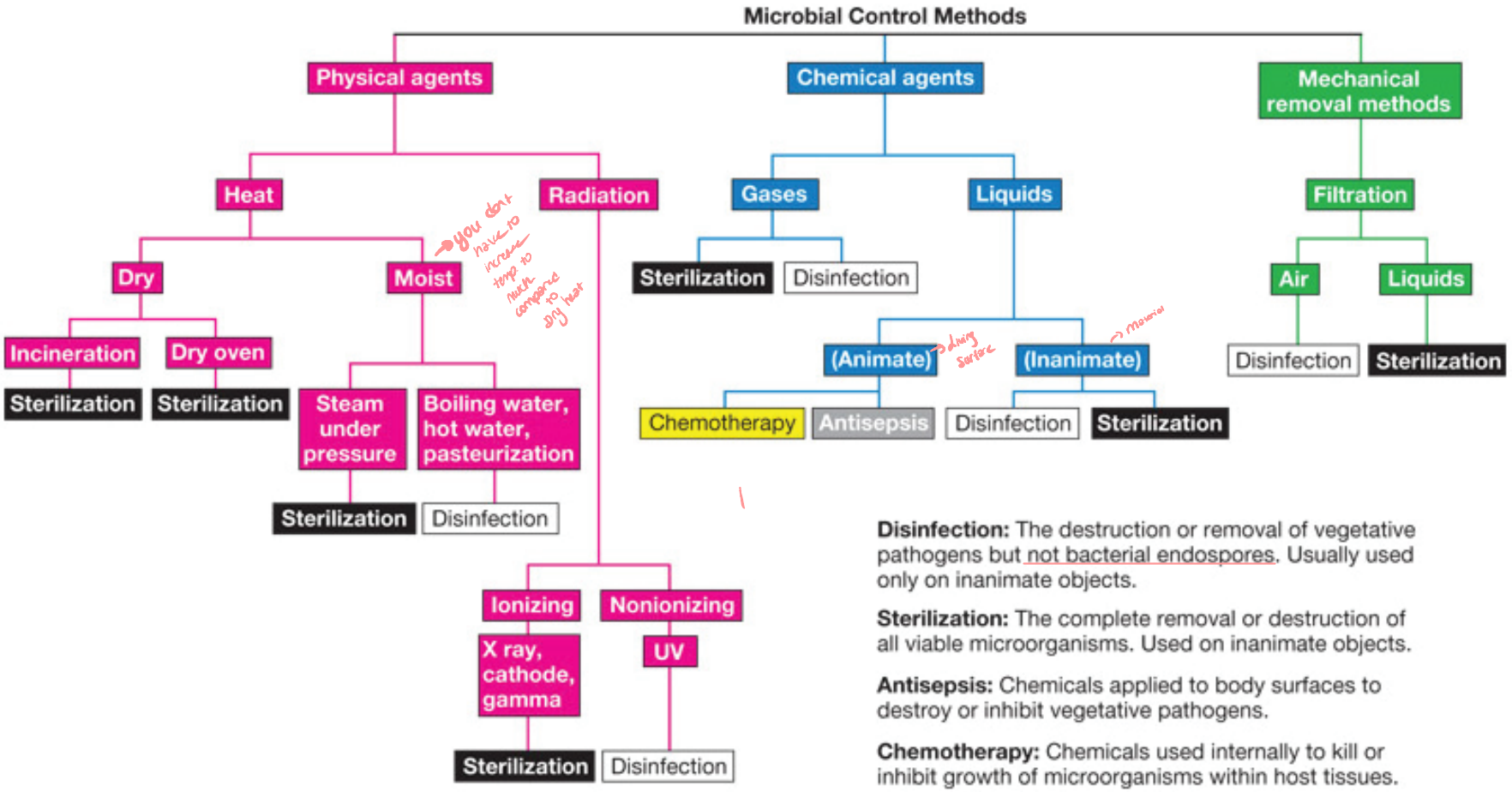
Environmental Microbiology

The Control of Microbial Growth

prepared by Prof. Bulent Icgen

The Control of Microbial Growth



Disinfection: The destruction or removal of vegetative pathogens but not bacterial endospores. Usually used only on inanimate objects.

Sterilization: The complete removal or destruction of all viable microorganisms. Used on inanimate objects.

Antisepsis: Chemicals applied to body surfaces to destroy or inhibit vegetative pathogens.

Chemotherapy: Chemicals used internally to kill or inhibit growth of microorganisms within host tissues.

The Control of Microbial Growth

Many bacteria cause disease and food spoilage: the need exists to kill or inhibit the growth of these bacteria

- Sterilization - removal or destruction of all living cells, viable spores, viruses and virions
- Disinfection - removal or destruction of pathogens (spores and some other microorganisms remain)
- Sanitization - reduction of microbial population to safe levels
- Antisepsis - prevention of infection (accomplished by antiseptics)
- Bactericide - substance that kills bacteria
- Bacteriostatic - substance that prevents growth of bacteria

The Pattern of Microbial Death

- Microorganisms are not killed instantly
- Population death usually occurs exponentially, slows down at later stages due to the survival of more resistant forms
- When do you consider a population to be dead?
  - microorganisms were previously considered to be dead when they did not reproduce in conditions that normally supported their reproduction
  - however we now know that organisms can be in a viable but non-culturable (VBNC) condition → we dont know the nutrients they need to grow (i.e. we can culture)
- Once they recover they may regain the ability to reproduce and cause infection

Physical Methods of Control

- Heat
- Low temperature
- Filtration
- Radiation

Heat

- Moist heat: steam sterilization → most effective.
  - Effective against all types of microorganisms; degrades nucleic acids, denatures proteins, and disrupts membranes
  - Autoclaves are used to kill endospores; uses steam under pressure to achieve temperatures above boiling

- Pasteurization: controlled heating at temperatures below boiling
  - Does not sterilize; kills pathogens and reduces levels of spoilage microorganisms, used for milk, beer, juice, etc.
  - Traditional method: 63 ºC for 30 minutes; flash pasteurization: 72 ºC for 15 seconds
  - Ultrahigh temperature (UHT) sterilization: milk heated at 140 to 150 ºC for 1 to 3 seconds. Products can be stored at room temperature for 1 to 2 months

- Dry heat sterilization → not common.
  - Less effective, requiring higher temperatures and longer exposure times (160-170°C for 2 to 3 hours)

Heat Killing

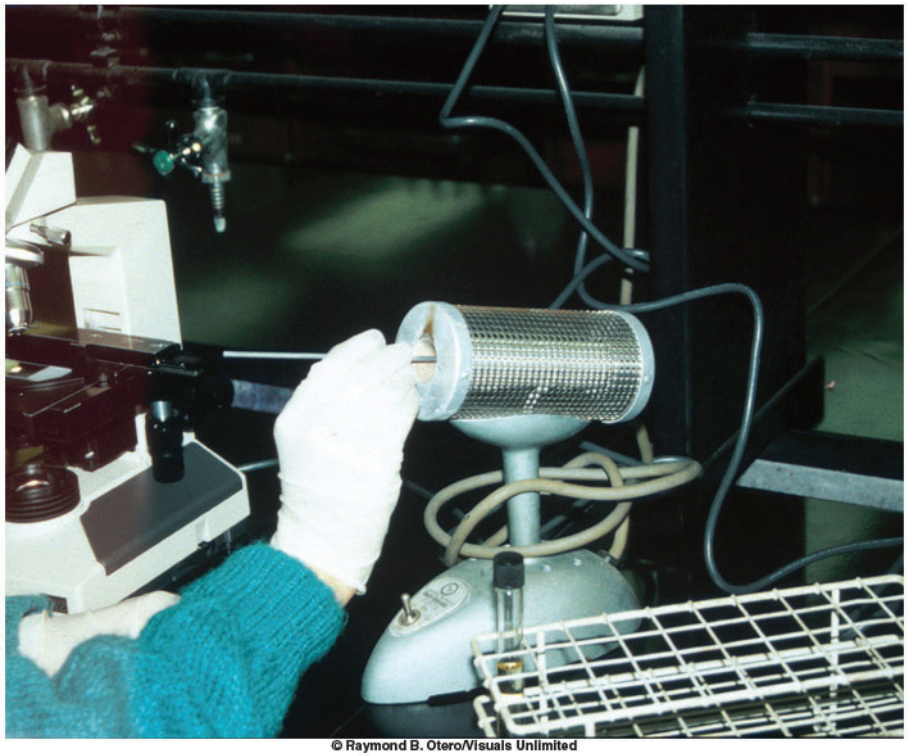
Table 7.2 Approximate Conditions for Moist Heat Killing

Organism	Vegetative Cells	Spores
Yeasts	5 minutes at 50–60°C	5 minutes at 70–80°C
Molds	30 minutes at 62°C	30 minutes at 80°C
Bacteria[a]	10 minutes at 60–70°C	2 to over 800 minutes at 100°C 0.5–12 minutes at 121°C
Viruses	30 minutes at 60°C	

[a]Conditions for mesophilic bacteria.

Dry Heat Incineration

- Bench top incinerators are used to sterilize inoculating loops used in microbiology laboratories



© Raymond B. Otero/Visuals Unlimited

Low Temperature → Preserving

↳ long term storing

- Refrigeration: storage at 4 ºC slows microbial growth (only used for short-term storage)

there is still growth but you can't notice

- Freezing: storage at - 20 ºC stops microbial growth (does not kill microorganisms)

- Freezing at -30 to -70 ºC used to preserve microbial samples

Filtration

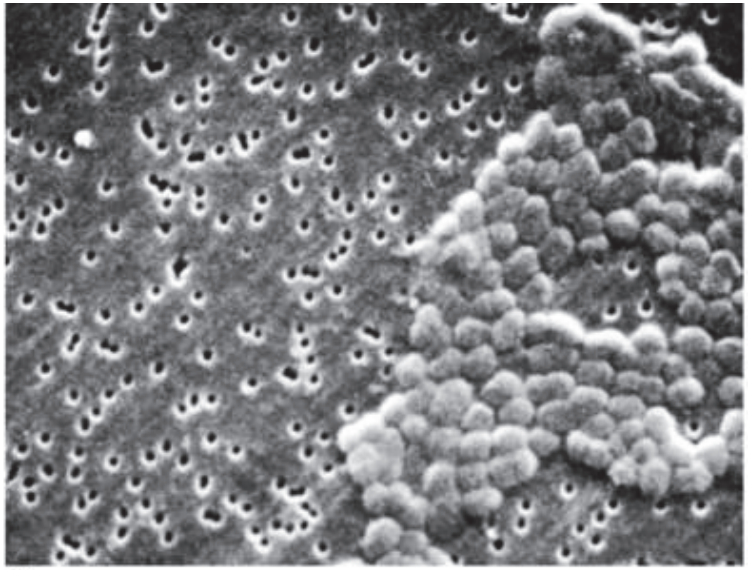
- Can be used to sterilized or reduce the microbial population of heat-sensitive liquids
- Removes microorganisms rather than destroying them !
- Solutions often forced through filters by pressure or a vacuum

(b)

Filtration

↳ under pressure

Membrane filters: Porous membrane about 0.1 mm thick; pore size of 0.2 um diameter removes most cells but not viruses



(b)

