INDUSTRIAL MICROBIOLOGY

Micro566 Industrial Microbiology

Presented by Prof. Nagwa Mohamed Aref

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Biology of Microorganisms
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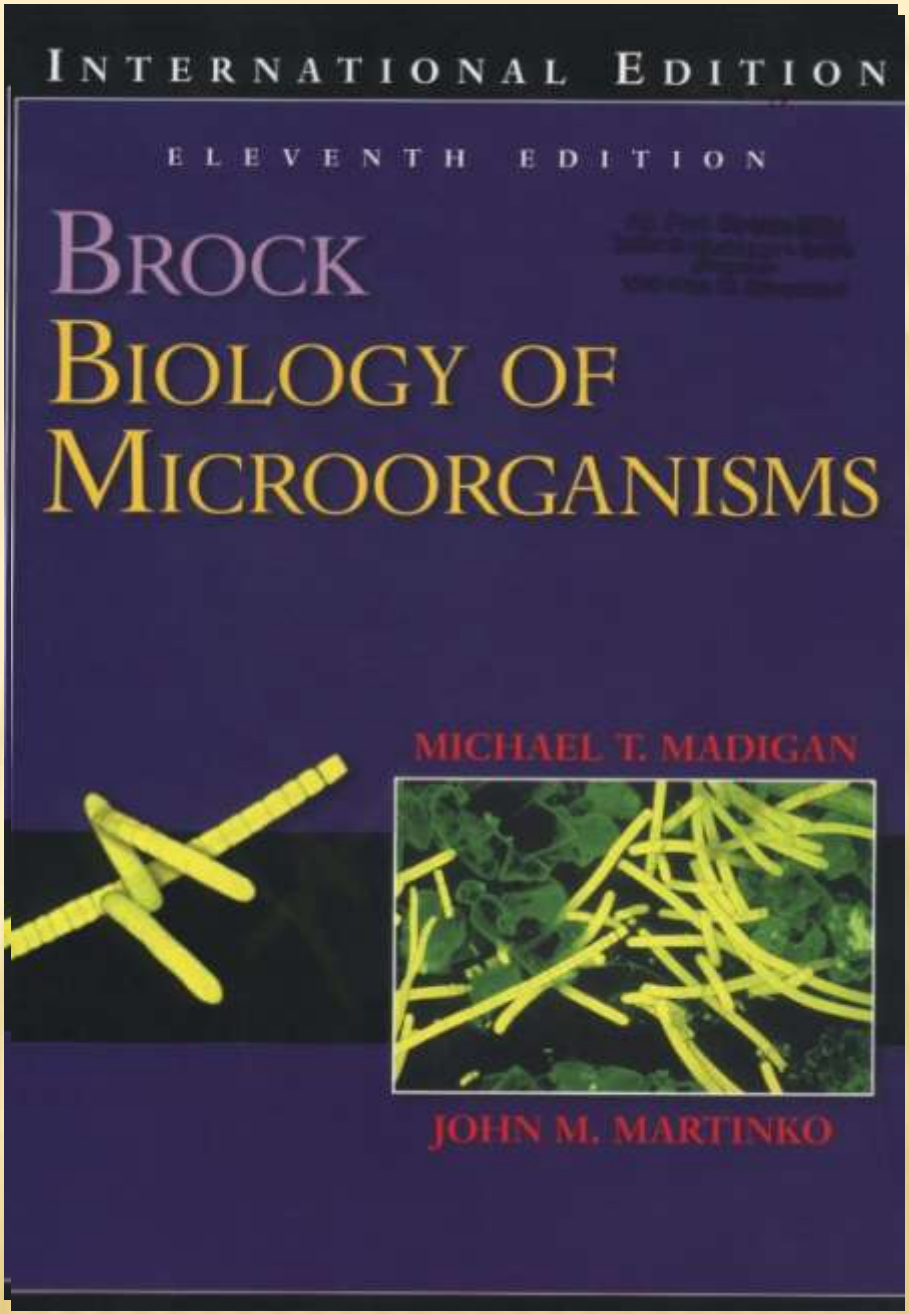
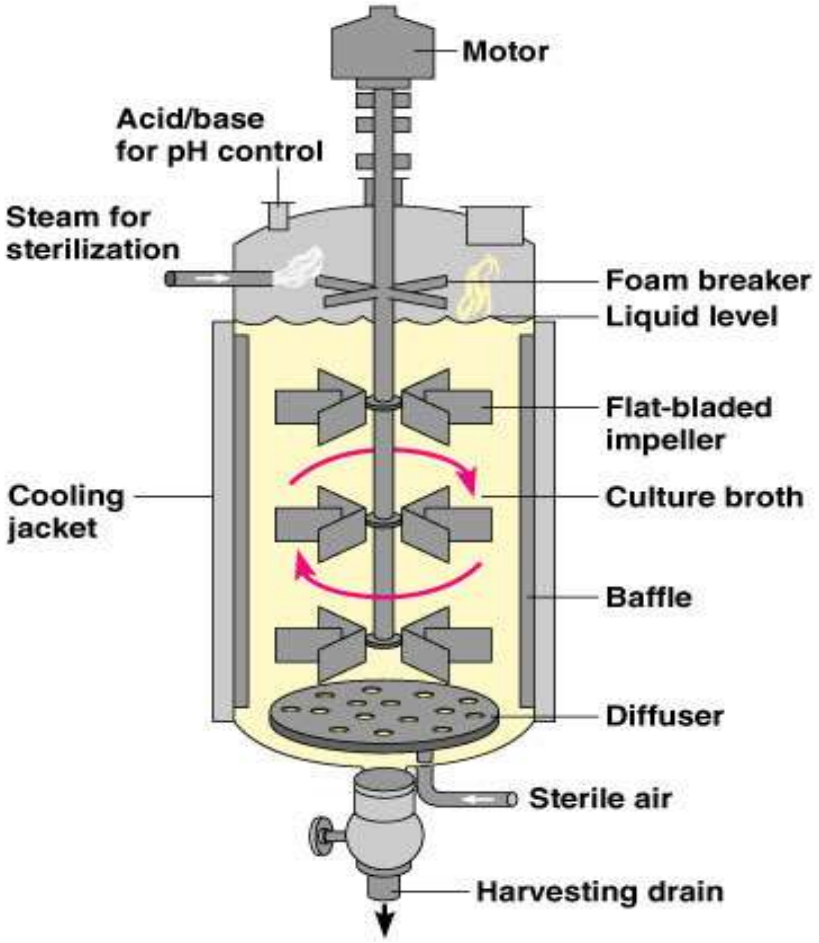


FERMENTATION TECHNOLOGY



(a) Section of a continuously stirred bioreactor

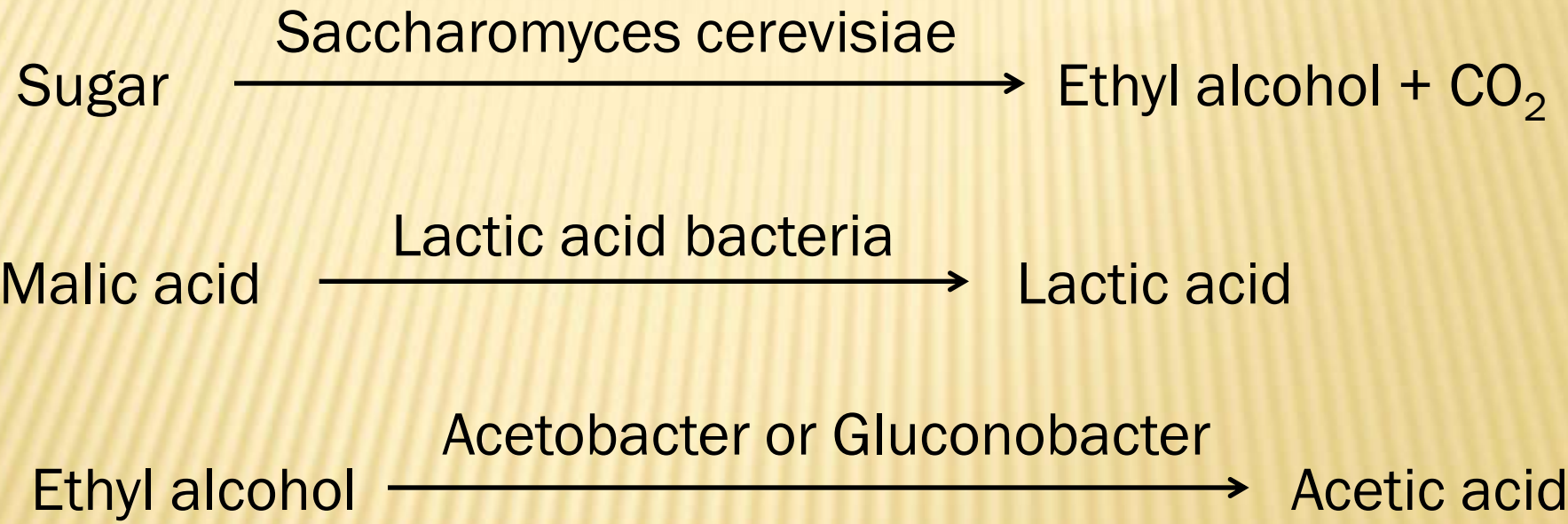
(b) A bioreactor tank is at the left.

Figure 28.10

INDUSTRIAL MICROBIOLOGY

- Amino acids
- Citric Acid
- Enzymes
- Vitamins
- Antibiotics
- Steroids

MICROBIAL METABOLISM



CHEESE

- Curd: solid casein from lactic acid bacteria and rennin
- Whey: liquid separated from curd
- Hard cheeses produced by lactic acid bacteria
- Semisoft cheeses ripened by Penicillium on surface

(a) The milk has been coagulated by the action of rennin (forming curd) and is inoculated with ripening bacteria for flavor and acidity. Here the workers are cutting the curd into slabs.

