use of organisms to produce food, pharmaceuticals and alcoholic beverages on a large scale industrial basis.
- For Microbiologist, “FERMENTATION” means
  - Mass cultivation of microbes under aerobic & anaerobic conditions.
  - Biological process occurs in absence of oxygen.
  - Spoilage of food by microbial activity.
  - Production of alcoholic beverages, vitamins, enzymes etc.
  - Partial oxidation of carbohydrates.

Basic principle

Organisms are grown under suitable conditions, by providing raw materials meeting all the necessary requirements such as carbon, nitrogen, salts, trace elements and vitamins.

End products

which are extracted for use by human being and that have a high commercial value.

Fermentation Methodology:

- Fermentation process is carried out in a container called the fermenter or bioreactor.
- The design and nature of the fermenter varies depending upon the type of fermentation carried out.
- Invariably all the fermenters have facilities to measure some of the fermentation parameters like temperature, pressure, pH, elapsed fermentation time, liquid level, mass etc.

THE PROCESS OF FERMENTATION

1. In bound logistic (the delivery & storage of raw material)
2. Up stream processing (USP)– processing of raw materials for fermentation.
3. The fermentation, where major conversion occurs.
4. Down streaming process (DSP) – purification and concentration of end product(s).

UPSTREAM PROCESSING

A) MICROORGANISMS

- Obtaining suitable micro organisms.
- Strain improvement to increase productivity and yield.
- Maintance of strain purity.
- Preparation of suitable inoculum.

B) FERMENTATION MEDIA.

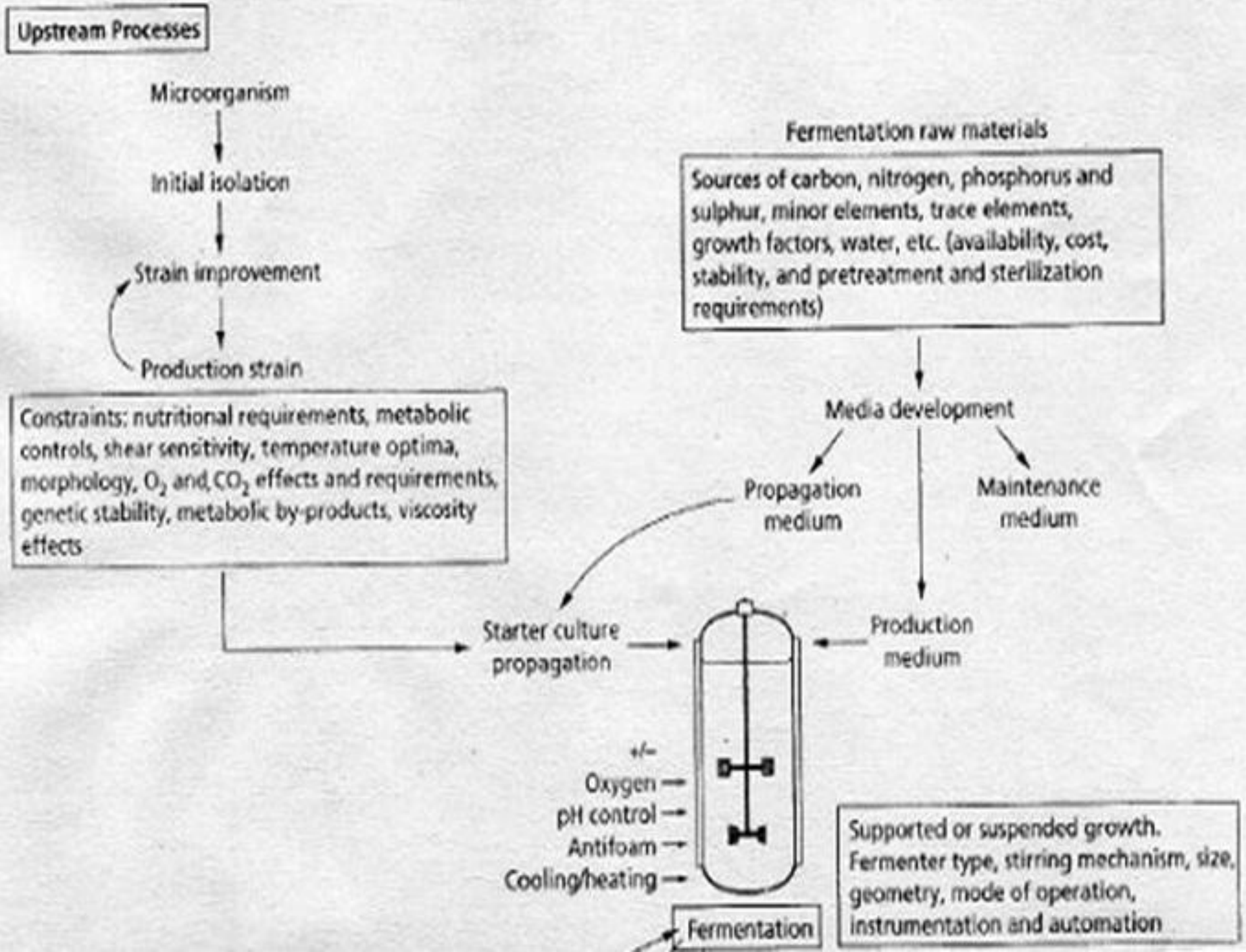
C) FERMENTATION PROCESS.