Air filtration

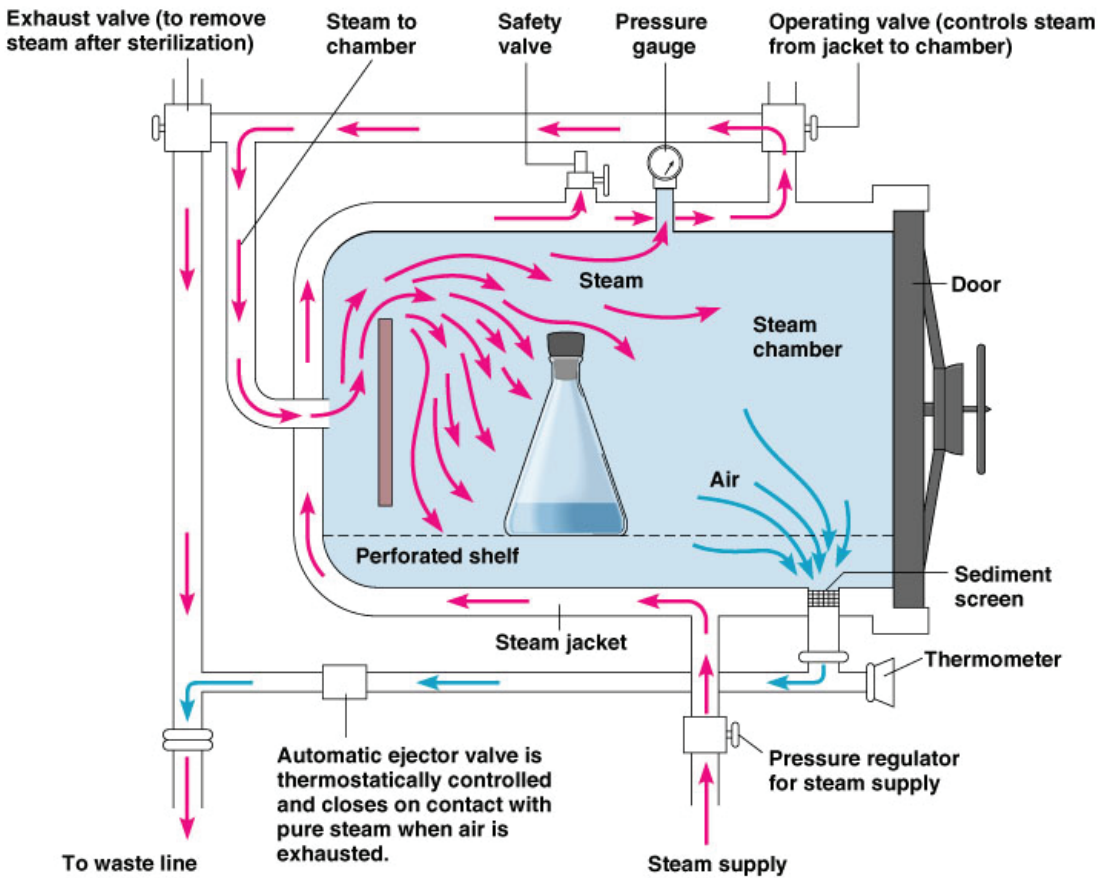
* Laminar flow biological safety cabinets: employ high efficiency particulate air (HEPA) filters that remove 99.97 % of particles larger than 0.3 um.

Actions of Microbial Control Agents

- Alternation of membrane permeability
- Damage to proteins
- Damage to nucleic acids

Heat

- Moist heat denatures proteins
- Autoclave: Steam under pressure



Physical Methods of Microbial Control

- Pasteurization reduces spoilage organisms and pathogens
- Equivalent treatments
  - 63°C for 30 min
  - High-temperature short-time 72°C for 15 sec
  - Ultra-high-temperature: 140°C for <1 sec
  - Thermoduric organisms survive

Physical Methods of Microbial Control

- Dry Heat Sterilization kills by oxidation
  - Flaming
  - Incineration
  - Hot-air sterilization → for bioreactors.

	Hot-air	Autoclave
Equivalent treatments	170˚C, 2 hr	121˚C, 15 min

Physical Methods of Microbial Control

- Filtration removes microbes
- Low temperature inhibits microbial growth
  - Refrigeration
  - Deep freezing
  - Lyophilization
- High pressure denatures proteins
- Desiccation prevents metabolism
- Osmotic pressure causes plasmolysis

Physical Methods of Microbial Control

- Radiation damages DNA
  - Ionizing radiation (X rays, gamma rays, electron beams)
  - Nonionizing radiation (UV)
  - (Microwaves kill by heat; not especially antimicrobial)

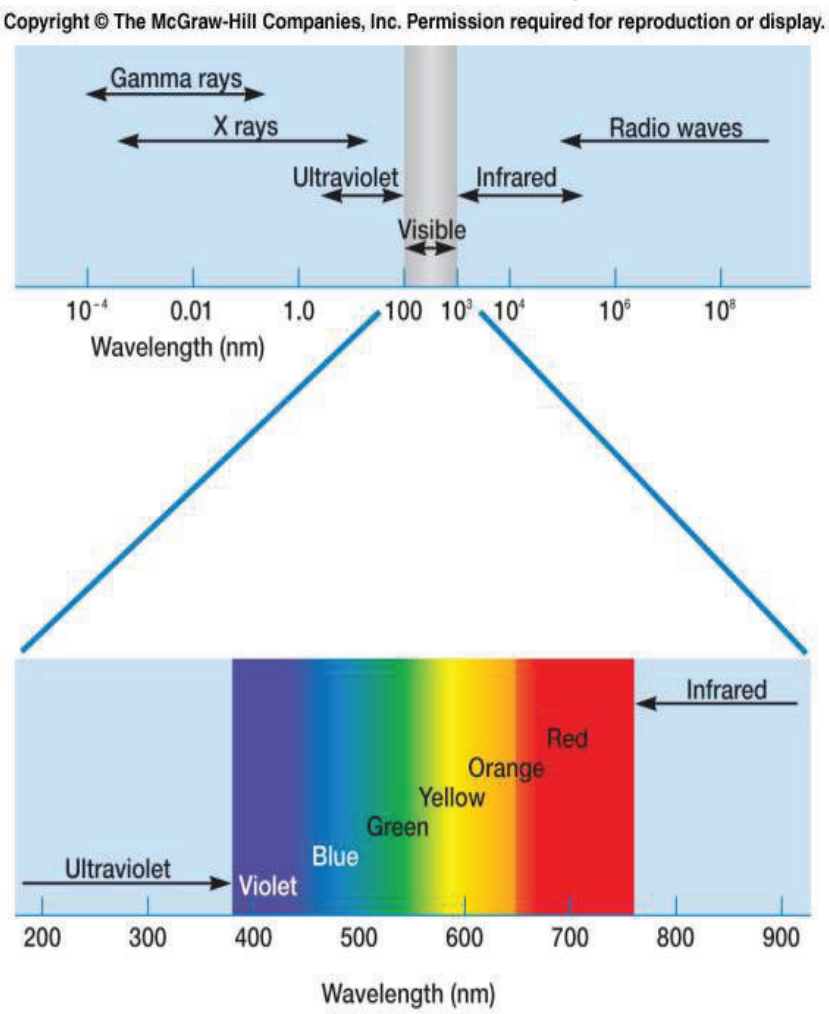
Radiation

Ultraviolet (UV) radiation

Near 260 nm; lethal but does not penetrate glass; used to sterilize air or exposed surfaces

Ionizing radiation

- Penetrates deep into objects, but not always effective against viruses
- Gamma radiation from Cobalt 60 often used
- Used to treat meat, fruits, vegetables and spices, antibiotics, hormones, plastic disposable supplies.



Chemical Methods of Microbial Control

- Principles of effective disinfection
  - Concentration of disinfectant
  - Organic matter
  - pH
  - Time

Commonly Used Agents for Disinfection and Antisepsis

Table 7.5

Relative Efficacy of Commonly Used Disinfectants and Antiseptics

Class	Disinfectant	Antiseptic	Comment
Gas			
Ethylene oxide	3–4[a]	0[a]	Sporicidal; toxic; good penetration; requires relative humidity of 30% or more; microbicidal activity varies with apparatus used; absorbed by porous material; dry spores highly resistant; moisture must be present, and presoaking is most desirable
Liquid			
Glutaraldehyde, aqueous	3	0	Sporicidal; active solution unstable; toxic
Stabilized hydrogen peroxide	3	0	Sporicidal; use solution stable up to 6 weeks; toxic orally and to eyes; mildly skin toxic; little inactivated by organic matter
Formaldehyde + alcohol	3	0	Sporicidal; noxious fumes; toxic; volatile
Formaldehyde, aqueous	1–2	0	Sporicidal; noxious fumes; toxic
Phenolic compounds	3	0	Stable; corrosive; little inactivation by organic matter; irritates skin
Chlorine compounds	1–2	0	Fast action; inactivation by organic matter; corrosive; irritates skin
Alcohol	1	3	Rapidly microbicidal except for bacterial spores and some viruses; volatile; flammable; dries and irritates skin
Iodine + alcohol	0	4	Corrosive; very rapidly microbicidal; causes staining; irritates skin; flammable
Iodophors	1–2	3	Somewhat unstable; relatively bland; staining temporary; corrosive
Iodine, aqueous	0	2	Rapidly microbicidal; corrosive; stains fabrics; stains and irritates skin
Quaternary ammonium compounds	1	0	Bland; inactivated by soap and anionics; compounds absorbed by fabrics; old or dilute solution can support growth of gram-negative bacteria
Hexachlorophene	0	2	Bland; insoluble in water, soluble in alcohol; not inactivated by soap; weakly bactericidal
Chlorhexidine	0	3	Bland; soluble in water and alcohol; weakly bactericidal
Mercurial compounds	0	±	Bland; much inactivated by organic matter; weakly bactericidal

Source: From Seymour S. Block, *Disinfection, Sterilization and Preservation.* Copyright © 1983 Lea & Febiger, Malvern, Pa. 1983. Reprinted by permission.

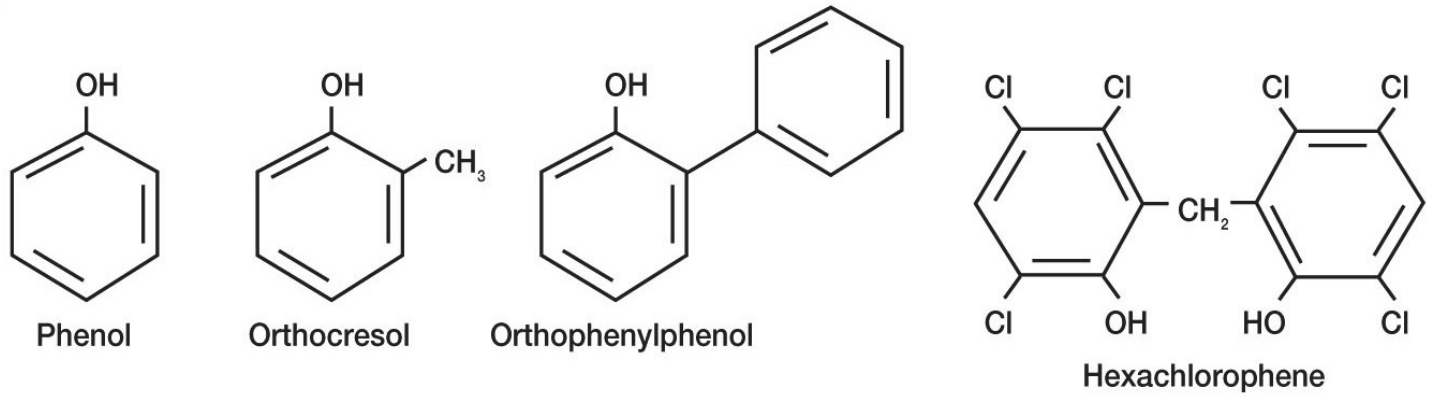
[a]Subjective ratings of practical usefulness in a hospital environment—4 is maximal usefulness; 0 is little or no usefulness; ± signifies that the substance is sometimes useful but not always.