Figure 28.8a

GROWTH IN BATCH CULTURE

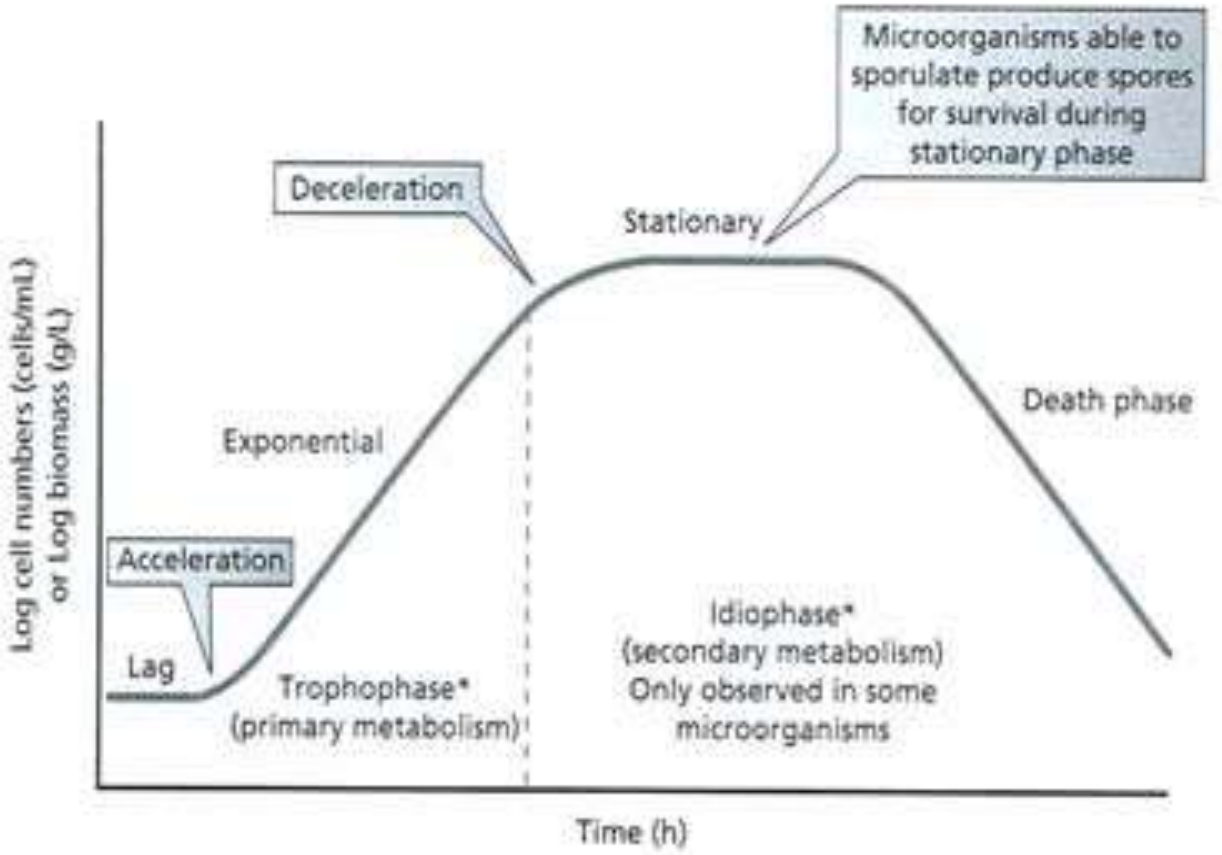
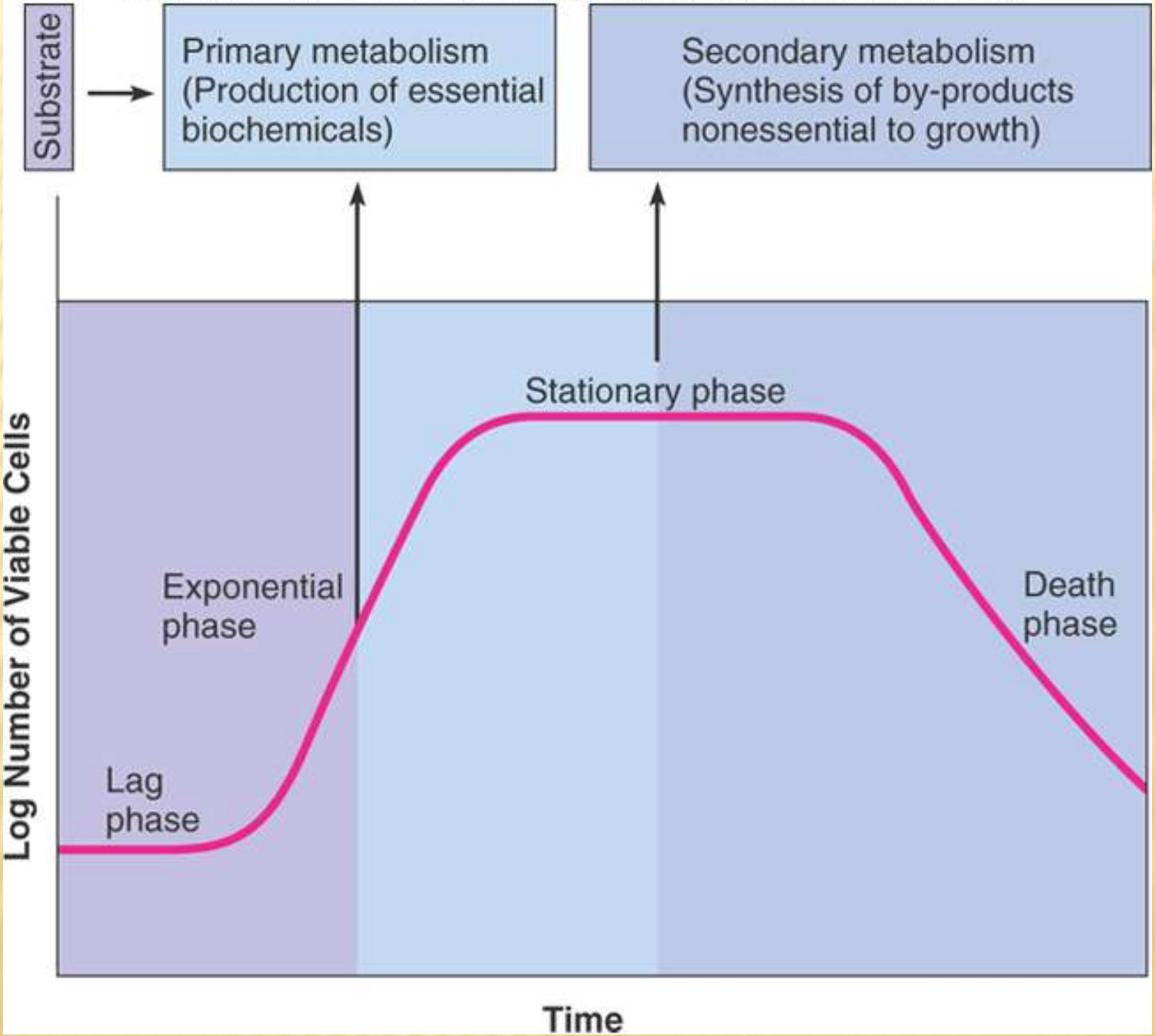
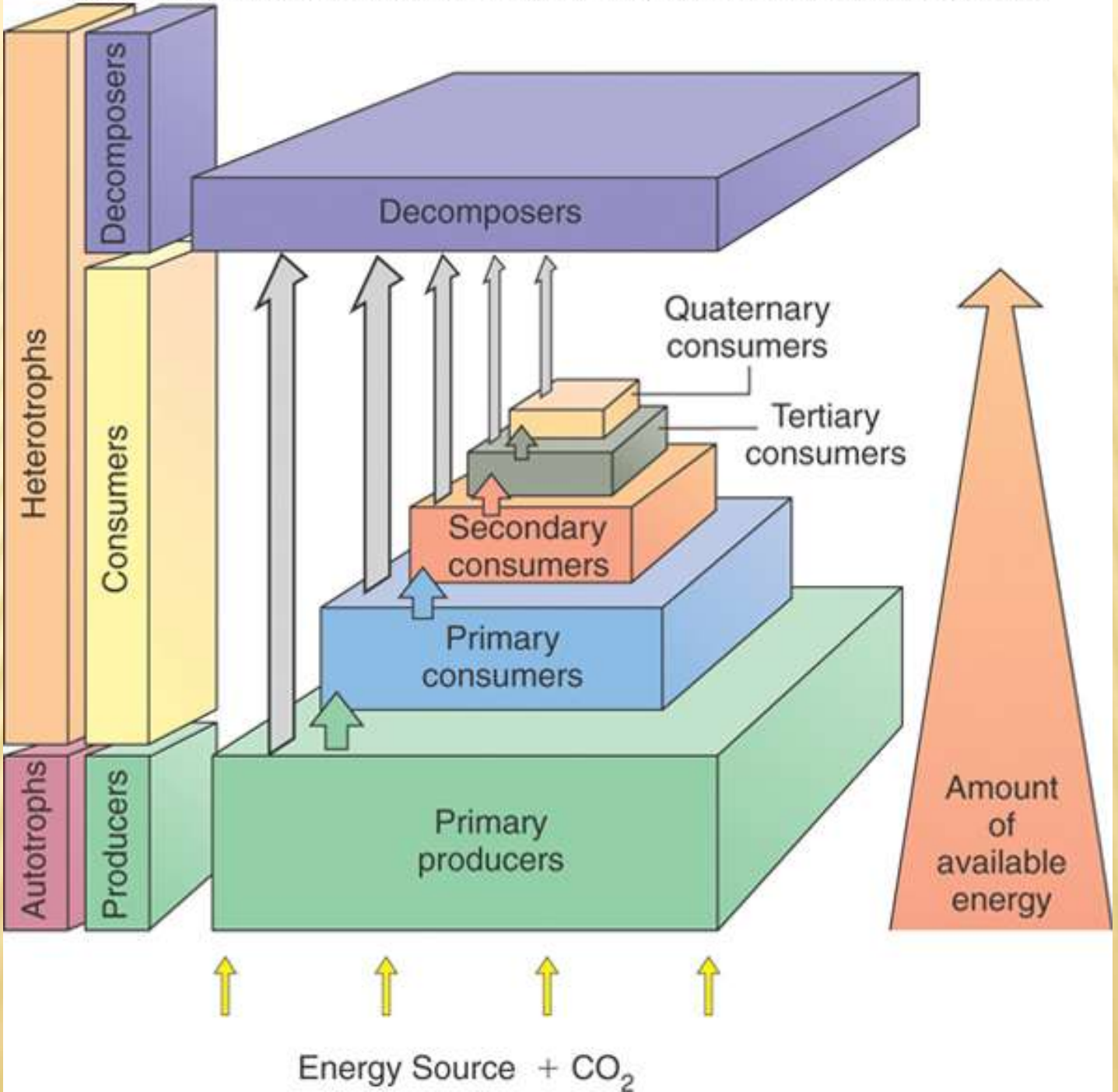
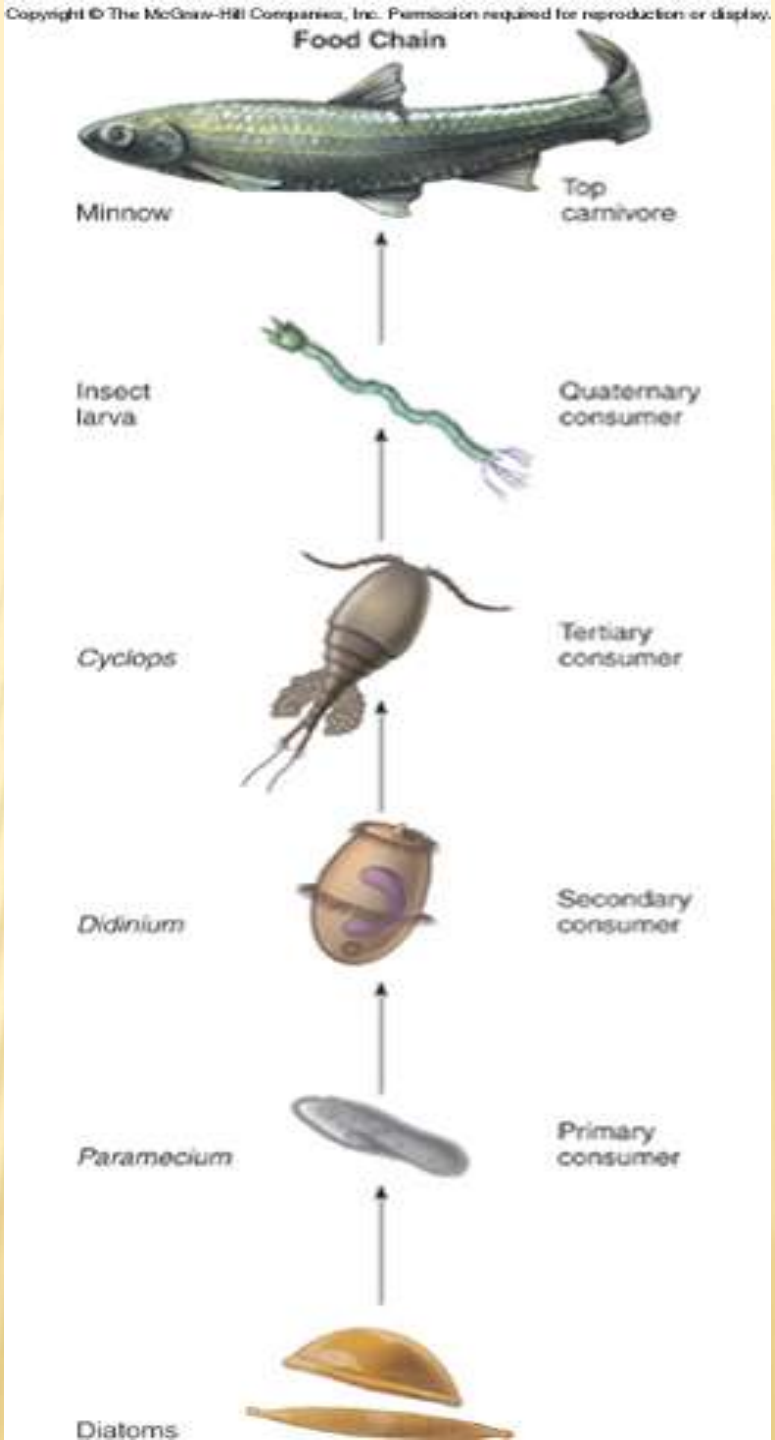