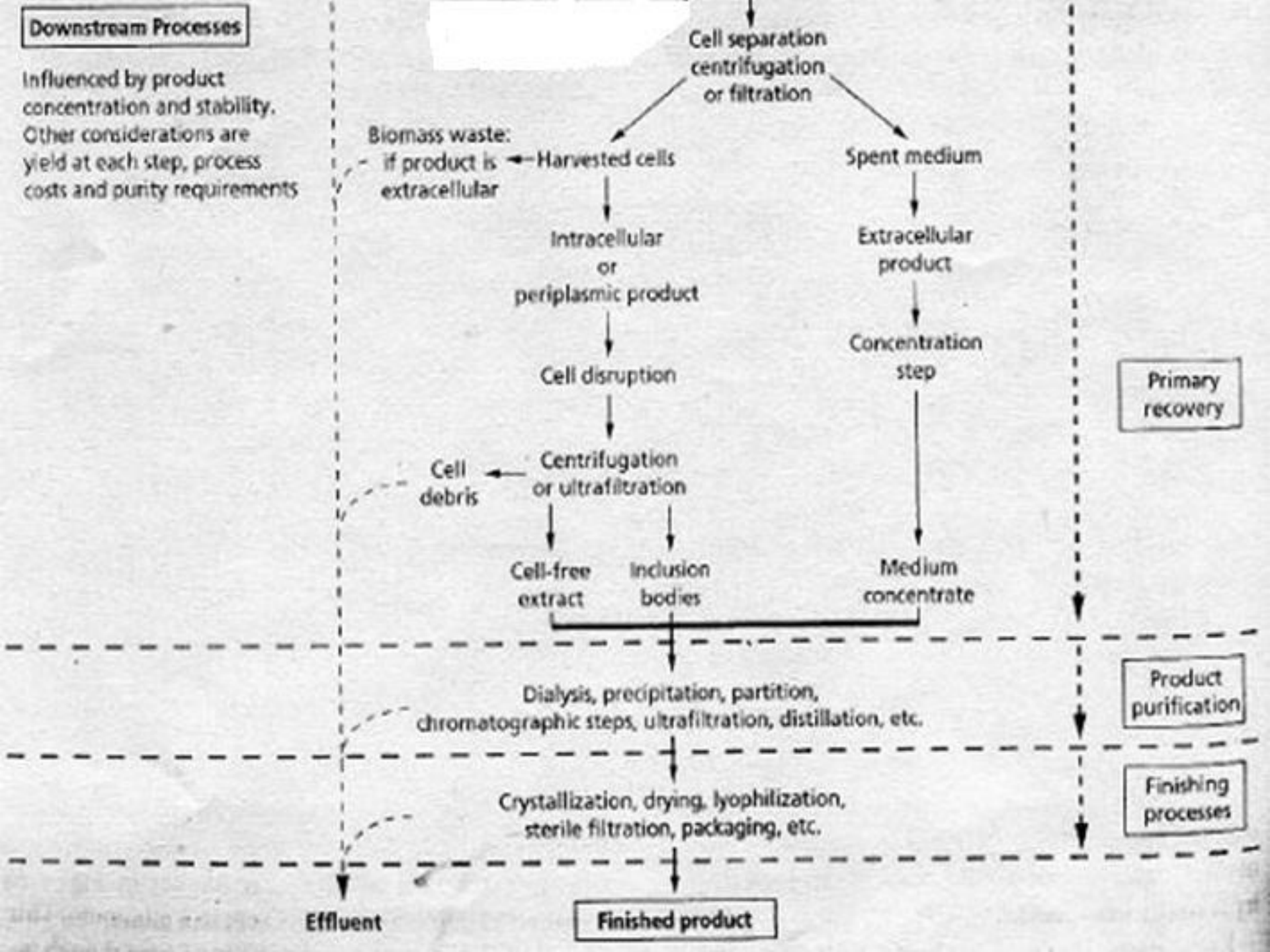
DOWN STREAM PROCESSING

A. Cell harvesting.

B. Cell disruption.

C. Product purification from extract or growth medium.


Upstream Processes
Microorganism
Initial isolation
Strain improvement
Production strain
Constraints: nutritional requirements, metabolic controls, shear sensitivity, temperature optima, morphology, O_2 and CO_2 effects and requirements, genetic stability, metabolic by-products, viscosity effects
Fermentation raw materials
Sources of carbon, nitrogen, phosphorus and sulphur, minor elements, trace elements, growth factors, water, etc. (availability, cost, stability, and pretreatment and sterilization requirements)
Media development
Propagation medium
Maintenance medium
Starter culture propagation
Production medium
+/−
Oxygen
pH control
Antifoam
Cooling/heating
Fermentation
Supported or suspended growth. Fermenter type, stirring mechanism, size, geometry, mode of operation, instrumentation and automation


Downstream Processes
Influenced by product concentration and stability. Other considerations are yield at each step, process costs and purity requirements
Cell separation centrifugation or filtration
Biomass waste: if product is extracellular
Harvested cells
Spent medium
Intracellular or periplasmic product
Extracellular product
Concentration step
Cell disruption
Primary recovery
Cell debris
Centrifugation or ultrafiltration
Cell-free extract
Inclusion bodies
Medium concentrate
Dialysis, precipitation, partition, chromatographic steps, ultrafiltration, distillation, etc.
Product purification
Crystallization, drying, lyophilization, sterile filtration, packaging, etc.
Finishing processes
Effluent
Finished product

TYPES OF FERMENTER

(1) External recycles airlift fermenter—for producing bacterial biomass, with methanol as substrate.

(2) Internal recycle airlift fermenter—for producing yeast with oil as substrate.

(3) Tubular tower fermenter—used for making beer, wine, vinegar etc.

(4) Nathan fermenter—used in brewing industry.

(5) Stirred fermenter—used for making antibiotics.