Chemical Methods of Microbial Control

- Evaluating a disinfectant
  - Use-dilution test
    1. Metal rings dipped in test bacteria are dried
    2. Dried cultures placed in disinfectant for 10 min at 20°C
    3. Rings transferred to culture media to determine whether bacteria survived treatment

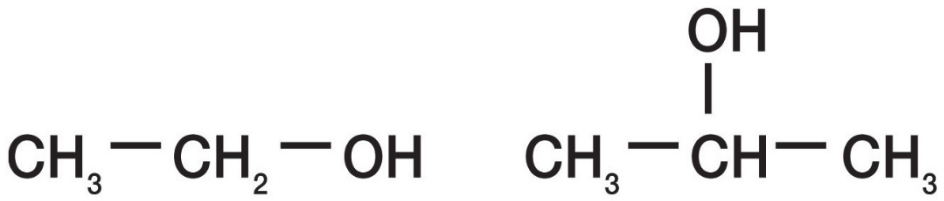
Phenolics

- Phenol first used by Lister
- Phenol and derivatives used as disinfectants in hospitals and labs
- Effective in the presence of organic material
- Can cause skin irritation
- Denature proteins and disrupt cell membranes



Alcohols

- Not effective against spores or lipid-containing viruses
- Ethanol and isopropanol most commonly used (at 70-80 %)
- Act by denaturing proteins and possibly dissolving membrane lipids



Ethanol

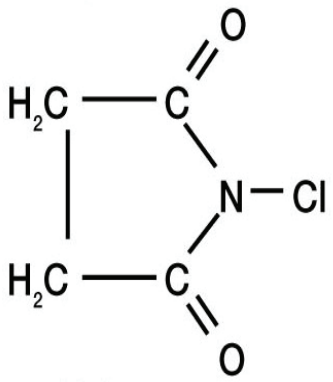
Isopropanol

Halogens

- Include fluorine, chlorine, bromine, iodine and astatine
- Iodine used as a skin disinfectant
- Chlorine used to disinfect water
- Both act by oxidizing cell material and iodinating or chlorinating molecules

-Iodophore

iodine complexed with organic carrier



Halazone

Halogens…

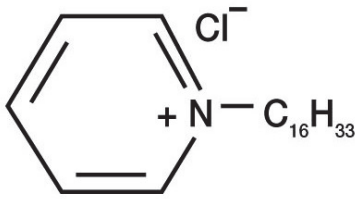
Chlorine

- oxidizes cell constituents
- important in disinfection of water supplies and swimming pools, used in dairy and food industries, effective household disinfectant
- destroys vegetative bacteria and fungi, but not spores
- can react with organic matter to form carcinogenic compounds

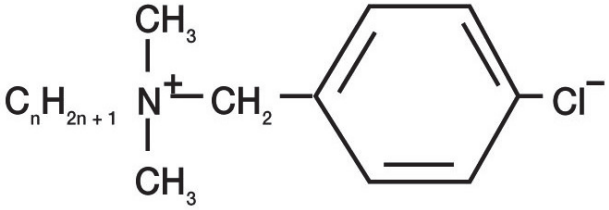
Heavy Metals

- e.g., ions of mercury, silver, arsenic, zinc, and copper
- effective but usually toxic
- combine with and inactivate proteins; may also precipitate proteins

Quaternary Ammonium Compounds



Cetylpyridinium chloride



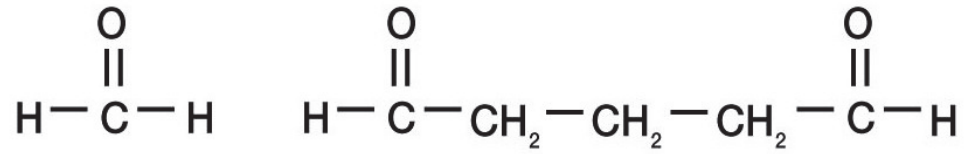
Benzalkonium chloride

- Detergents
  - organic molecules with hydrophilic and hydrophobic ends
  - act as wetting agents and emulsifiers
- Cationic detergents are effective disinfectants
  - kill most bacteria, but not *Mycobacterium tuberculosis* or endospores
  - safe and easy to use, but inactivated by hard water and soap

→ they don't have cell wall

Aldehydes

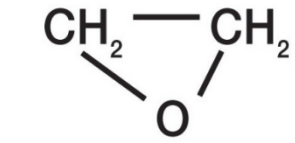
- Formaldehyde and glutaraldehyde are the most commonly used
- Are highly reactive molecules
- Inactivate proteins and DNA by cross-linking alkylating molecules



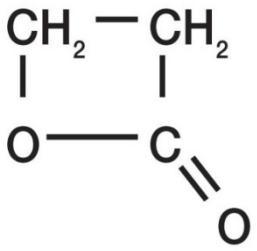
Formaldehyde

Glutaraldehyde

Sterilizing Gases



Ethylene oxide



Betapropiolactone

- Used to sterilize heat-sensitive materials
- Microbicidal and sporicidal
- Combine with and inactivate proteins

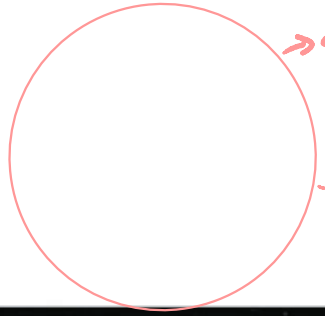
Types of Disinfectants

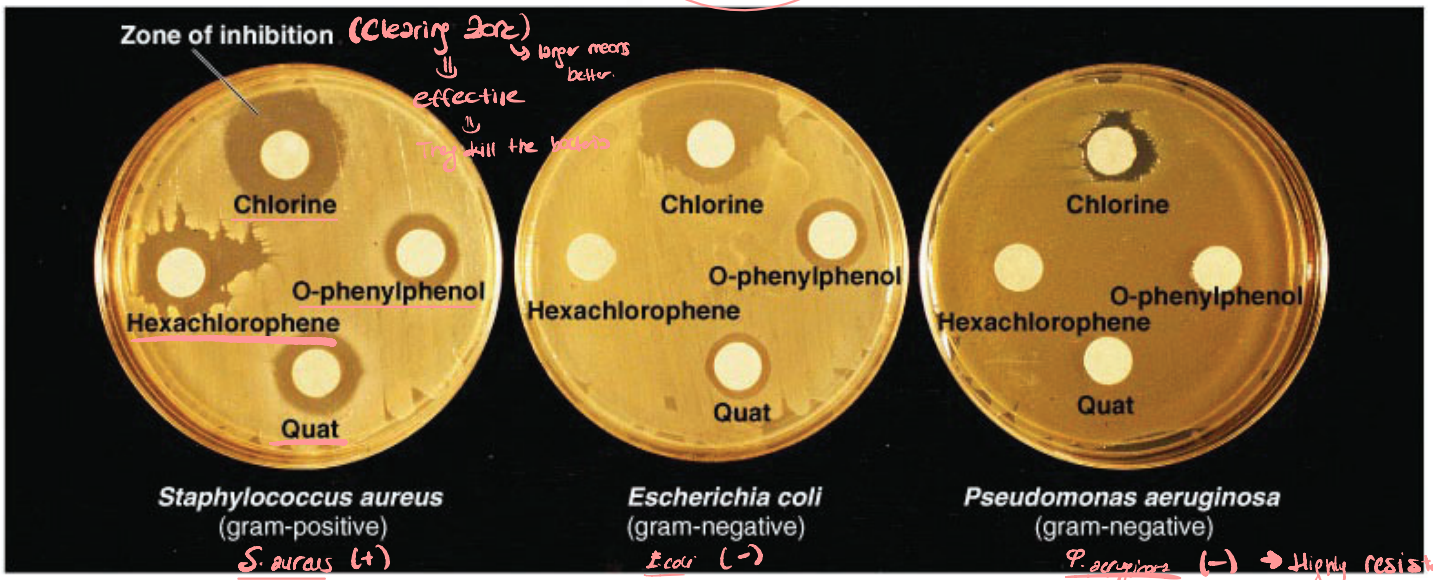
- Peroxygens
  - Oxidizing agents
  - O_3, H_2O_2, peracetic acid