Fig. 2.1 Growth of a microorganism in a batch culture. *Trophophase and idiophase: see Chapter 3, Secondary metabolism.







SECONDARY FERMENTATION

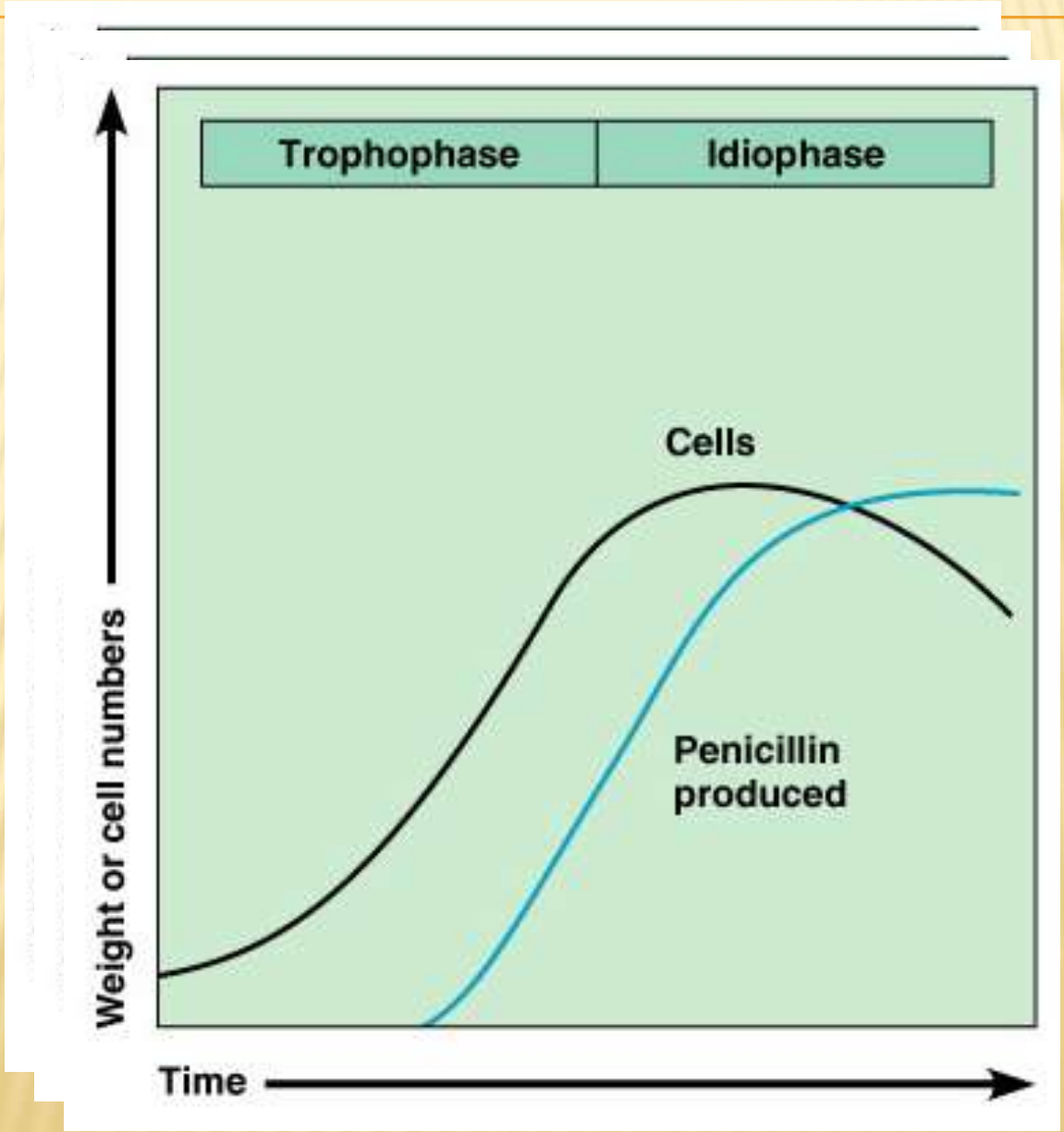


Figure 28.11b

PRIMARY FERMENTATION

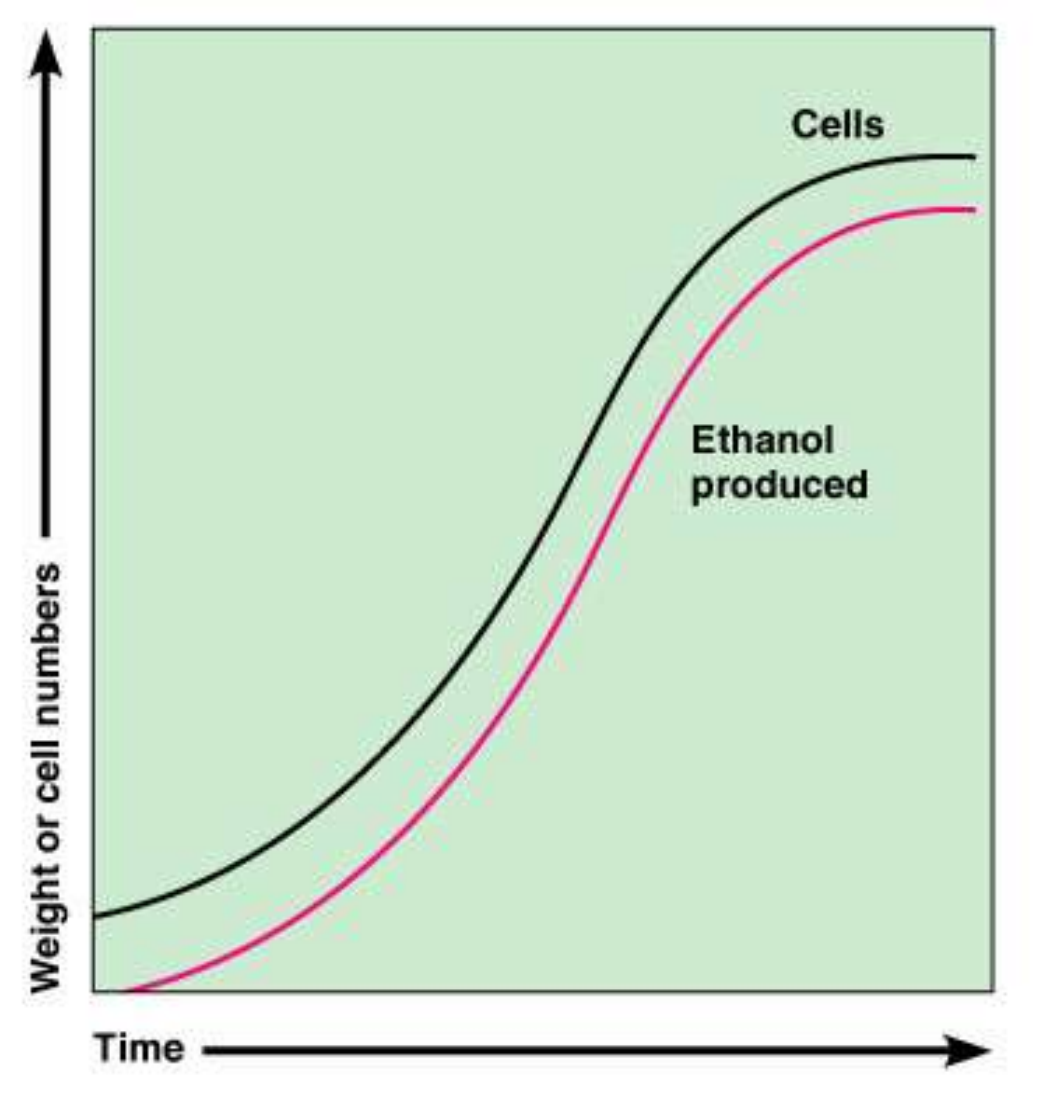
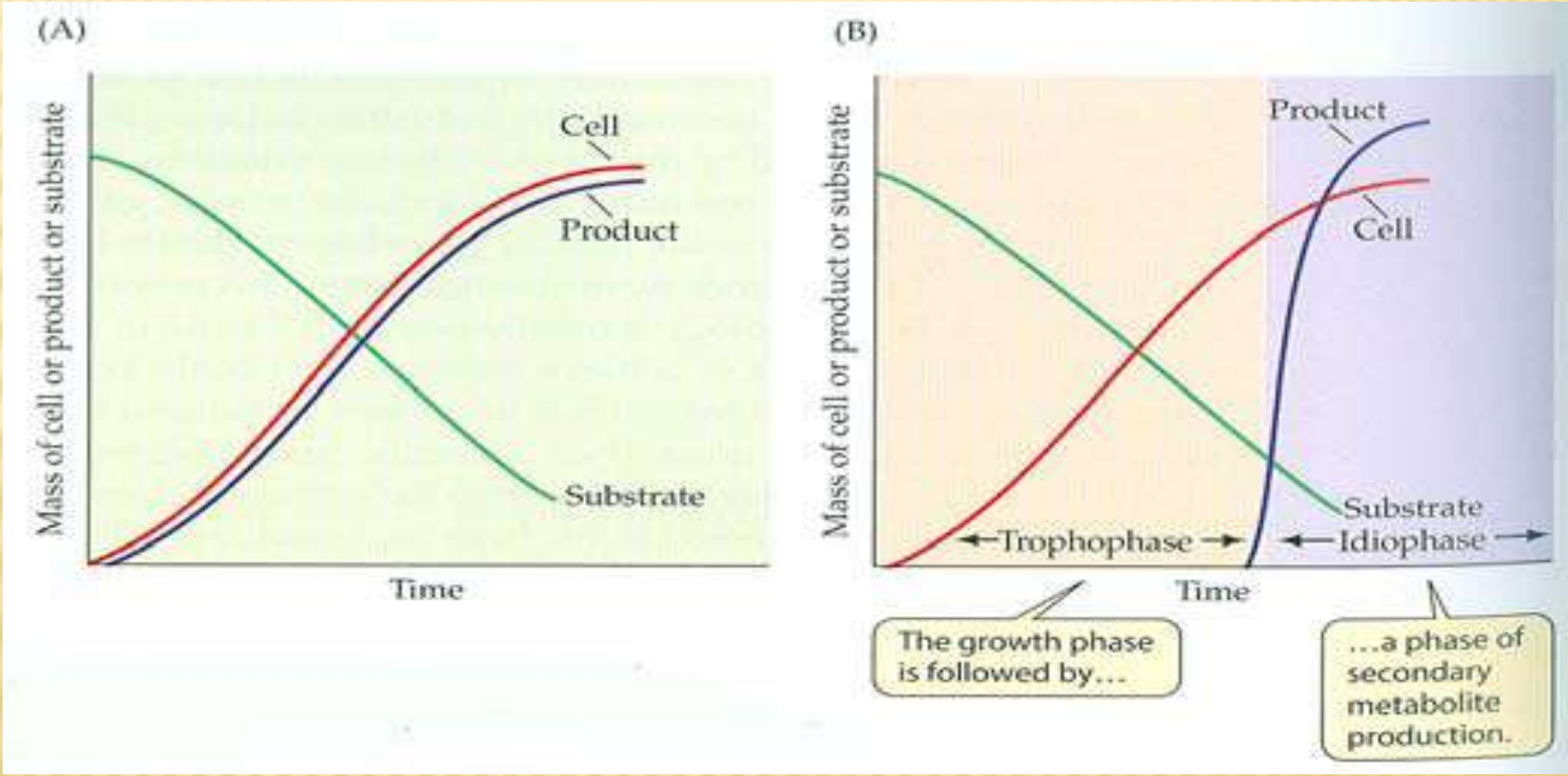


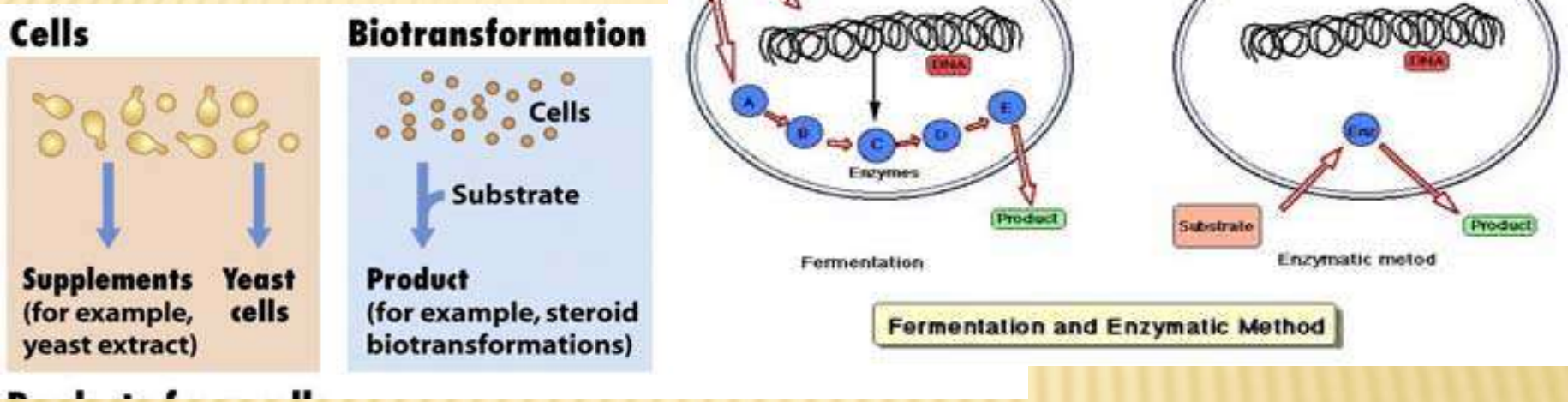
Figure 28.11a

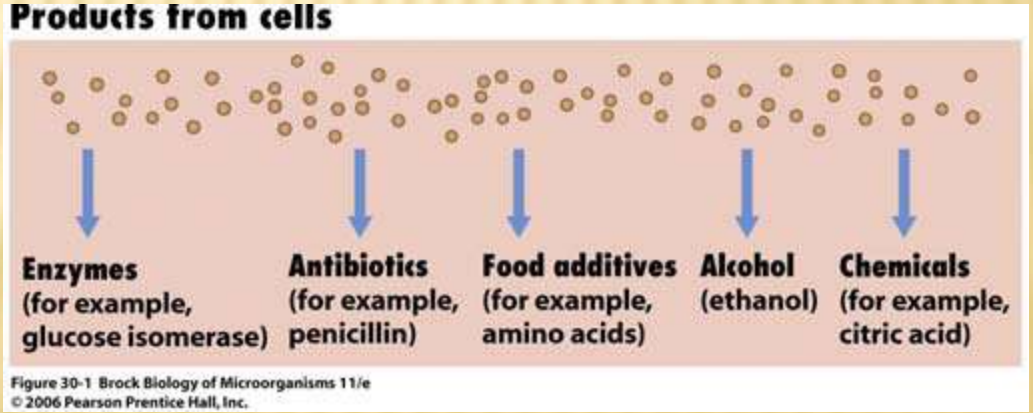
Primary metabolite

Secondary metabolite



1.Microbial cells or cell products
2. Enzymatic biotransformation products
3.Fermentation products (de novo synthesis)