TYPES OF FERMENTATION PROCESS

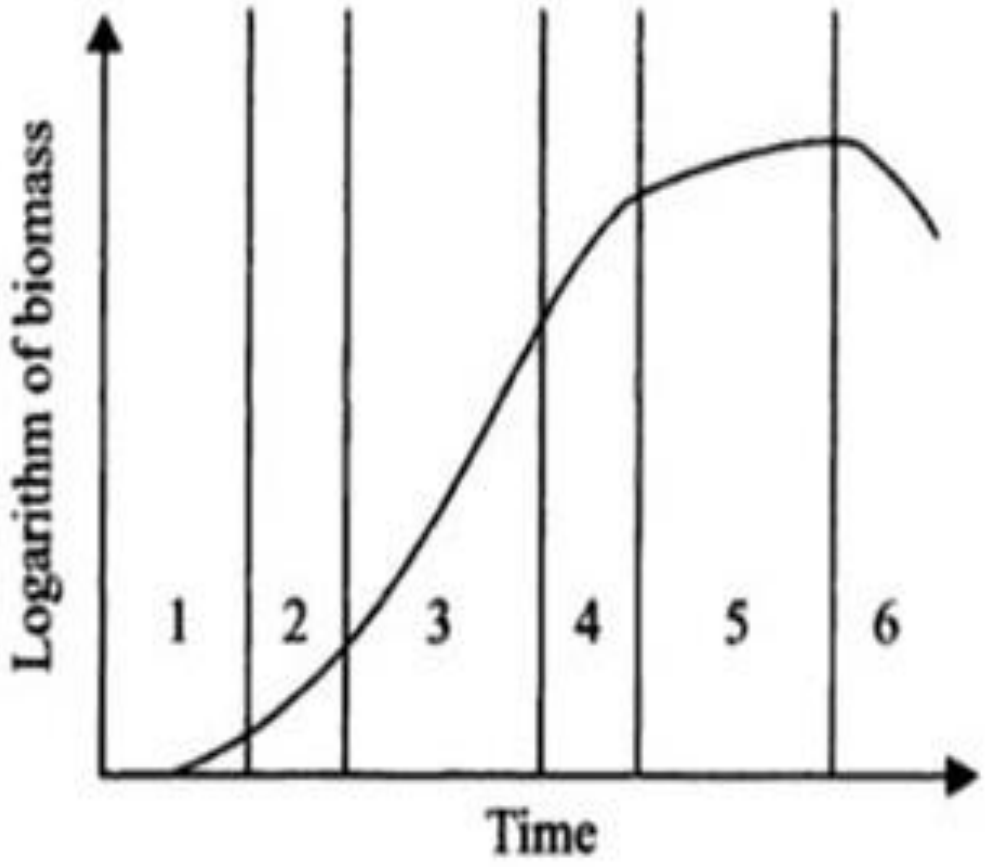
(1) Batch fermentation

(2) Feb-batch fermentation &

(3) Continuous culture.

1) Batch fermentation:

- Type of fermentation wherein there is change in
  - Culture medium,
  - Number of microorganisms and
  - The amount of the product produced (i.e. the metabolite or target protein).
- In batch fermentation six phases of the microbial growth are seen.



(1). Lag Phase
(2). Transient acceleration
(3). Exponential phase
(4). Deceleration phase
(5). Stationary phase
(6). Death phase

Characteristic Growth Curve of Microbes in Batch Culture

FACTORS INFLUENCING IN LAG PHASE

1) CHEMICAL COMPOSITION.
2) AGE OF INOCULLUM.
3) CONCENTRATION OF INOCULUM.
4) VIABILITY & MORPHOLOGY OF INOCULLUM.

At Exponential phase:

Cells divides with maximum frequency to reach maximum growth rate (μ max).

MATHEMATICAL EXPRESSION OF GROWTH

It can be based on cell mass (X) or cell number (N).

Rate of cell growth based on cell mass:

Rate of change of biomass with given time given by:

$$dx/dt = \mu x \text{ --------(1)}$$

$$\mu = 1/x^* dx/dt \text{ -------(2)}$$

Where,

X – Concentration of biomass (g/L)

μ - Specific growth rate.

t - time (hr)

MATHEMATICAL EXPRESSION OF GROWTH

On integrating equation 1 & 2

$$X_t = x_0 + \mu t \text{ --------(3)}$$

Where,

X_t _ biomass concentration after time t.

X_0 _ biomass concentration at start of exponential phase.

Taking natural log (ln) in eqn (3)