ozone hydrogen peroxide

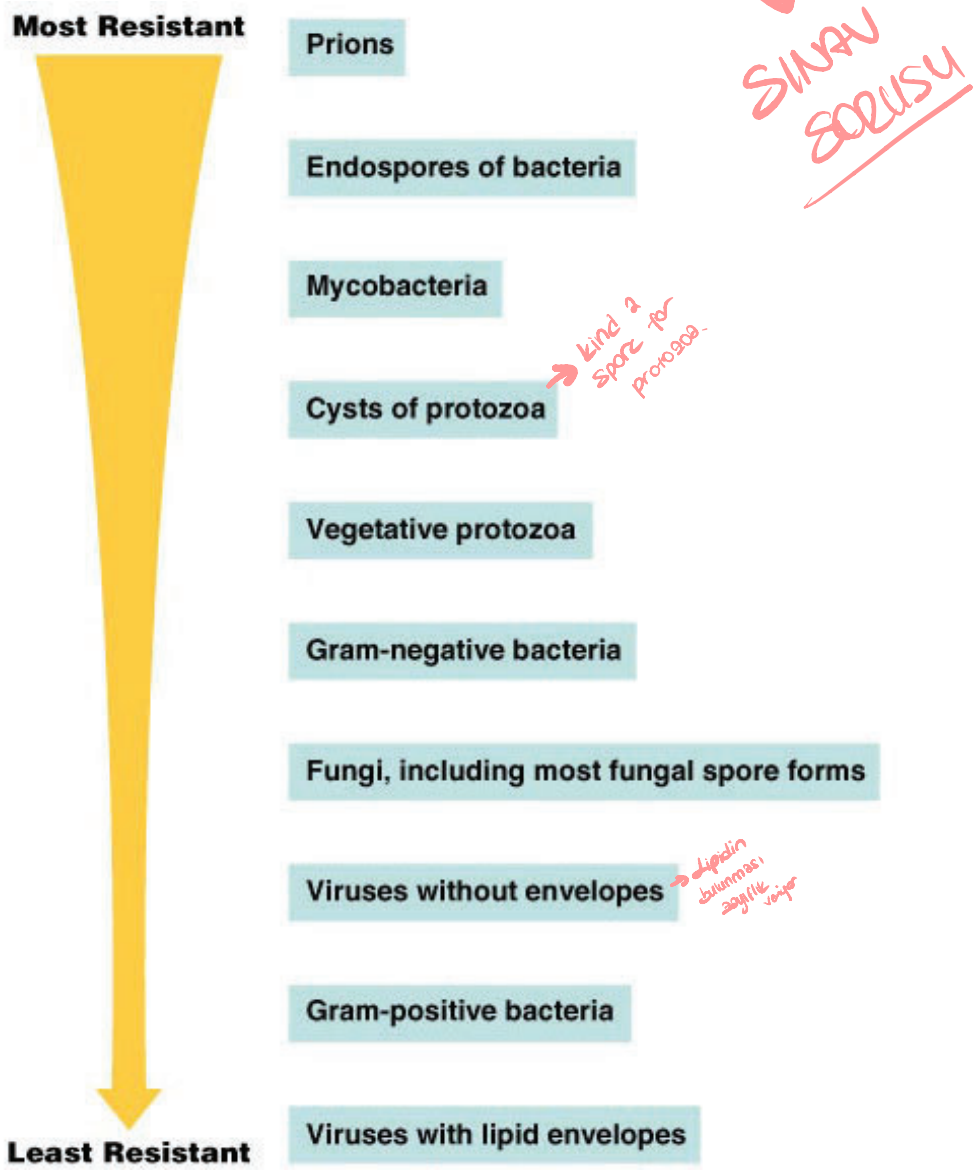
Chemical Methods of Microbial Control

- Evaluating a disinfectant
  - Disk-diffusion method





Microbial Characteristics and Microbial Control

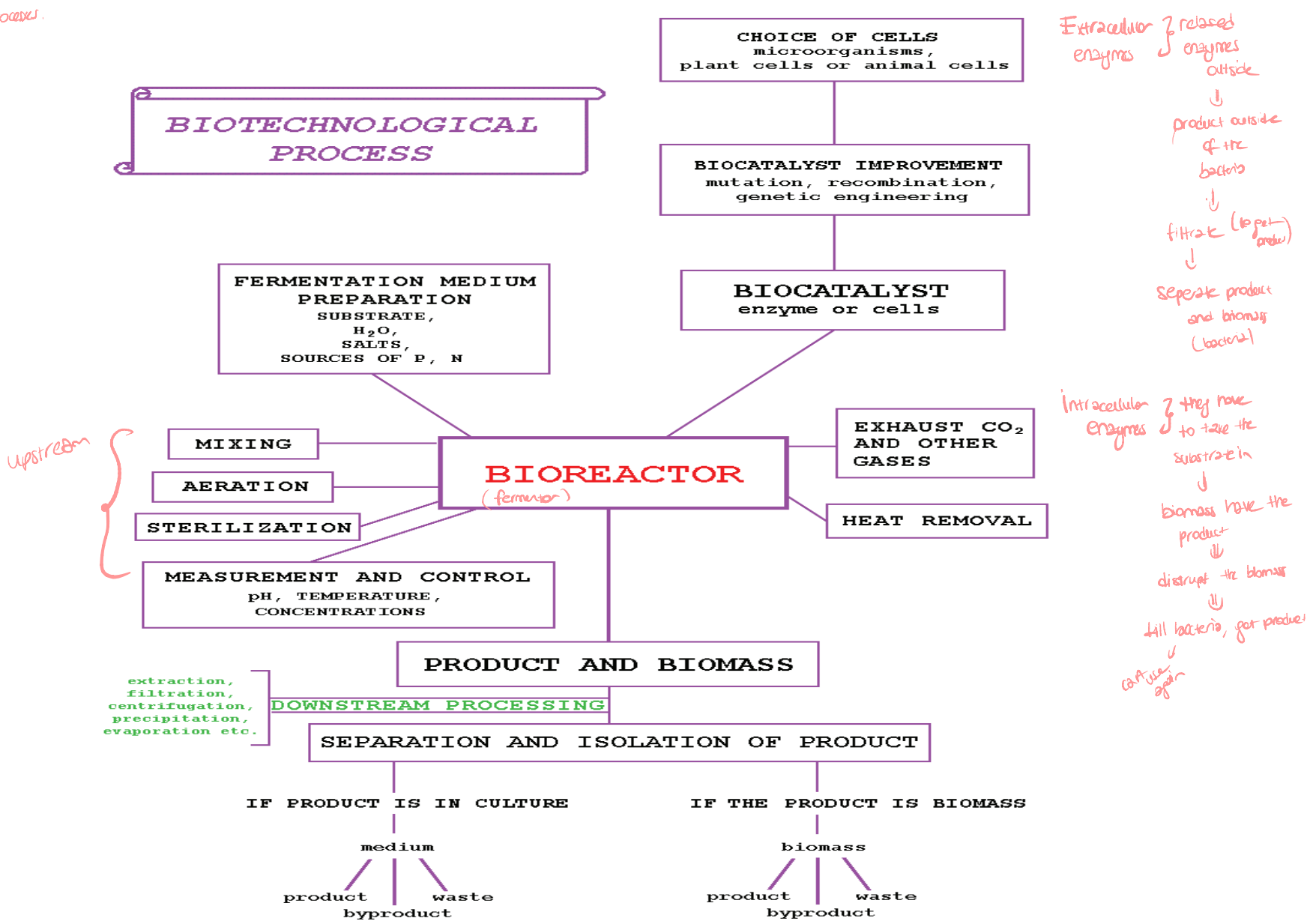


Environmental Microbiology

Basis Operations in Industrial Fermentations

prepared by Prof. Bulent Icgen

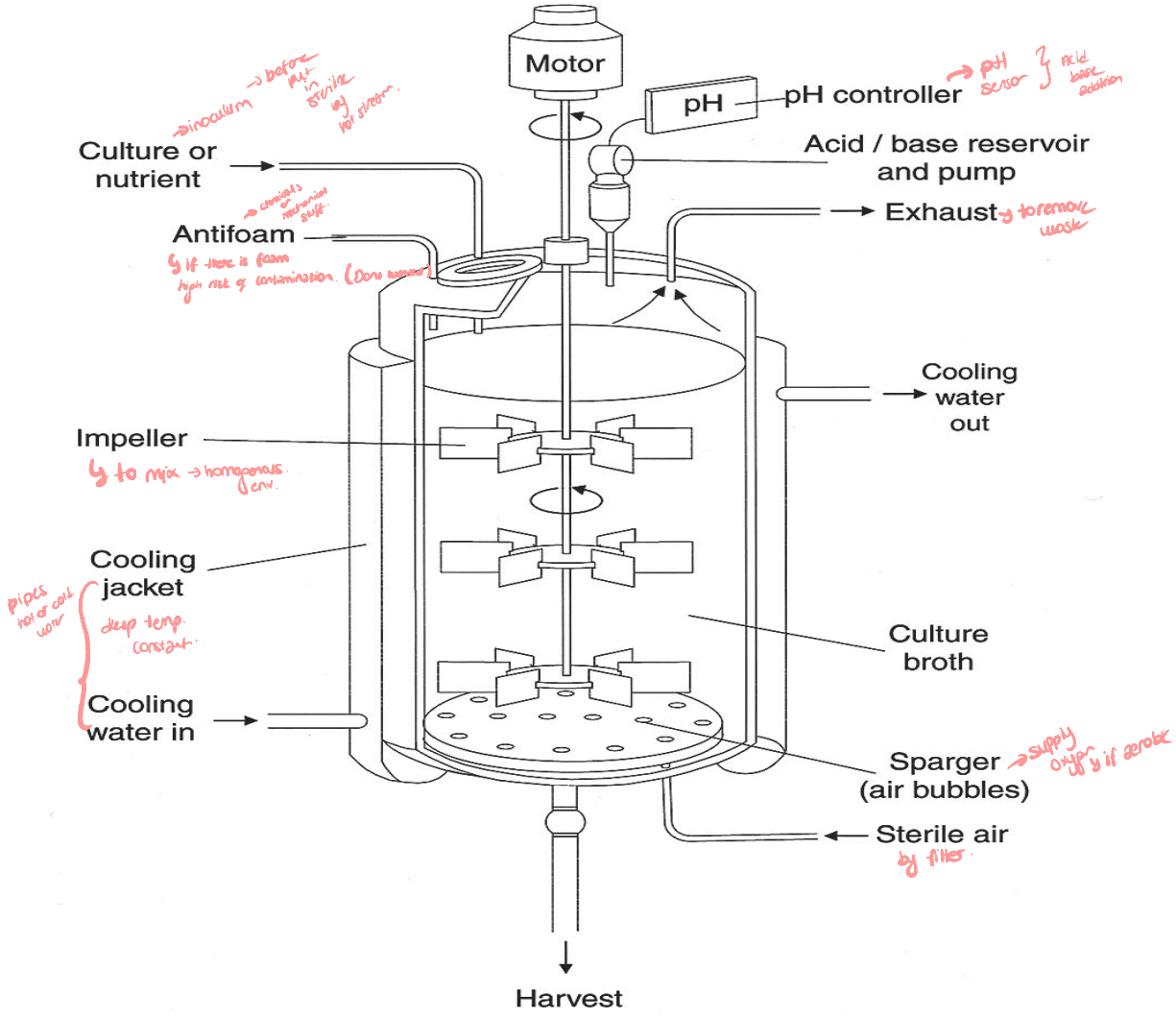
Basis Operations in Industrial Fermentations



What Is a Bioreactor?

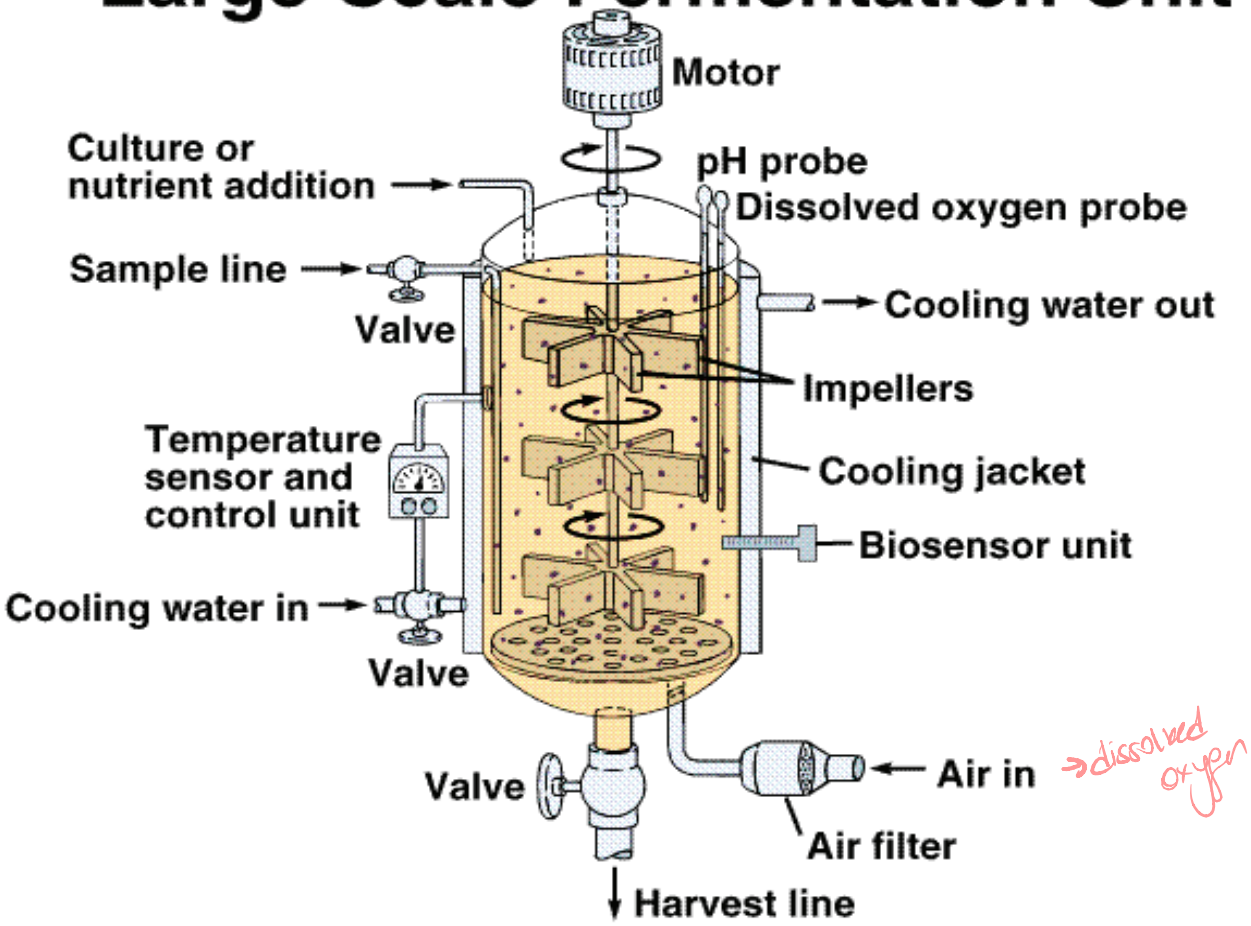
Bioreactor or fermentor is a container in which substrate will turn into product by the action of biocatalyst. It can be described as the heart of a bioprocess since conversion occurs here.