CONCEPT OF INDUSTRIAL FERMENTATION

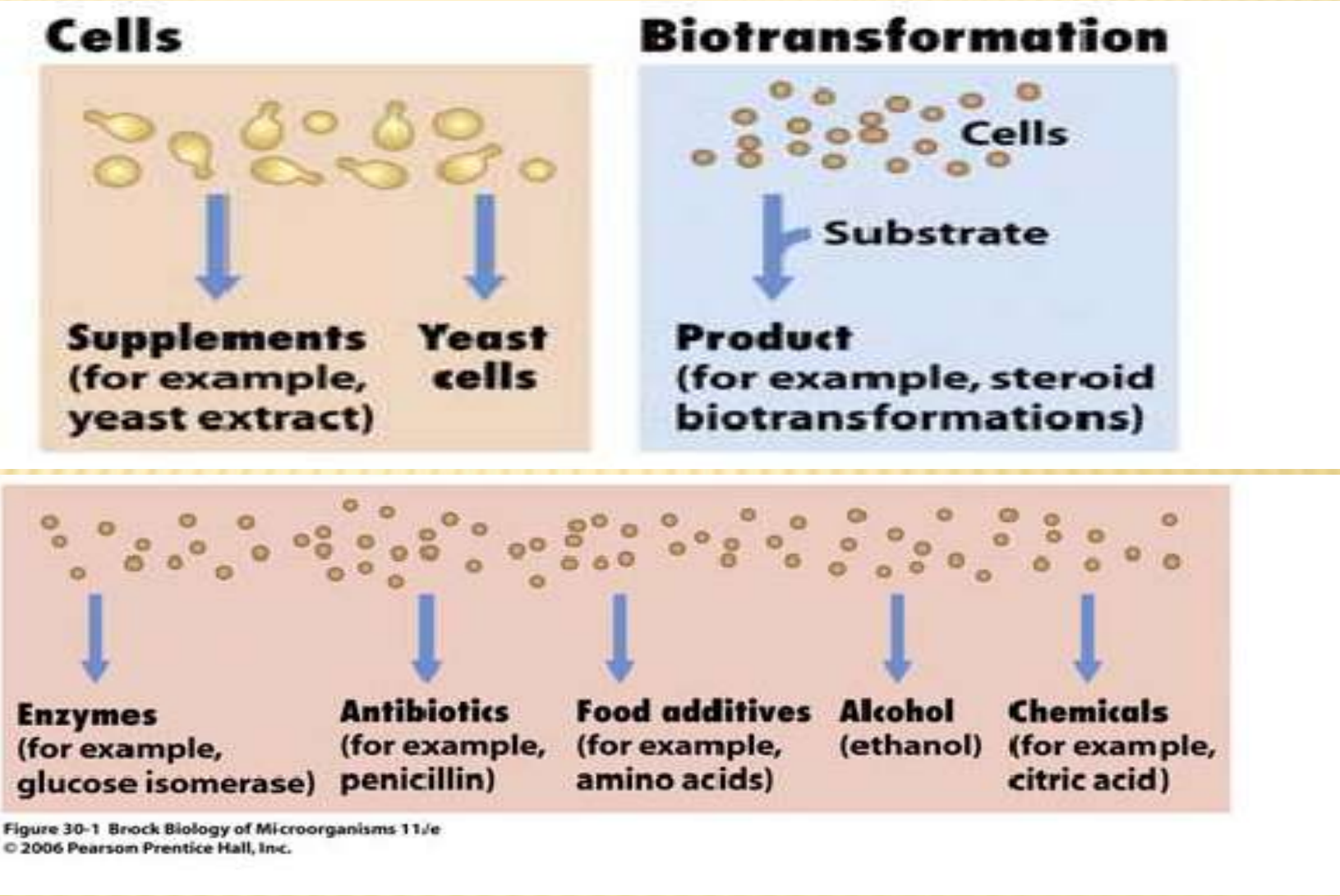


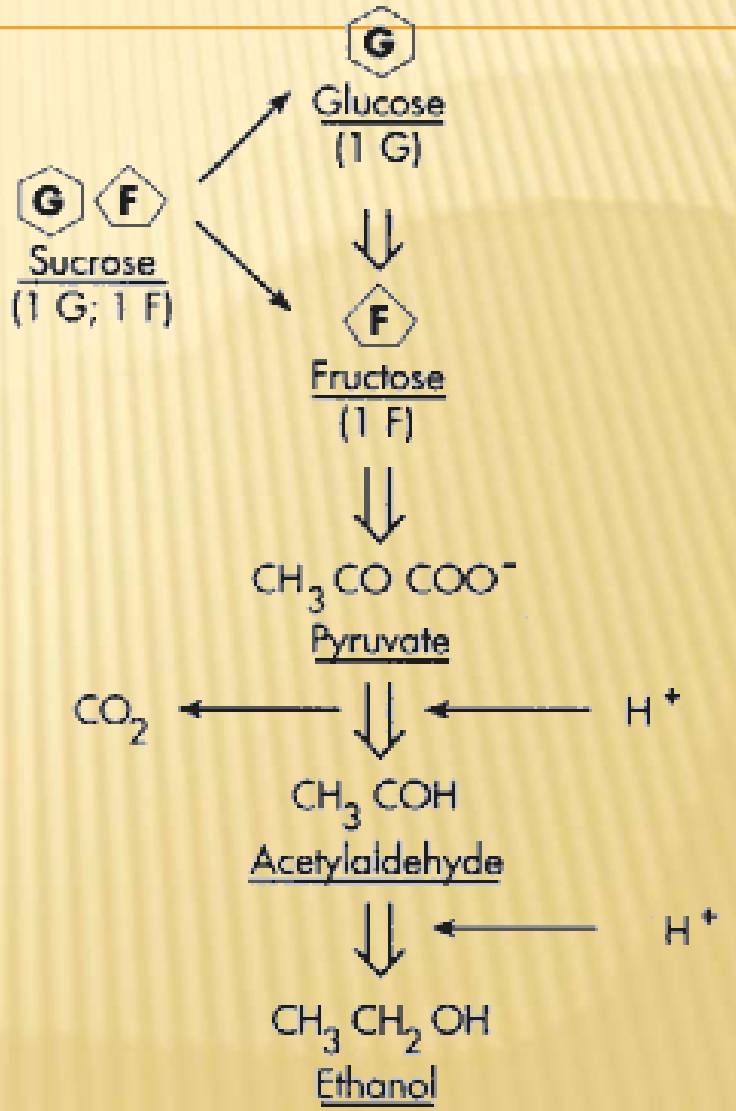
Figure 30-1 Brock Biology of Microorganisms 11/e

WHAT IS FERMENTATION?

- Pasteur's definition: "life without air", anaerobe red ox reactions in organisms
- New definition: a form of metabolism in which the end products could be further oxidized
- Any Microbe requires Water, Oxygen, Energy source, Carbon source, Nitrogen source and Micronutrients for the growth.
- **Carbon & Energy source + Nitrogen source + O2 + other requirements → Biomass + Product + byproducts + CO2 + H2O + heat**

DEFINITION OF FERMENTATION:

- Fermentation technology is the oldest of all biotechnological processes.
- The term is derived from the **Latin verb *fevere*, to boil**--the appearance of fruit extracts or malted grain acted upon by yeast, during the production of alcohol.
- Fermentation is a process of **chemical change** caused by organisms or their products, usually producing effervescence and heat.
- Microbiologists consider fermentation as 'any process for the production of a product by means of mass culture of micro-organisms'.
- Biochemists consider fermentation as **'an energy-generating process in which organic compounds act both as electron donors and** acceptors';
- Hence fermentation is 'an **anaerobic process where energy is produced without the participation of oxygen or other inorganic electron acceptors'.**