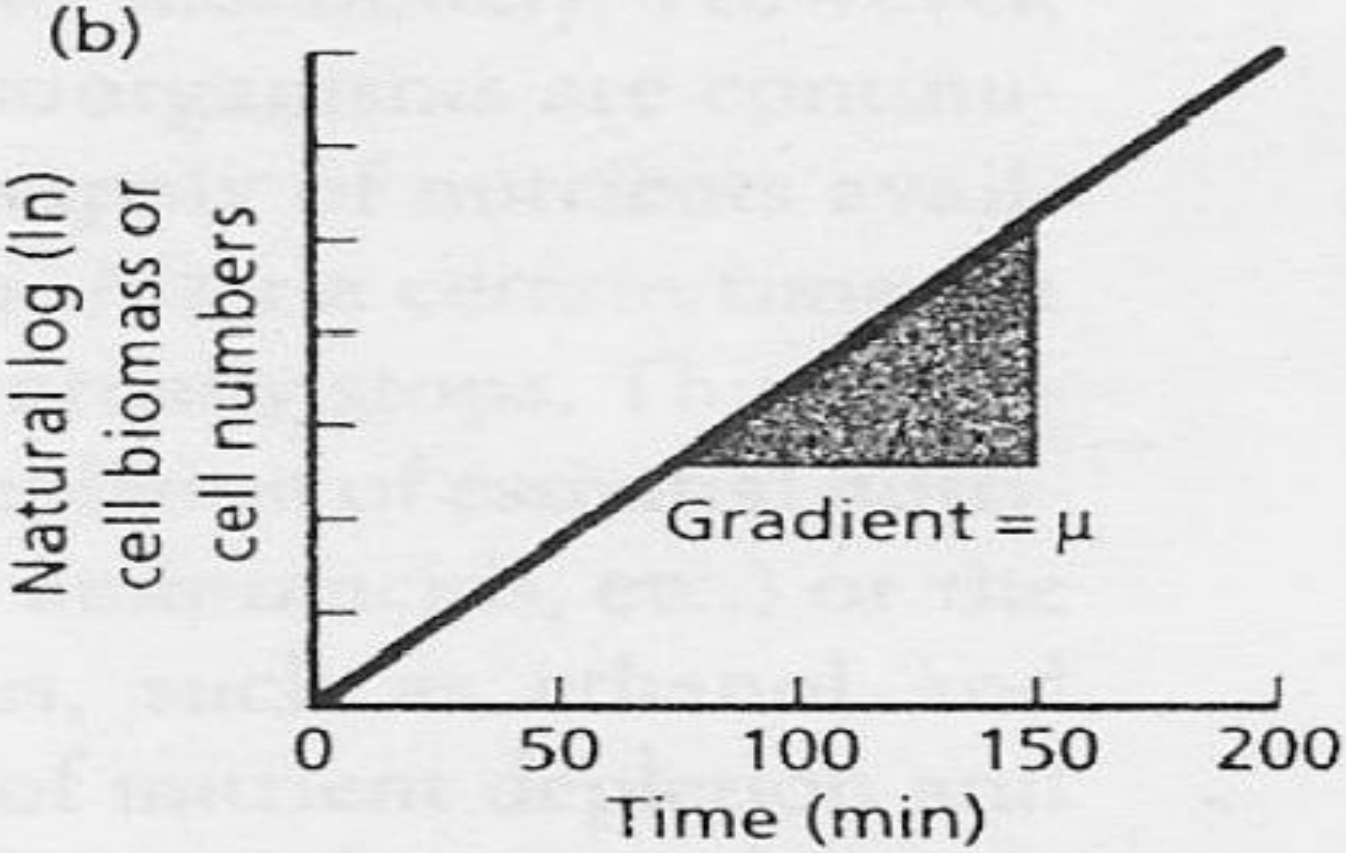
$$\text{In } X_t = \text{In } x_0 + \mu t \text{ ------(4)}$$

$$\mu = (\text{In } X_t - \text{In } x_0) / t$$

This equation in the form of y= c +mx

For cells in exponential phase, a plot of natural log of biomass concentration against time should yield a straight line with a slope (gradient) equal to μ.

Mathematical expression of Growth



Advantages:

- Versatile: can be used for different reactions every day.
- Safe: can be properly sterilized.
- Little risk of infection or strain mutation
- Complete conversion of substrate is possible.

Disadvantages:

- High labor cost: skilled labor is required
- Much idle time: Sterilization, growth of inoculums, cleaning after the fermentation
- Safety problems: when filling, emptying, cleaning.

Example of batch fermentation technology

The fermenter or bioreactor: Mud pot or steel cooking vessel or a dish or a cup

The substrate: Milk ; The specific temperature: 37°C

The organism: Preformed curd (containing the microbe Lactobacillus cecai)

The incubation: At 37° C for 6-8 hours ; The aeration: The process is anaerobic

The process:

- Curd has microbes that utilize lactose present in the milk.
- Lactose is hydrolysed into glucose and galactose. Galactose is converted to glucose. Glucose is broken down to lactic acid by glycolytic pathway.
- The lactic acid produced, lowers the pH of milk from 6.6 to 4.5. The isoelectric pH of milk protein-casein is 4.5.
- At this pH, casein precipitates forming fine micelles in the milk thereby curdling it.
- The container/fermenter is not disturbed (no stirring is taken up) so that the precipitation is uniform.