- Bioreactors (or fermentors) may be **liquid** (**submerged)** or **solid state** (**surface)**.
- Most fermentors used in industry are of the submerged type, because the submerged fermentor saves space and is more amenable to engineering control and design.
- The discussions in most of the applications will be therefore be on submerged fermentors; solid state fermentors will be discussed at the end of the chapter.



The basic components of a fermenter that would be employed in commercial production of antibiotics. Fermenters range from a few litres in size to 100,000 litres.

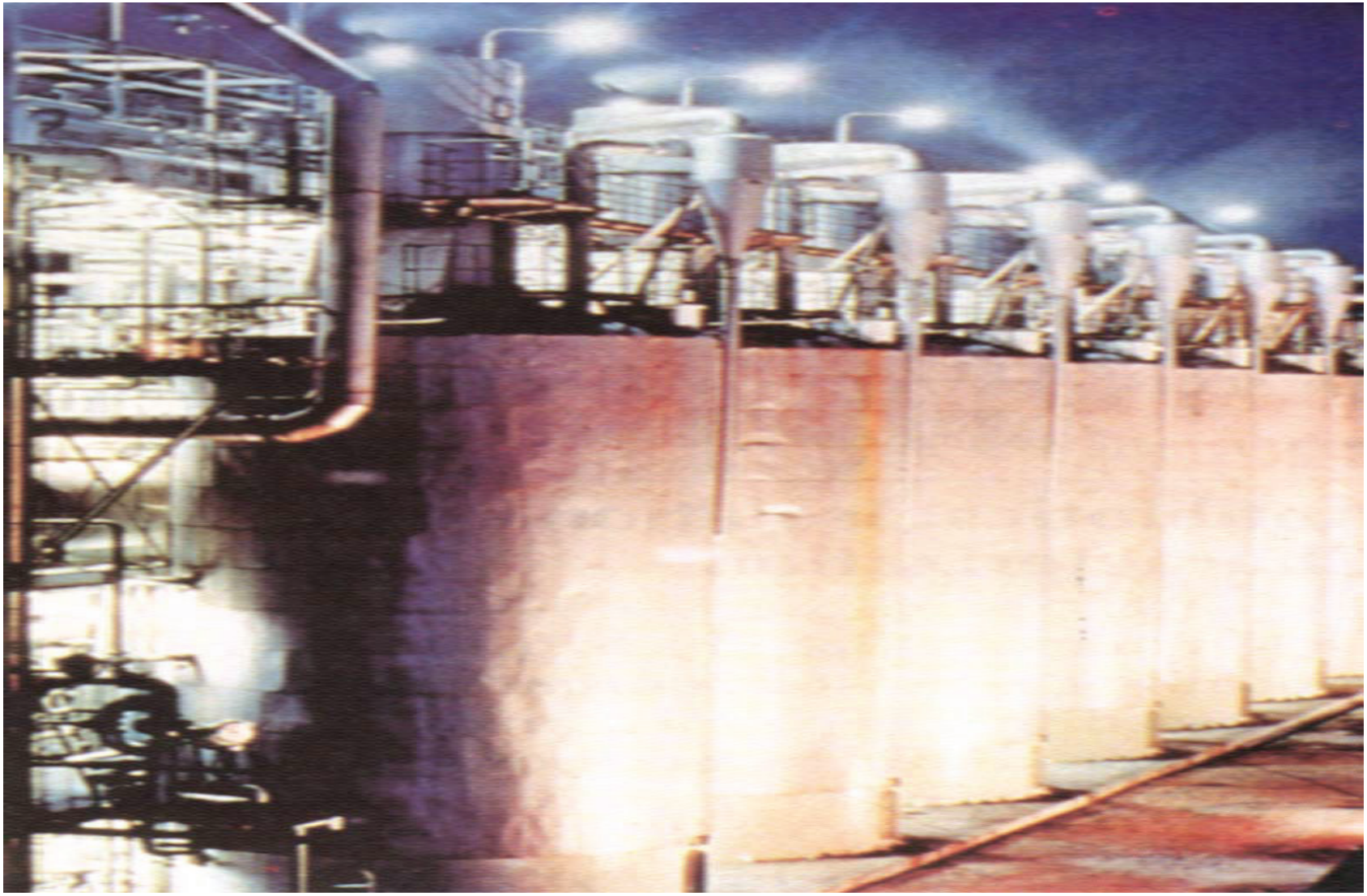
Large-Scale Fermentation Unit



→ dissolved oxygen → you supply in gas but you need in liquid form.

Lab scale fermentor

Large Scale Fermentor

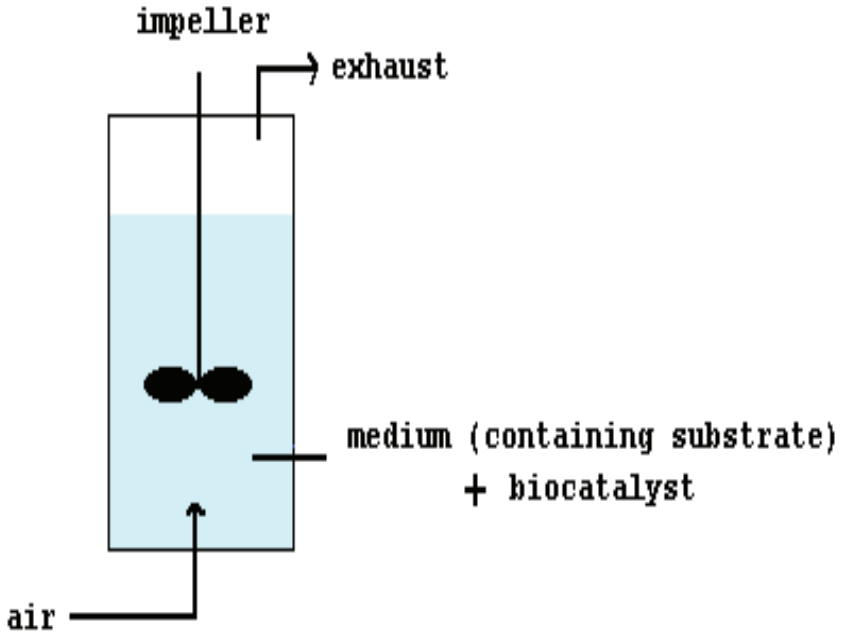


The Mode of Bioprocess Operations

- Batch operations
- Fed-batch operation
- Continuous operations
- Semi-continuous operation

Batch Operations

In batch operations, reactor is filled with medium and then inoculated with biocatalyst (enzyme or cells). Reaction proceeds, substrate is consumed and products are formed. Finally reactor is opened, product is taken and purified.



Batch Operations

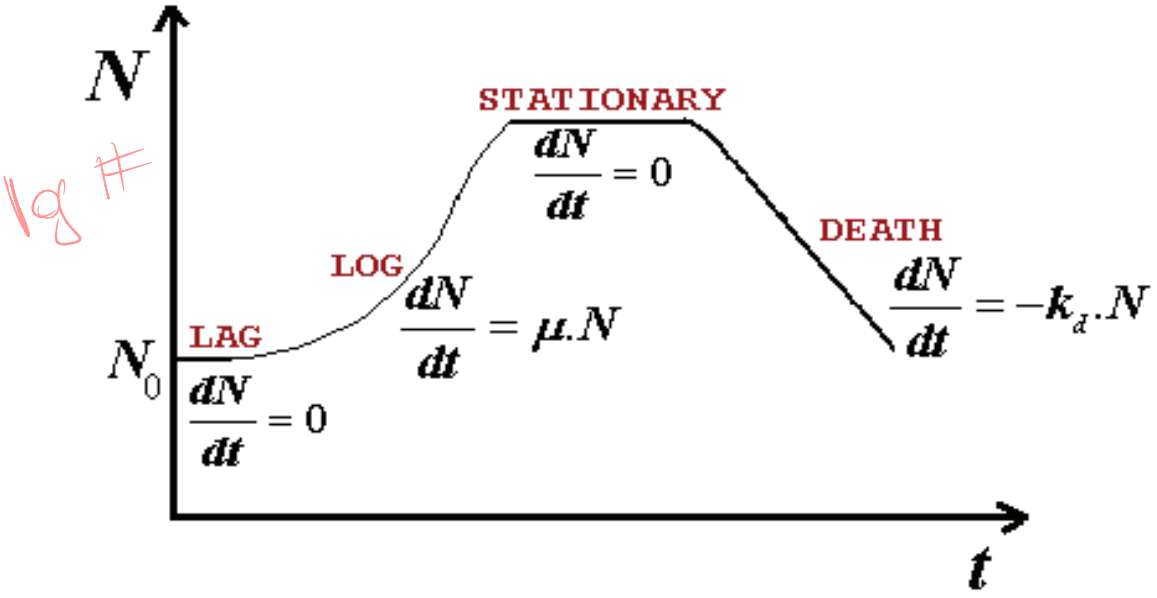
•Once operation started there is no inlet or outlet component. Contents are mixed to provide homogeneity. However, if it is aerobic system there should be gas inlet (aeration) and gas outlet (exhaust).

•Between each batch run, operation stopped in order to harvest products. This is called as "down" time. During down time bioreactor is empty, cleaned, sterilized and refilled. This lowers the efficiency of operation

Batch Operations

Growth kinetics of batch culture

The number of living cells varies with time in a batch system as shown below:



where;

N = number of bacteria at any time t in the reactor

t = time

N_0 = initial number of bacteria in the reactor after inoculation

Batch Operations

Lag Phase:

Number of bacteria does not change with time in lag phase.

$$\frac{dN}{dt} = 0$$

Log Phase:

Number of bacteria increases exponentially in log phase.

$$\frac{dN}{dt} = \mu N$$

where;

μ is specific growth rate

Batch Operations

Stationary Phase:

There is no net change in number of bacteria with time in stationary phase. Bacteria divide but also die at equal rate. Most of the important biological products (especially secondary metabolites like antibiotics) are produced during this phase.