INDUSTRIAL UNIT

OUTLINE OF A FERMENTATION PROCESS

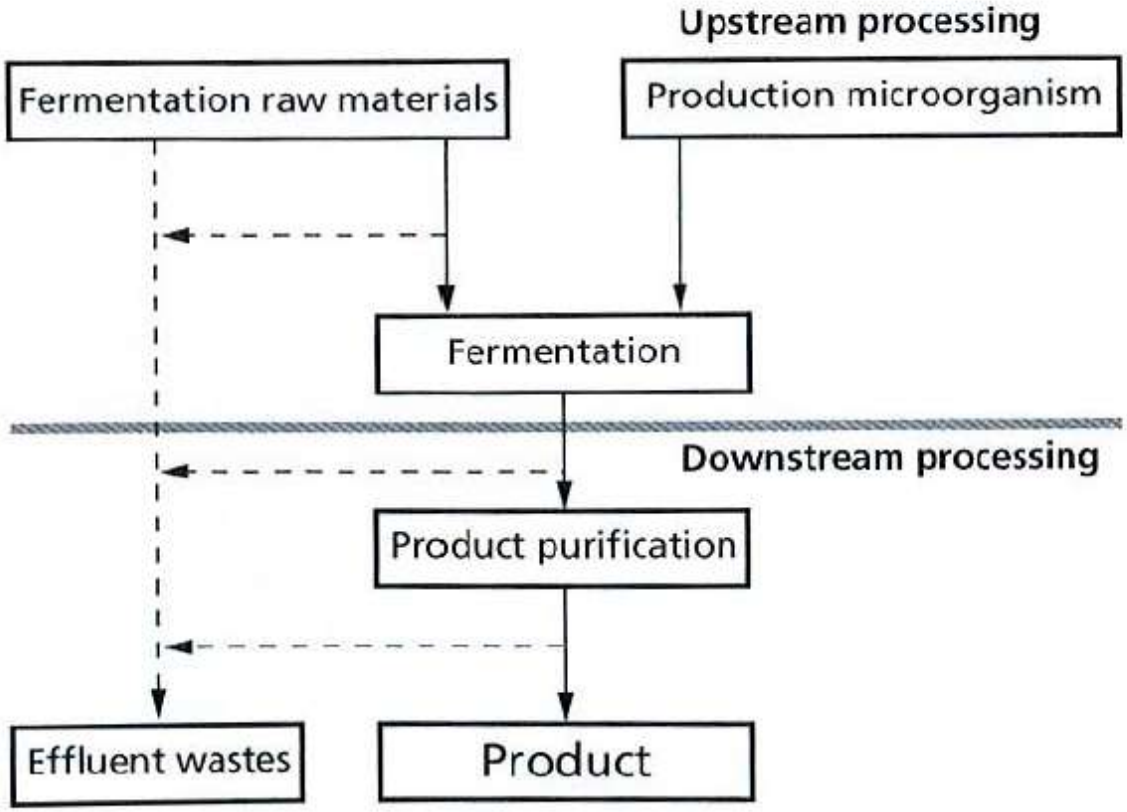
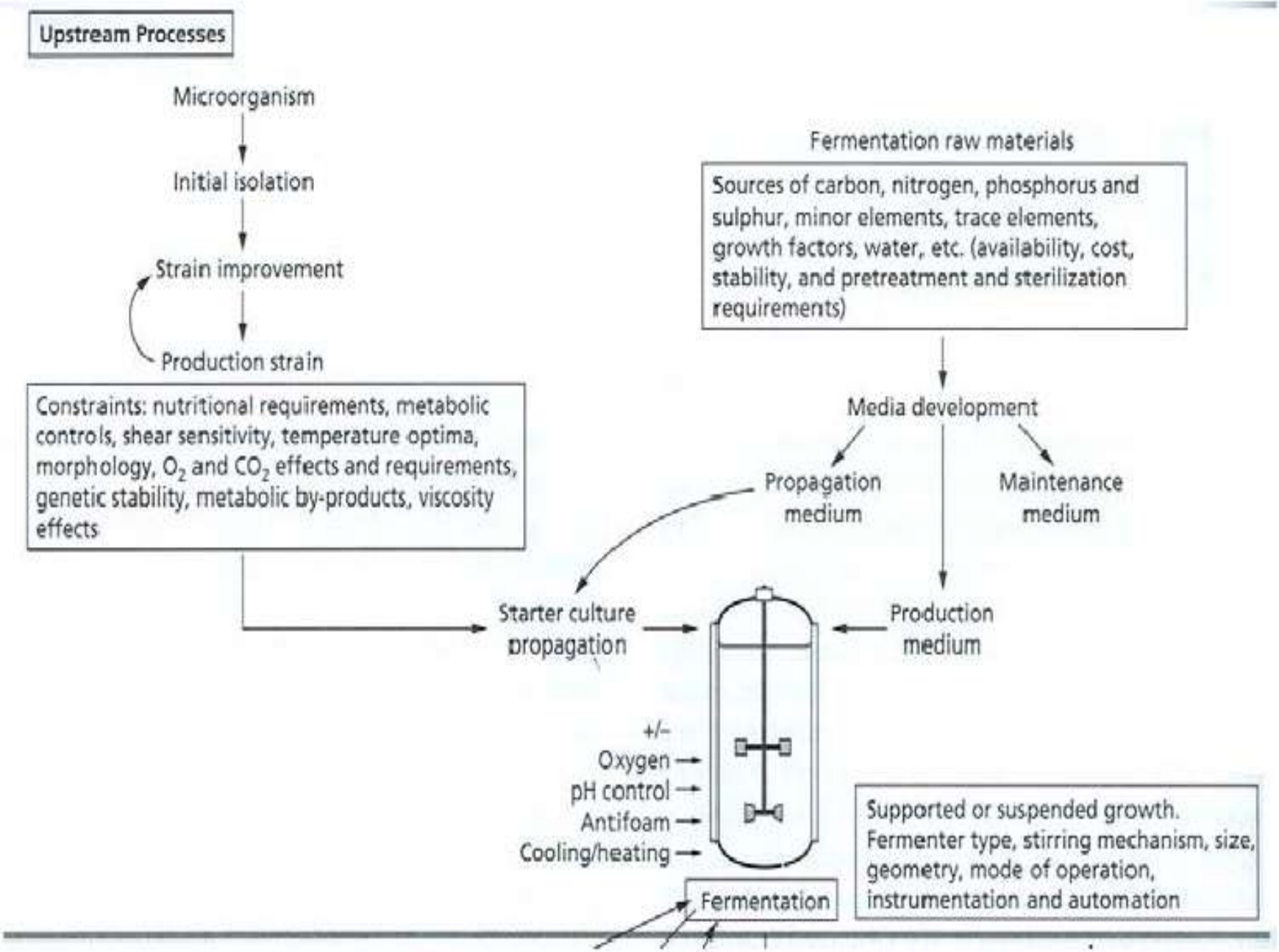
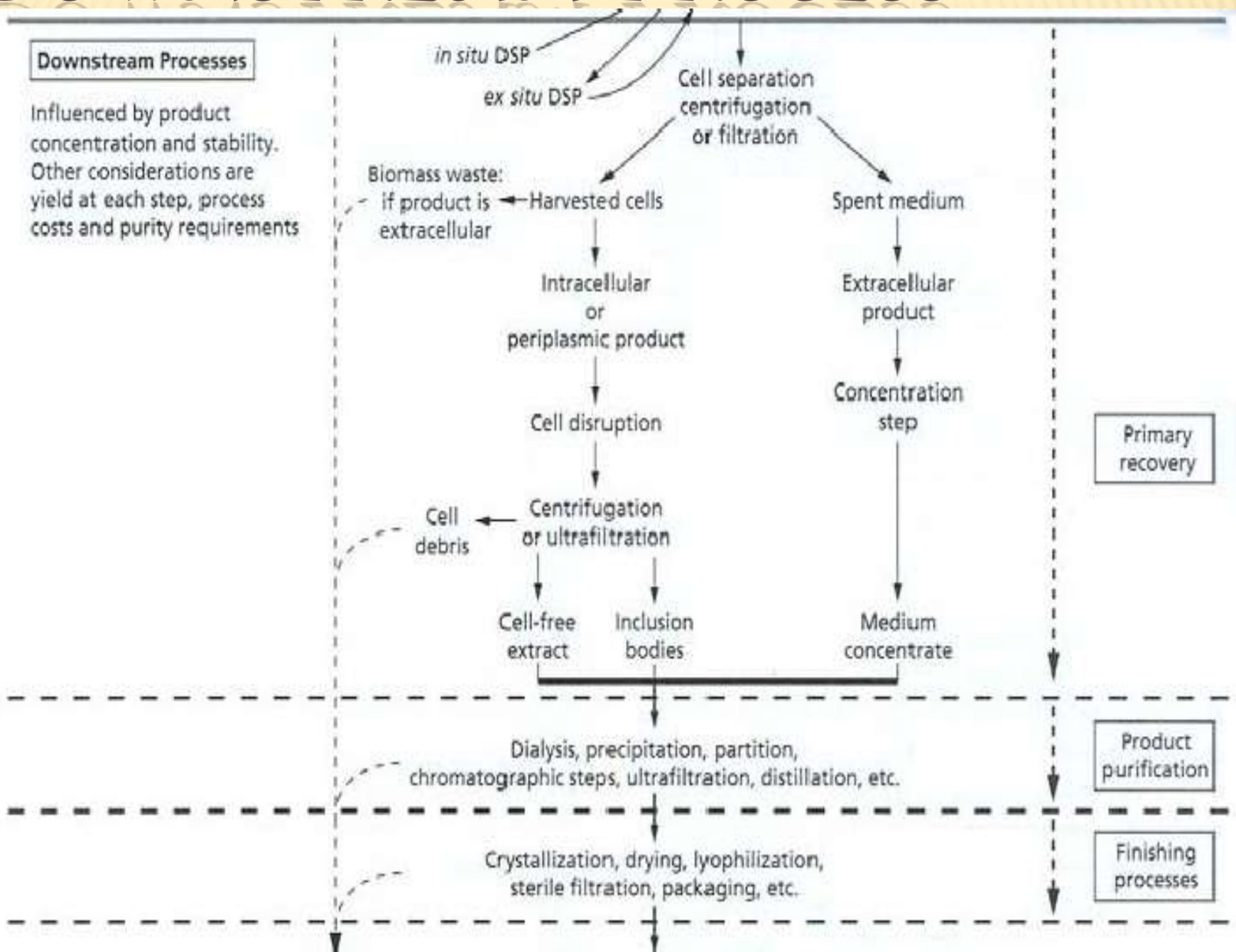


Fig. i Outline of a fermentation process.