- The elapsed time i.e. the time required to form the curd is crucial; it depends upon the atmospheric temperature.
- During summer within 4-6 hours, whereas during rainy season it takes 6-8 hours and in winter it takes almost 8-12 hours.
- The process is anaerobic hence it is better to keep the vessel closed.
- Though it cannot be air tight but still the surface of the milk that contains fat prevents air from penetrating in the liquid, furthermore the metabolizing microbes replace the oxygen with carbon dioxide released.

2) Feb-batch fermentation:

- Freshly prepared culture media is added at regular intervals without removing the culture fluid.
- This increases the volume of the fermentation culture.
- This type of fermentation is used for production of proteins from recombinant microorganisms.

Advantages:

- Production of high cell densities due to extension of working time (particularly important in the production of growth-associated products)
- Controlled conditions in the provision of substrates during the fermentation, particularly regarding the concentration of specific substrates as for ex. the carbon source

- Control over the production of by-products or catabolite repression effects due to limited provision of substrates.
- Allows the replacement of water loss by evaporation
- Alternative mode of operation for fermentations leading with toxic substrates.
- Increase of antibiotic-marked plasmid stability by providing the correspondent antibiotic during the time span of the fermentation

Disadvantages:

- It requires previous physiology analysis of the microorganism.
- Requires a substantial amount of operator skill for the set-up, and development.
- In a cyclic fed-batch culture, care should be taken in the design of the process to ensure that toxins do not accumulate to inhibitory levels.
- Also, if many cycles are run, the accumulation of non-producing or low-producing variants may result.
- The quantities of the components to control must be above the detection limits of the available measuring equipment.

3) Continuous fermentation:

- The products are removed continuously along with the cells and the same is replenished with the cell growth and addition of fresh culture media.
- The concept of control in fermentation has its root from chemostat.
- Initially start as batch culture.
- when reaches exponential phase, extended by continuous addition fresh fermentation medium.
- The reactor is continually stirred and constant volume maintained results rate of microbial cell growth equals to rate at which cells displaced from vessel.
- Fermentation growth rate is proportional to dilution rate of the medium.
- Used in the production of SCP, Antibiotics, Organic solvents.