Death Phase:

Number of bacteria decreases with time.

$$\frac{dN}{dt} = -k_d . N$$

Batch Operations

During log phase the number of organisms in the reactor at any time t can be calculated, by using rate equation shown below:

$$\frac{dN}{dt} = \mu . N$$

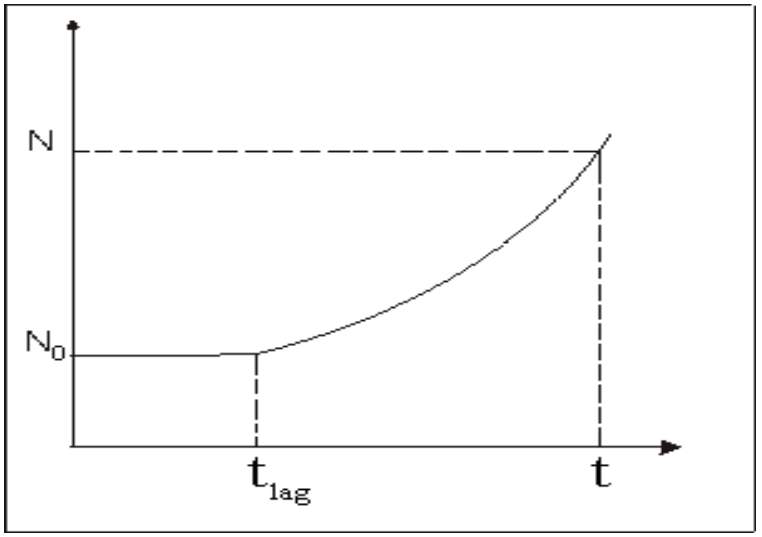
Batch Operations

This rate equation can be integrated:

$$\int_{N_0}^{N} \frac{dN}{N} = \int_{t_{lag}}^{t} \mu . dt$$

$$ln \frac{N}{N_0} = \mu.(t-t_{lag})$$

$$N = N_0.e^{\mu.(t-t_{lag})}$$



where;

N_0 : initial number of bacteria at t_0 (starting time)

t_{lag} : time where lag phase ends

According to last equation, number of bacteria in the reactor at any time t during log phase can be calculated, as it is seen in the graph.

Batch Operations

$$\int_{N_0}^{N} \frac{dN}{N} = \int_{t_0}^{t} \mu . dt$$

$$ln \frac{N}{N_0} = \mu . t$$

Doubling time:

Time required for doubling the number of bacteria in the reactor.

t_d : doubling time

$N = 2N_0 \Longrightarrow \ln 2 = \mu . t_d$

Therefore;

$$t_d = \frac{ln2}{\mu}$$

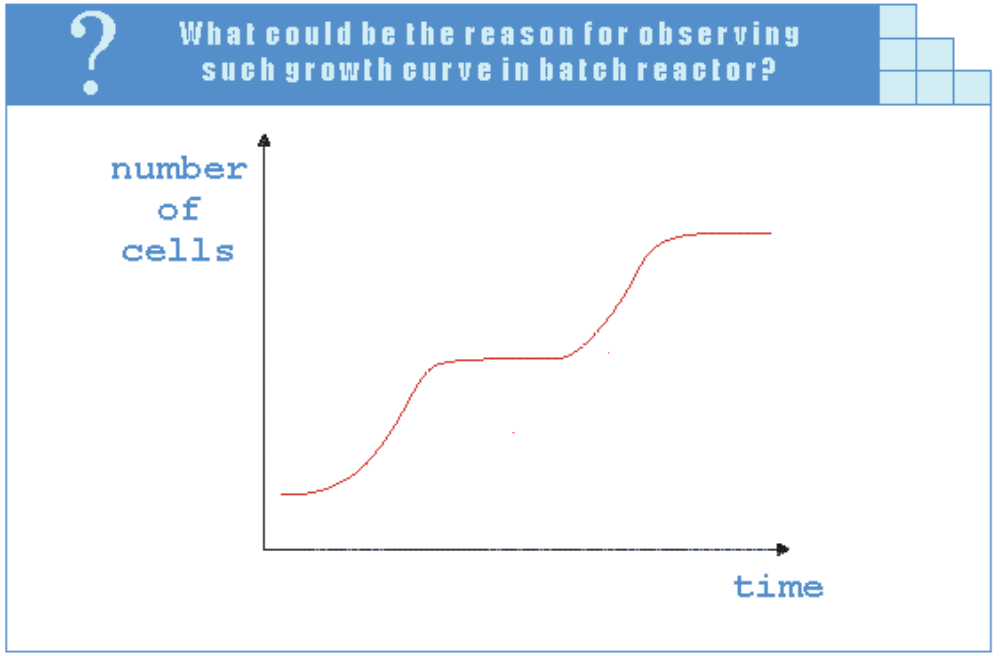
μ can be calculated from this equation when t_d is determined experimentally.

Fed-Batch Cultivation

- Fed-batch cultivation is a modification of batch cultivation in which the nutrient is added intermittently to a batch culture.
- It was developed out of cultivation of yeasts on malt, where it was noticed that too high a malt concentration lead to excessively high yeast growth leading to anaerobic conditions and the production of ethanol instead of yeast cells.

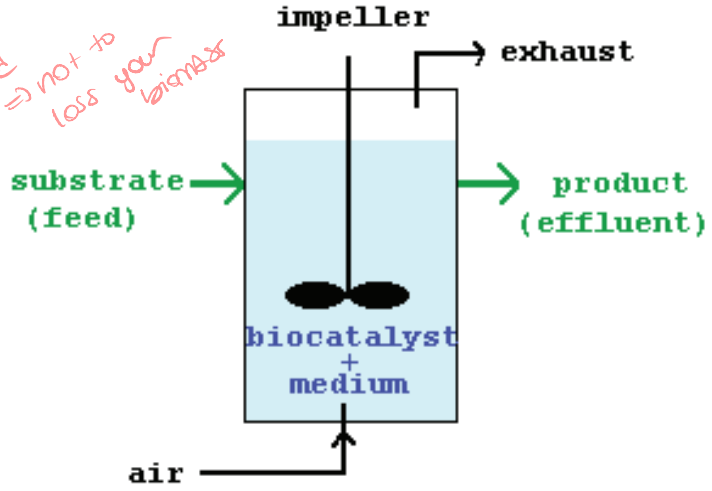
Diauxic Growth

caused by shift in metabolic patterns in the middle of the growth. After one carbon source is utilized, the cell divert its energy for synthesizing the enzymes necessary to utilize the other carbon source. Therefore, a second lag phase is observed .



Continuous Operations

In continuous operations, there is a continuous medium flow through the reactor. Incoming stream (feed) contains the substrate and leaving stream (effluent) contains the product.



Enzyme or cells can be immobilized inside the reactor, therefore they do not need to be added in the feed and also they don't leave the reactor in the effluent.

Types of Continuous Culture

Continuous culture: an open-system microbial culture of fixed volume

- Chemostat: most common type of continuous culture device

Both growth rate and population density of culture can be controlled independently and simultaneously. Dilution rate: rate at which fresh medium is pumped in and spent medium is pumped out(mean cell residence time or hydraulic retention time 'HRT')

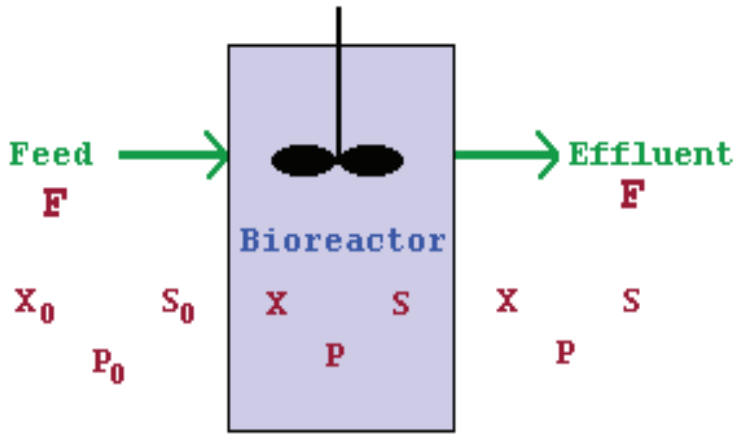
- Turbidostat - regulated by biomass using optical density (photoelectric cell)
- Biostat - regulated by biomass other than optical density (e.g CO_2 production)

Fermenter Configuration

- STR → Stirred Tank Reactor — most common
- Up-flow
- Plug-flow
- Single-stage
- Multi-stage
- Cell recycle

Continuously Stirred Tank Reactor (CSTR)

(STR)



F : volumetric flow rate of feed and effluent

S_0 : initial substrate concentration

P_0 : initial product concentration

S : substrate concentration at any time in the reactor

P : product concentration at any time in the reactor

X_0 : viable cell concentration in feed stream (i.e. g / l)

X : viable cell concentration in effluent and in reactor (i.e. g / l)

Continuous Operations

There are two assumptions for continuous operations

1- Mixing is vigorous such that concentrations are uniform throughout the reactor.

2- Effluent composition is same with reactor contents.

- Such continuous configurations for cultivation of microorganisms are called chemostat.
- After system operated, conditions in the vessel does not change through time, this is called as steady state.

Continuous Operations

In steady state continuous operations, there is no accumulation of components, because volumetric rates of feed and effluent are equal.

Otherwise; (Not steady state)

more feed than effluent = accumulation

more effluent than feed = depletion of source

- In the steady state where all concentrations within the vessel are "independent of time", the mass balance can be applied to any component of the system:
- **rate of addition to the system - rate of removal from system+ rate of production within the system = 0**

Advantages and Disadvantages of Batch and Continuous Operations

Batch Systems

- easy to operate and control
- genetic stability of organism could be controlled if it is genetically engineered biocatalyst.
- lower contamination risk
- non-productive down time is a disadvantage
- batch to batch variability is problem
- accumulation of inhibitory products is problem

Continuous Systems

- degeneration of biocatalyst
- higher contamination risk is a disadvantage
- efficient, higher productivity
- product is obtained with uniform characteristics; quality of the product is almost same from time to time
- no accumulation of inhibitory products

- Continuous systems are more efficient than batch operations
- Batch systems are preferred especially for pharmaceutical products because of ability to control bioreactor much closely; including the genetic stability of the organism used, if it's modified by rDNA techniques.

Kinetics And Technology of Nutrient Limitation

Type of culture;

- *Batch*; μ varies during culture cycle
- *Fed-batch*; μ is controlled or regulated after a certain time
- *Continuous*; μ is controlled

μ reflects the physiological state or intracellular environment

$\therefore$ control $\mu \Rightarrow$ control intracellular environment

Microbial Growth in a Chemostat

wash-out ⇒ losing biomass
(happens if you dilute system too much)
↓ to get avoid keep system steady-state condition