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 PROCESS



STEPS IN FERMENTATION

- Fermentor selection
- Microbial Strain selection
- Fermentation Media Selection
- Fermentation process
- Upstream processing
- Downstream processing
- Quality Control & Assurance
- Product Recovery
- Packaging

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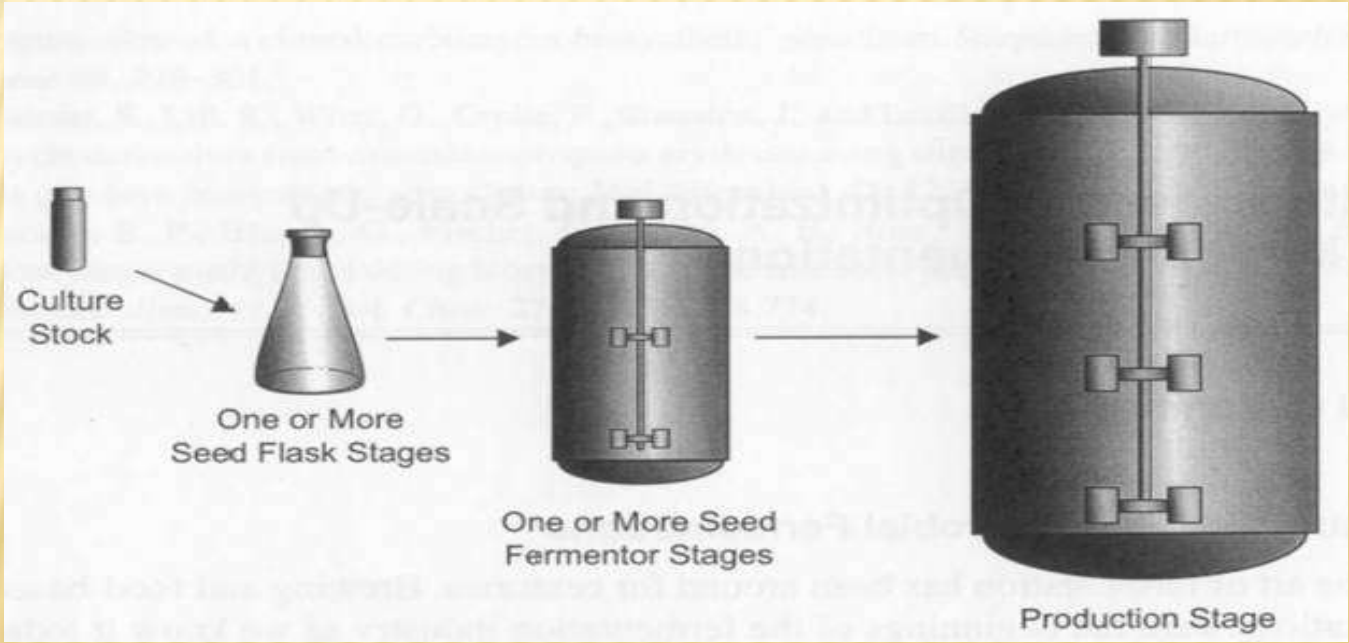
FERMENTOR SELECTION