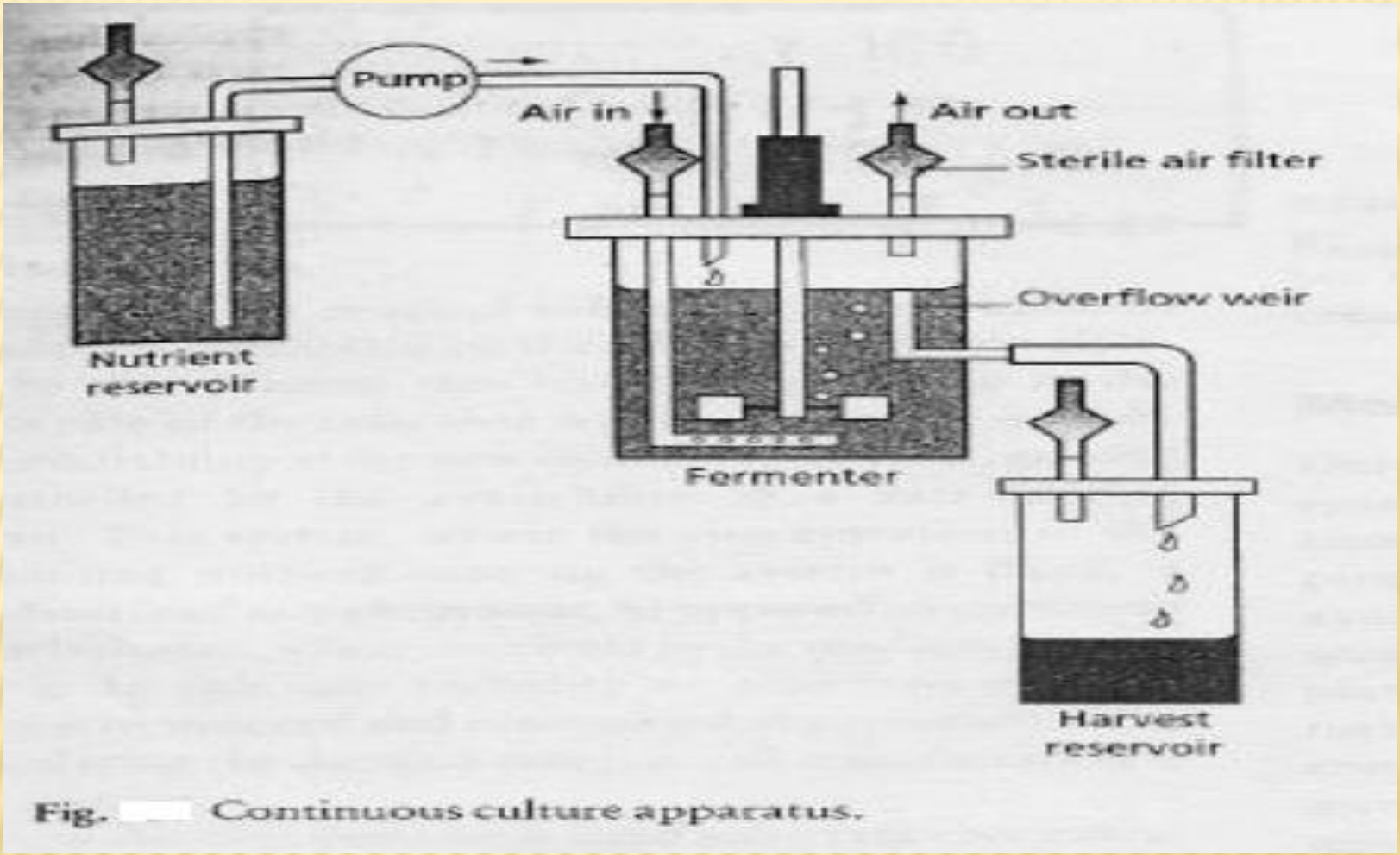


Fig. Continuous culture apparatus.

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

This equals number of reactor volumes passing through reactor per unit time and is expressed in reciprocal time per hour.

$D = F/V$

Where,

D – Dilution rate (per hour)

F – Flow (l/h)

V – reactor volume (L)

Addition of medium controlled at constant level, so growth rate and cell lose determined.

Under steady state condition, net bio mass balance described as

$dx / dt = \mu x - Dx$

$\mu x = Dx$ (bcoz at steady stae growth rate = loss of cells, $dx/dt=0$)

$\mu = D$

i.e, growth rate depends on dilution rate maximum upto μmax

Advantages:

1.Shorter fermentation time. No yeast lag phase .

2.Improved alpha acid utilization (less loss to yeast) 10-20%.

3.Very consistent product, Less system downtime.

4.Savings on cleaning.

Disadvantages:

1. less scope for different products

2. Less scope to respond to seasonal and short term variations in demand

3. Difficult to maintain microbiological purity but very important to do so.

4. Risk of yeast culture changing overtime is greater (can’t go back to pure culture every tenth batch or so).

5. has to operate seven days a week.

Example of continuous fermentation technology

The fermenter or bioreactor: Stomach (G.I. tract)
The substrate: Food ; The specific temperature: 37°C ; The organism: Enzymes, microbes, acids etc. ; The Incubation: 37°C for 3-4 hours ;The aeration: Anaerobic

The process:

- The food eaten by us is stored in the stomach where HC1 and some enzymes are secreted that convert food into chyme (semi solid).
- The food stays for 3-4 hours at 37° C.
- The digested food is absorbed in the blood and the undigested food is excreted.
- This is a continuous fermentation because the substrate (food) is continuously added and the products (digested/undigested material) are continuously removed.
- Stomach-the fermenter is a stirred type i.e. the peristaltic movement of the gastro-intestinal tract mixes the food.

CATEGORIES OF FERMENTATION TECHNOLOGY

1. MICROBIAL BIOMASS PRODUCTION:

- Four types of microorganisms are used to produce biomass: bacteria, yeasts, fungi and algae.
- Choice of a microorganism depends on numerous criteria, the most important of which is the nature of the raw material available.
- The other criteria are:
  - nutritional: energy value, protein content, amino acid balance;
  - technological: type of culture, nutritional requirements, type of separation;
  - toxicological.