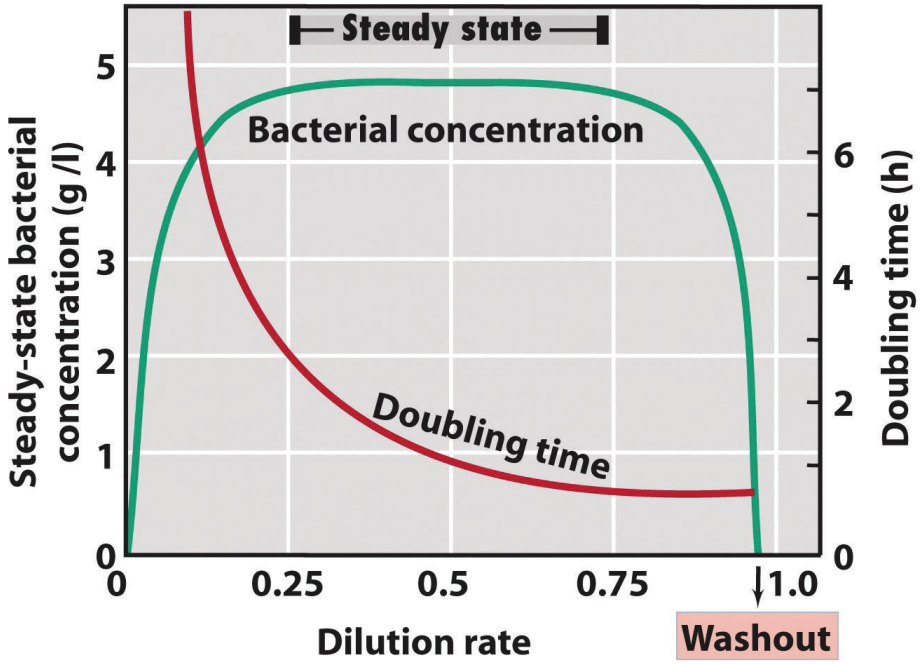
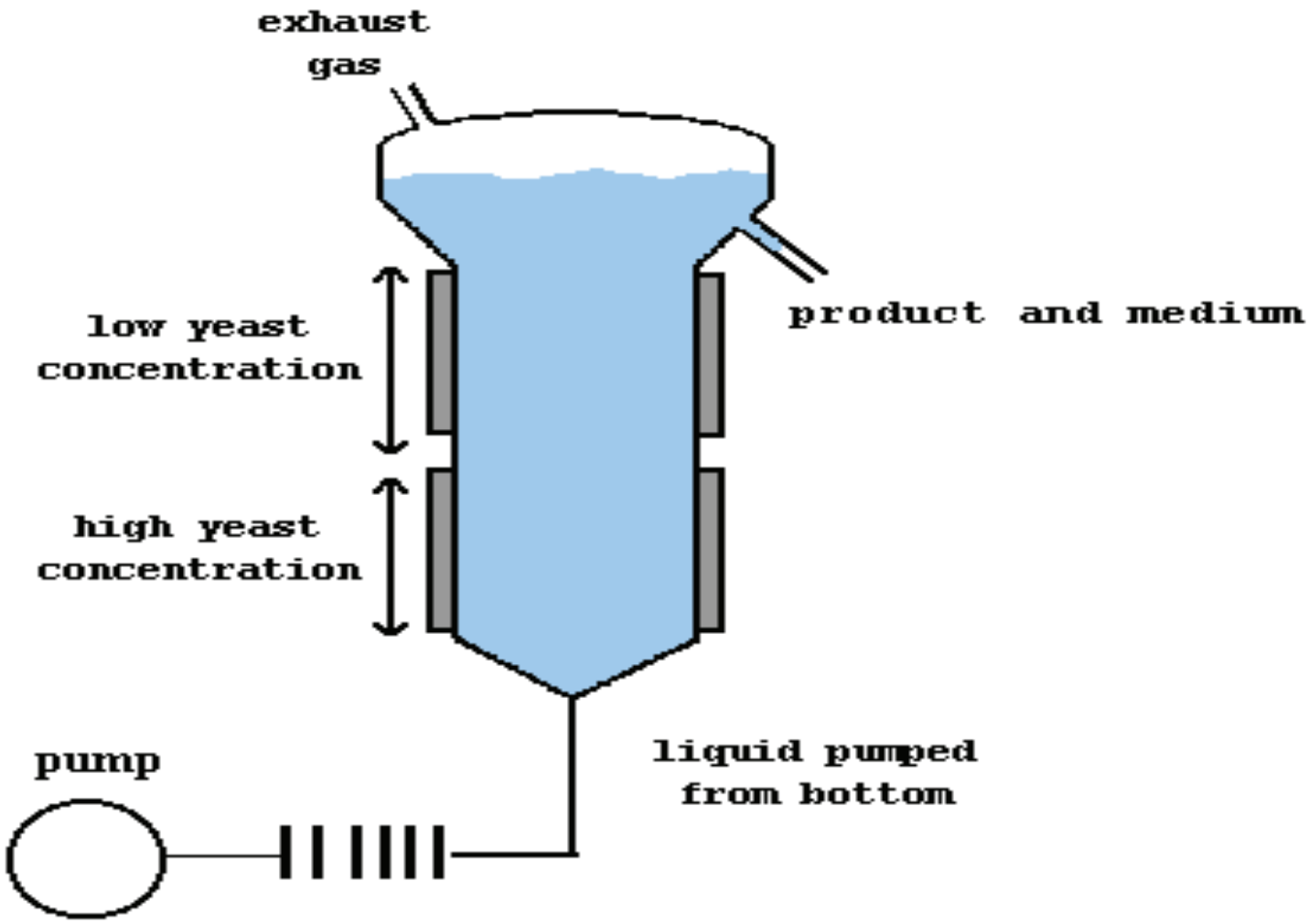


Figure 6-15 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

Chemostat cultures are sensitive to the dilution rate and limiting nutrient concentration

- At too high a dilution rate, the organism is washed out
- At too low a dilution rate, the cells may die from starvation
- Increasing concentration of a limiting nutrient results in greater biomass but same growth rate

Tower Reactor



- medium and gases moves upward, due to action of pump at the bottom.
- Concentration of microorganism is high at lower parts of the bioreactor due to gravity, whereas lower at the top of the bioreactor.
- Foaming may be a problem caused by proteins in the medium, if it becomes excessive microorganisms and medium might escape with exhaust gases. In this case antifoaming agents are used. These agents can be chemical agents (lard oil, polymethylsiloxane, polyethylene glycol etc.) or mechanical foam breakers.

Semi-Continuous Fermentations

- In semi-continuous fermentations, simultaneous nutrient addition and outflow withdrawal are carried out intermittently, rather than continuously.
- There are two types of semi-continuous fermentation, namely;

  (i) ‘cyclic-continuous’; (ii) ‘cell reuse’.
- In *Cyclic-continuous,* a single vessel is usually employed, although a series of vessels may be used.

Semi-Continuous Fermentations

- Fermentation proceeds to completion or near completion and a volume of the fermentation broth is removed.
- Fresh medium of a volume equivalent to that withdrawn is introduced into the vessel.
- As the size of the fresh medium is reduced, the time taken to complete the fermentation cycle is reduced until eventually the intermittent feeding becomes continuous.
- In cell reuse, cells are centrifuged from the fermentation broth and used to reinoculate fresh medium.
- It is continuous only in the sense that cells are reused; in essence it is a batch fermentation.

Stages needed for transferring an industrial process from the laboratory to the commercial fermentor

"Scaleing -Up"

Name of the process

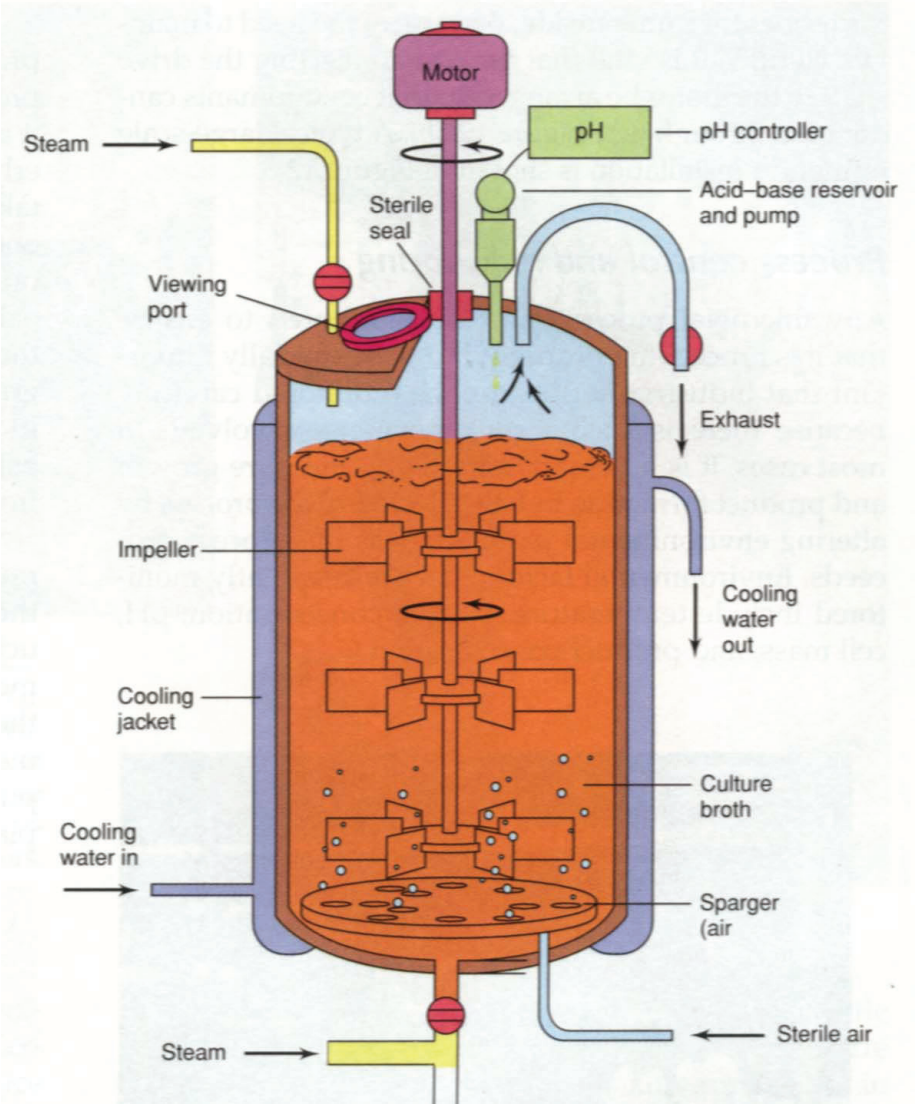
- Shake flask Experiments
- Lab scale fermentor (1-10 L)
- Pilot scale fermentor (100-10000 L)
- Commercial (industrial) fermentor (10,000-500,000 L)

Laboratory Process Development Shake Flask Experiments

Optimization of conditions for cell growth and product formation using shake flask experiments:

1. pH
2. Temperature
3. Dissolved oxygen (DO)
4. Substrate choice
5. Maximal and optimal substrate concentration
6. Others

Laboratory Process Development Fermentor Experiments

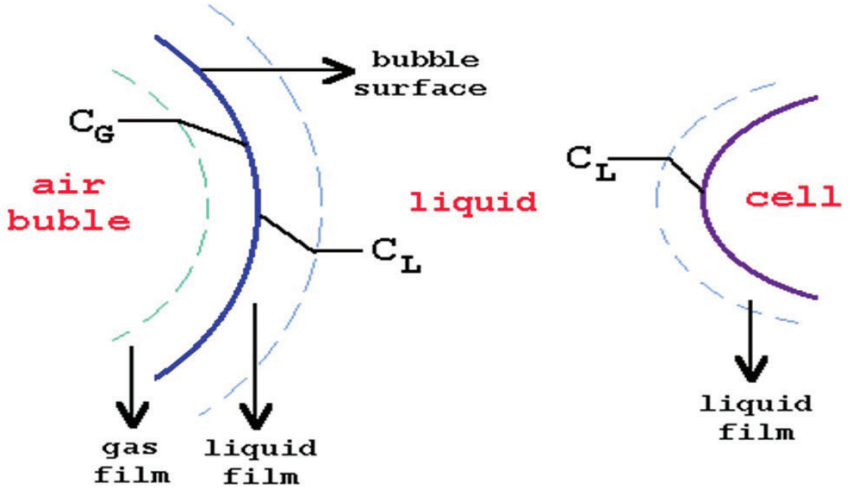


- Agitation
- Cooling and heating
- Air inlet and outlet
- pH control
- Nutrient addition
- Inoculation
- Viewing port

Oxygen Transfer

- For aerobic fermentation, oxygen is essential requirement. 1 m^3 aqueous culture medium contains about 7-8.10^{-3} kg dissolved oxygen. For concentrated microbial population, this amount of oxygen is depleted only in a few seconds. For this reason, oxygen should be supplied continuously, it should be dissolved in liquid, enough gas-liquid mass transfer (transfer of O_2 or other gases, from one concentration to another) should be achieved and dissolved gas should be transferred to organism.