Earlier times

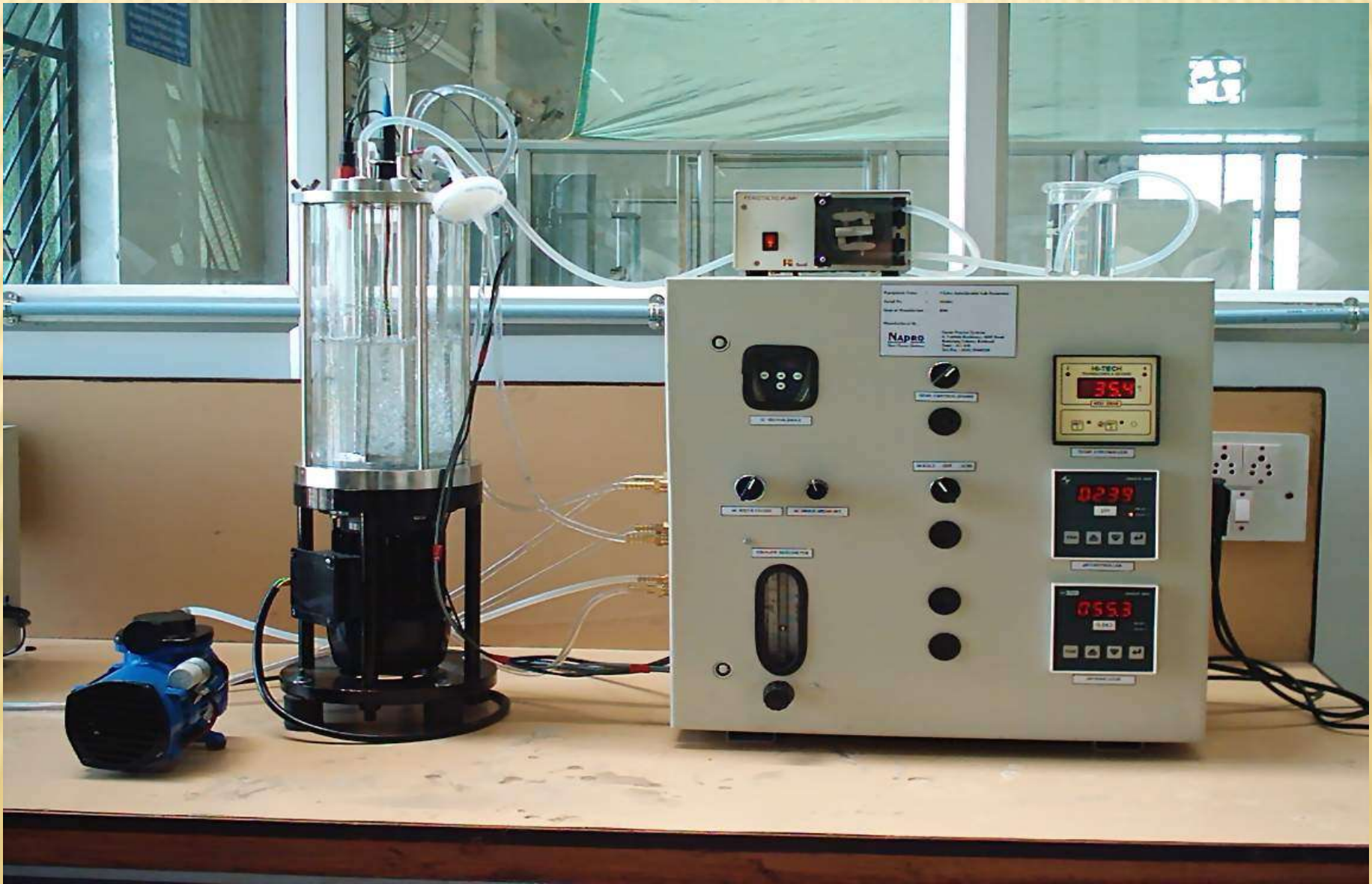
- 

FERMENTOR STAGE

SHAPE
MATERIAL
SIZE



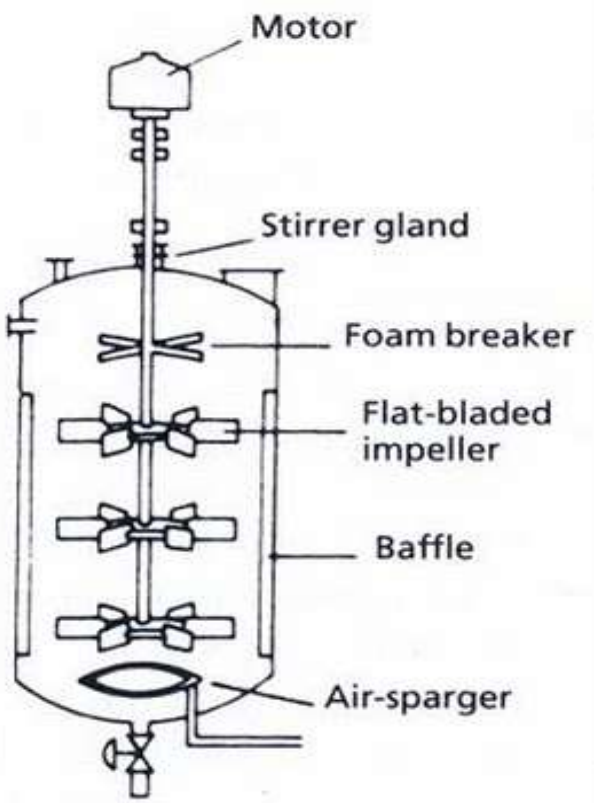
LABSCALE FERMENTOR



PILOT SCALE FERMENTOR

PRODUCTION TANKS

Basic Design



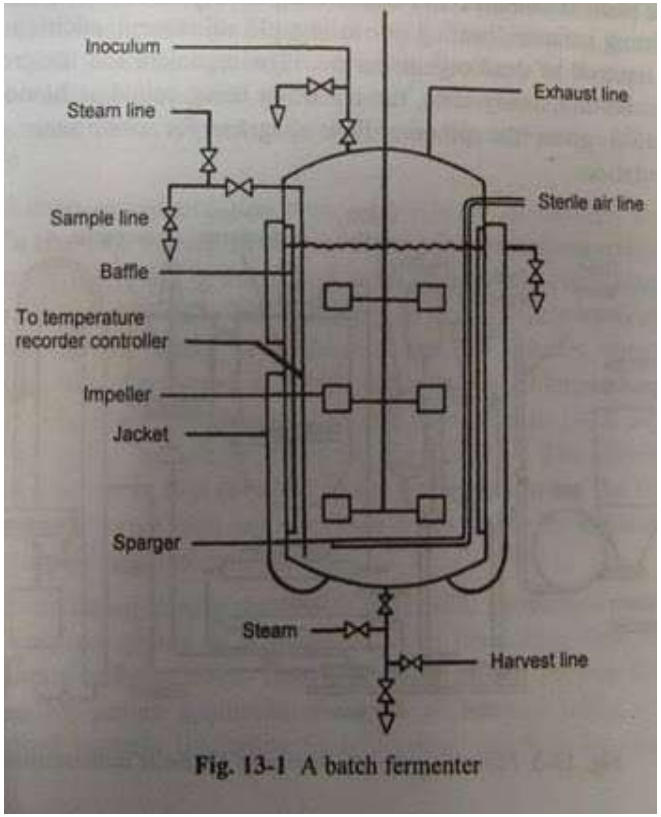
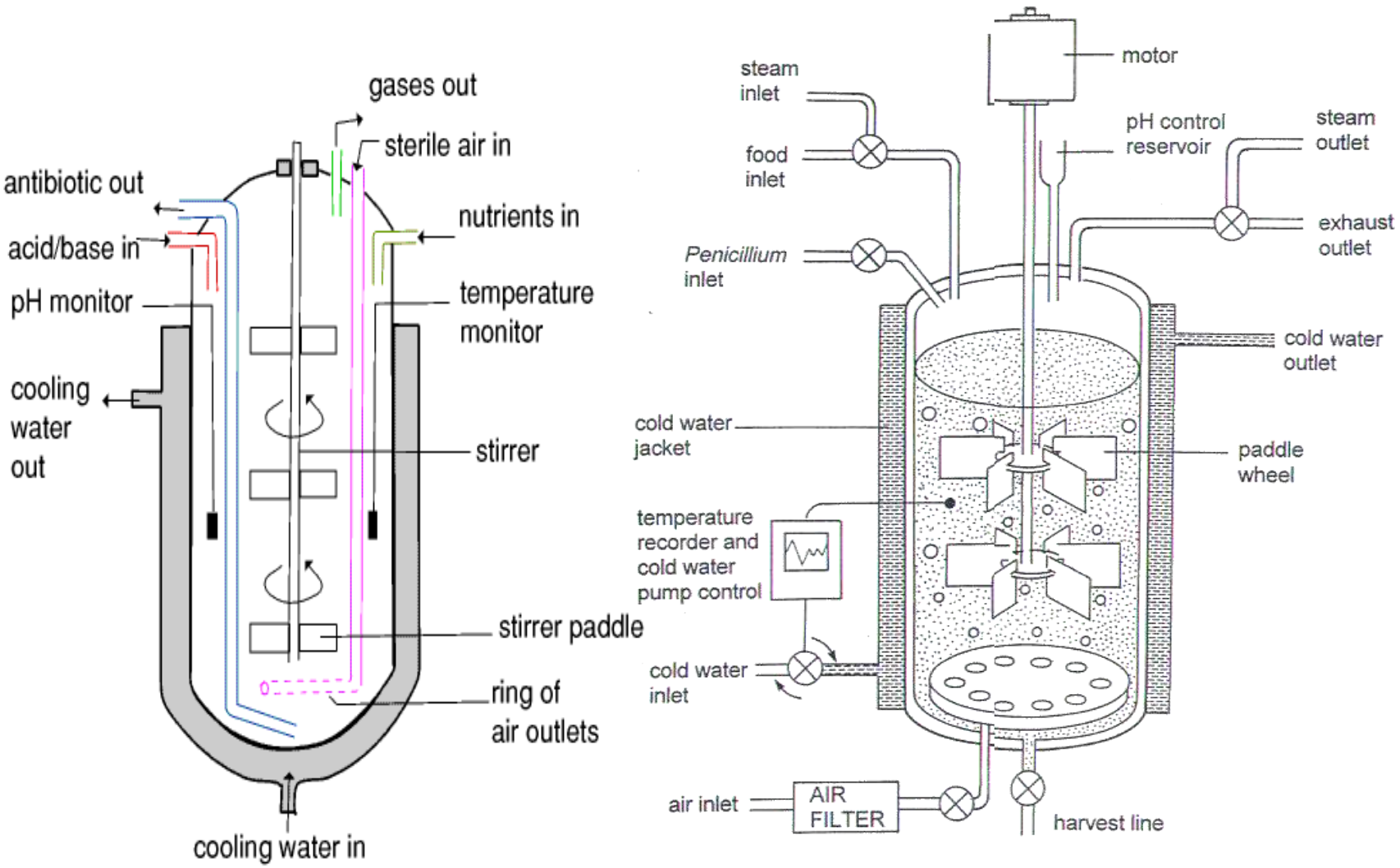


Fig. 13-1 A batch fermenter



Cross section of a fermenter for Penicillin production

A **BIOREACTOR** DIFFERS FROM A FERMENTER IN THAT THE FORMER IS USED FOR THE MASS CULTURE OF PLANT OR ANIMAL CELLS, INSTEAD OF MICRO-ORGANISMS

Parts of a fermentor

- Aeration & agitation system
- Impeller
- Sparger
- Baffles
- Load cells
- Inlet & exit gas analyser
- ph meter
- Flow cell
- Steam line

PARTS OF A FERMENTOR IMPELLER

- Impeller blades

AGITATION

- Instead of a traditional propeller agitator a **new vibromixer** is used. A strong motor moves one or more stirring discs up and down. The major advantage is an **efficient mixing** and aeration of the culture medium together with very **complete separation** of the inside of the vessel from the outside by a low cost silicone membrane.
- **No vortex** is built up and **baffles are eliminated.**This type of **agitation is also gentler on the cells** and **foaming is reduced with max mixing efficiency** .
- The frequency of agitation is controlle by a microprocessor and can be varied through a broad range.

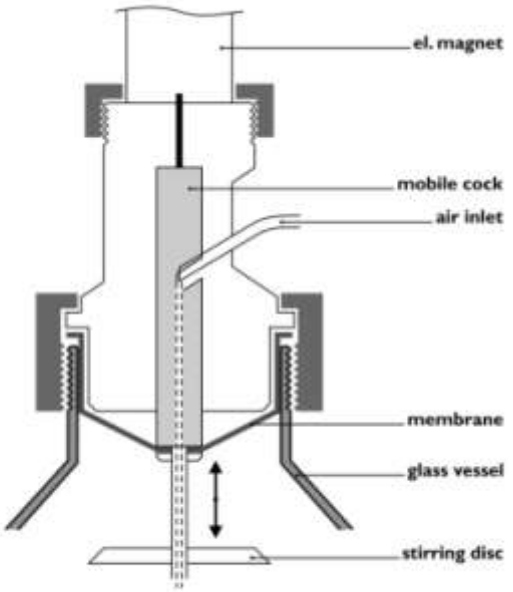


Figure 1. Fermentor head

AIR INPUT/SPARGER

- The flow rate can be set from 0 to 5 l/min in 0.1 l/min steps. A **precise mass-flow meter** is used.
- Commonly used floating ball capillaries (rotameters) give accurate readings in this case.
- A **self-cleaning elastic air micro-sparger** has been developed. Its special construction allows an automatic elimination of salt deposits, which would block the airflow in normal spargers. This is important particularly for micro-spargers having very narrow channels.





LOAD CELL

- A **load cell** is a transducer that is used to convert a force into electrical signal. The strain gauge converts the deformation (strain) to electrical signals.
- A load cell usually consists of four strain gauges in a Wheatstone bridge configuration.The electrical signal output is in millivolts and requires amplification by an instrumentation amplifier.