The ideal microorganism should possess the following technological characteristics:

– high specific growth rate (m) and bio-mass yield (Yx/s);

– high affinity for the substrate;

– low nutritional requirements, i.e., few indispensable growth factors;

– ability to use complex substrates;

– ability to develop high cell density;

– stability during multiplication;

– capacity for genetic modification;

– good tolerance to temperature and pH.

In addition, it should have a balanced protein and lipid composition. It must have a low nucleic acid content, good digestibility and be non-toxic

In Yeasts:

- Their protein content rarely exceeds 60 %, their concentration in essential amino acids such as lysine (6 to 9 %), tryptophan and threonine is satisfactory.
- In contrast, they contain small amounts of the sulfur-containing amino acids methionine and cysteine their nucleic acid content ranges from 4 to 10 %.
- They can be used in a raw state.However, their specific growth rate is relatively slow (generation time 2 to 5 hours)

In Bacteria

- The specific growth rate and biomass yield of bacteria are greater than those of the other categories of microorganisms.
- Total protein content may reach 80 %. Their amino acid profile is balanced and their sulfur-containing amino acid and lysine concentrations are high.
- In contrast, their nucleic acid content(10 to 16 %) is greater than that of yeasts.
- A limited number of bacterial species can be used in foodstuffs as many are pathogenic.

In Fungi

- Their protein content (50 %) is often smaller than that of yeasts and bacteria, and they are deficient in sulfur-containing amino acids.
- There are also problems of wall digestibility.

In Algae

- Algae have a low sulfur-containing amino acid content.
- Their nucleic acid content is about 4 to 6 %. They are easy to recover, but multiplication is very slow.

2. Microbial metabolites:

- During the metabolism of microbial cells a number of compounds are produced and many are secreted out of the cell, which can be easily extracted and are very useful to man and animals.
- Therefore fermentation by microbial cells is carried out on an industrial scale, in order to get various metabolites.

(a) Primary metabolites:

- Metabolites which are produced by the metabolism required for the maintenance of the minimum life process of a microbe are known as primary metabolites.
- The primary metabolites are produced in abundance at an early stage of growth.
- Examples of primary metabolites are ethanol, citric, acid, glutamic acid, lactic acid, acetic acid, acetone, formic acid, butanol, propionic acid, dihydroxy-acetone, glycerol etc.
- These metabolites are produced by fermentation technology applying different microbes under varying conditions of fermentation.

(b) Secondary metabolites:

- Secondary metabolites are those metabolites, which are not produced directly by the metabolism required for the vital life process of microbes, instead are produced by some specialized metabolic process.
- However most of the secondary metabolites are derived from the primary metabolites.
- The secondary metabolites include the antibiotics, alkaloids, toxic pigments, vitamins etc.

3. Microbial enzymes:

- When microbes are cultured, they secrete some enzymes into the media.
- These enzymes are extracted and widely used in several industries like detergent, food processing, brewing and pharmaceutical.
- They are also used for diagnostic, scientific and analytical purposes.

- Some of the enzymes produced by fermenting microbes are Glucose oxidase, protease glucoamylase, amylase, glucose isomerase, rennin, pectinase, superox-ide dismutase, cellulase, invertase, lactase and lipase.
- Some thermophile bacteria produce enzymes that are thermo-stable and which can be used in indus-trial processes at high temperatures, ex. glyceraldehyde-3-phosphate dehydrogenase, phosphofructo-kinase, alcohol dehydrogenase, superoxide dismutase and restriction endonucleases.

4. Bioconversion

- The fermenting microbes have got the capacity to convert an added substrate into some more valuable product.
- Example: Conversion of ethanol to acetic acid (vinegar),

  isopropanol to acetone,

  glucose to gluconic acid,

  sorbitol to sorbose (this is used in the manufacture of vitamin C), sterols to steroids.

DOWN STREAM PROCESS (DSP)

- First of all, the broth is conditioned i.e. the cells are aggregated and form large clumps, which makes the separation easier.
- The conditioning is done by heating, freezing, pH change, antigen-antibody reactions etc.
- Then the conditioned broth is used for the separation of the constituents for which techniques like sedimen-tation, floatation, filtration, ultra-filtration, centrifugation and micro-filtration are applied.
- The final product is recovered.

THANK YOU