Resistance (Gas Film, Liquid Films and Gas Liquid Interface) in Oxygen Transfer Pathway



Factors Affecting Oxygen Transfer

bioreactor	diameter capacity power aeration rate aeration system
nutrient solution	density viscosity composition of nutrient salts
structure of microorganisms	
antifoaming agent	
temperature	

- Antifoaming agents decrease surface tension
- Aeration of filamentous fungi or agglomerated cells are hard
- If aeration rate increases too much or decreases, efficiency of aeration decreased, bubbles may stick to impeller.
- As temperature increases, dissolved oxygen decreases, therefore temperature should be adjusted optimally.
- Increasing pressure cause an increase in gas solubility. At deeper parts of fermentors more oxygen is dissolved, at higher parts less O_2 is dissolved. As viscosity increases, decreases.
- If density increases, decreases.
- Nutrient salts prevents bubble coalescence, ionic strength and increases.
- Mixing is helpful in aeration. In continuous reactors, when medium is added, quick mixing is needed, otherwise they are excluded from reactor.

Mixing Is Necessary to

- maintain homogenity,
- attain rapid dispersion and mixing of components injected into fermenter,
- enhance heat transfer and temperature control,
- enhance mass transfer.

Design of Bioreactors and Operation

Choice of bioreactors should be done according to these criteria:

1-prefer a reactor, which you have experienced before.

2-be simple, make no more complications than necessary, because purpose is to lower cost of production.

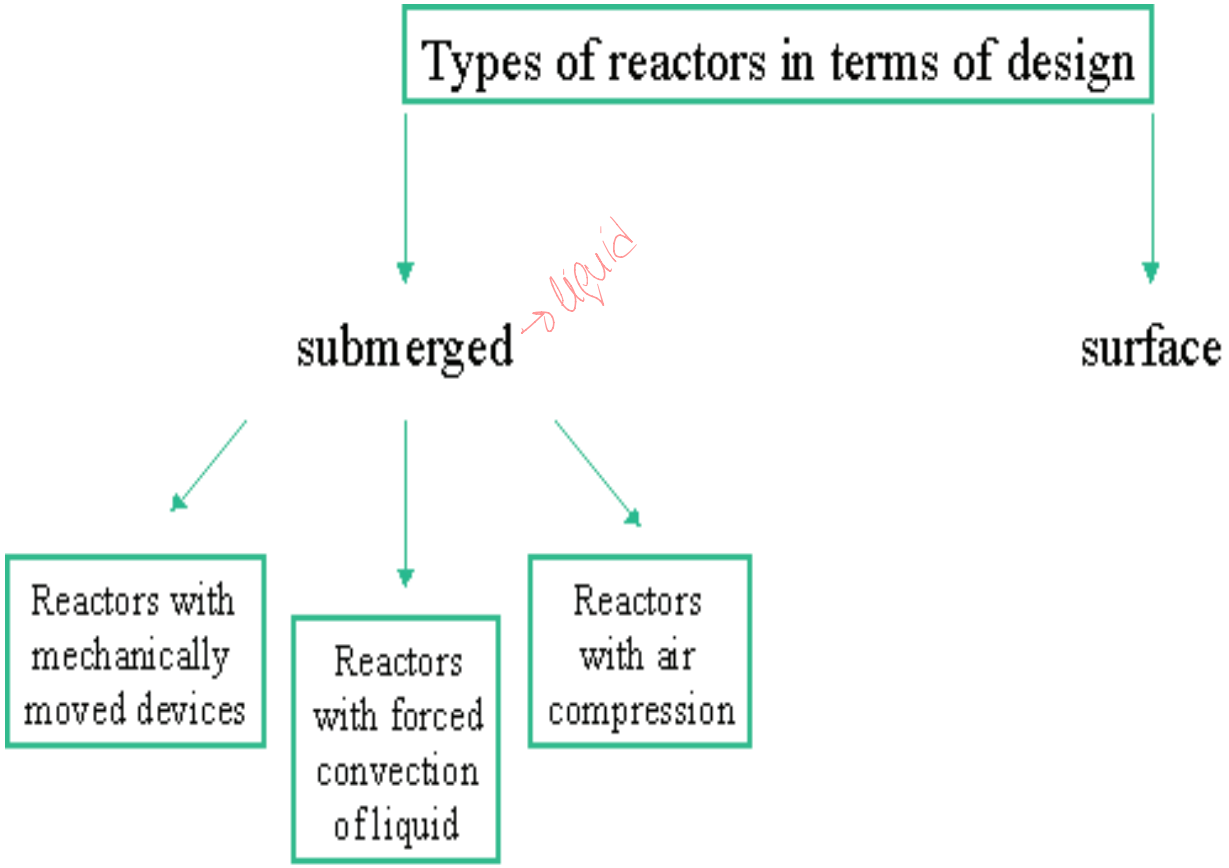
3-main demand is sterile conditions. To maintain sterility, keep valves, pumps, joints, probe insertions, sample ports, gas inlets as few as possible.

Stagnant regions, air pockets, pipe branches, crevices increase risk of contamination.

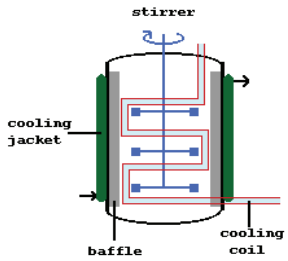
- Total Capacity of the Plant Determined by Expected Demand of Product and Productivity:

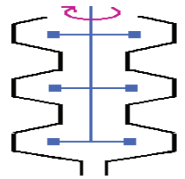
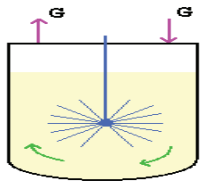
high volume	intermediate value	amino acids, organic acids, polymeric substance
high volume	low value	methanol, ethanol, beer
low volume	high value	drugs, antibiotics

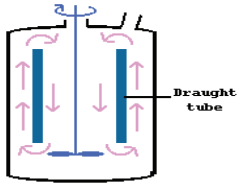
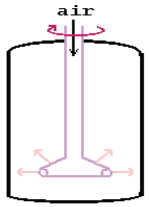
- System can be designed with one or two large reactor, or several small units.
- Initial cost of first type is less than the other, whereas if any problem occurs in one of the reactors operation stops.
- Second type's disadvantage is that more utility is needed for operation.

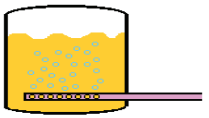
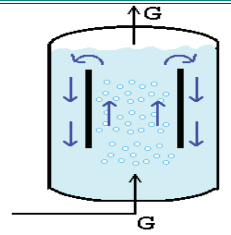
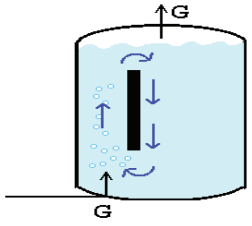

Types of reactors in terms of design
submerged
liquid
surface
Reactors with mechanically moved devices
Reactors with forced convection ofliquid
Reactors with air compression

Submerged Reactors

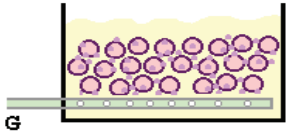
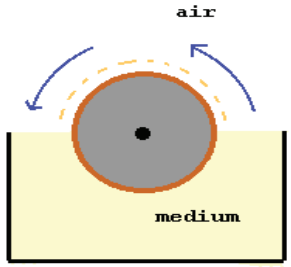
Reactors with mechanically moved devices	
Mechanically stirred	

Stirred cascade reactor	
Paddle reactor	

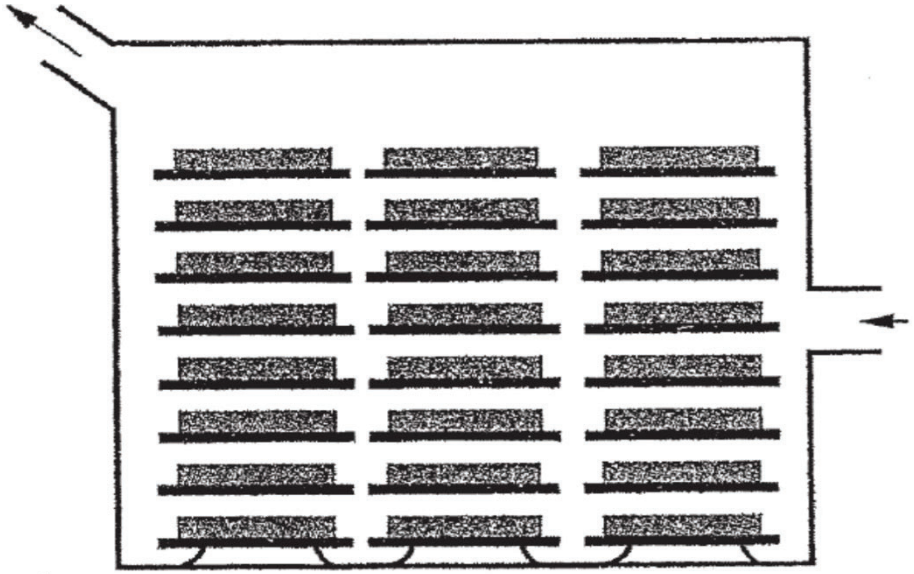
Mechanically stirred draught tube reactors	
Gas aspiring reactor	

Power input by gas compression	
Single stage buble column	
Single stage air lift tower loop reactor Figure 1.	
Single stage air lift tower loop reactor Figure 2.	

Surface Reactors

Fluidized bed reactor	
Immersing surface reactor	

Tray Fermentor



Inoculum Preparation

- Cells to be used must be actively growing, young and vigorous and must therefore be in the phase of logarithmic growth.
- Since organisms used in most fermentations are aerobes, the inocula will usually be vigorously aerated in order to encourage maximum cell development
- The chemical composition of the medium may differ in the inoculum and production stages.
- The inoculum usually forms 5-20% of the final size of the fermentation to shorten the production time.
- The initial source of the inoculum is usually a single lyophilized tube.
- If the content of such a tube were introduced directly into a 100,000 liter fermentor, it would take very long time to achieve a production population.
- Inocula are prepared in several stages of increasing volume.
- When the lyophilized vial is initially plated out and shown to be pure, the entire plate is scraped off and transferred to the shake flask so as to avoid picking mutants

Materials for Bioreactor Construction

While deciding on construction material of bioreactor these properties of material should be taken into account:

- mechanical properties
- corrosion resistance
- ease of fabrication
- availability
- cost

- If aseptic conditions are **not** necessary for operation (like in the case of bakers' yeast) concrete, brick or glass reinforced plastics can be used as construction materials.
- If aseptic conditions are considered as necessary, stainless steel is one of materials preferred. It is strong; no other supporting material is needed for construction. However, it is more expensive and it is more difficult to fabricate.
- Thin chromium oxide film on surface, 3% Molybdenum in alloy provides resistance to corrosion, oxidation and reduction. To provide stabilisation of chromium oxide film titanium is included in alloy.
- 18/8 stainless steel which contains 18% chromium and 8% nickel can also be used as construction material.
- In addition mild steel can be used for bioreactor construction, if lined with glass, synthetic resin, rubber or stainless steel layer. These additional materials are used for protection of steel.