- The output of the transducer is plugged into an algorithm to calculate the force applied to the transducer.





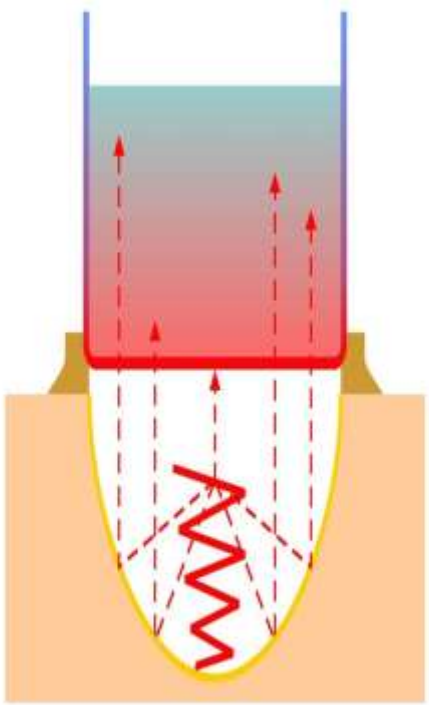
BAFFLES

TEMPERATURE CONTROL

A **new infrared (IR) radiator** with a gilded parabolic reflector is used to warm the culture broth. The heat radiation (150W) is concentrated on the **bottom of the vessel** where it is absorbed by the medium in a similar way to the sun heating water.

There is **no overheating at any volume** of the culture. overshooting of the temperature is reduced & the **Temperature can be controlled more precisely.**

The temperature **sensor is placed directly in the pH sensor** and is used at the same time for an **automatic correction of pH and pO_2 electrodes.**



PH MEASUREMENT AND CONTROL

- pH is measured by a combined, sterilizable pH electrode with incorporated temperature sensor.
- Due to multiple Variopin plug it can be sterilized without any protection.
- The pH value has an automatic temperature correction.
- The addition of acid or base is controlled by a microprocessor.

PO_2 MEASUREMENT & REGULATION

A sterilizable Clark-type electrode with large cathode measures the concentration of dissolved oxygen with a glass reinforced TEFLON membrane giving fast response times and short polarization. The microprocessor performs a semiautomatic two-point calibration with automatic temperature compensation. The regulation of dissolved oxygen is obtained by a variation of the flow rate of air.

CONTINUOUS FERMENTATION (CHEMOSTAT)

A **scale adaptor** allows weighing of the fermentor. It is simply placed under the front part of the fermentor body and connected to the X-channel input of the fermentor. By means of a pump connected to the fermentor, the weight (volume) of the culture can be kept constant. This allows the running of **continuous cultures at low cost.**

GAS FLOW CONTROLLER

Flow controller system specially designed for the use together with laboratory bioreactors and fermentors. It allows the control of pH of cell culture by controlled addition of gaseous CO_2, control of nitrogen or of any other gas with suitable controller.

A **high quality laminar mass flow sensor** measures the flow rate given by the digital display controlled by a microprocessor.

The **flow rate can be programmed & volume totalized.**

HIGH-QUALITY PERISTALTIC PUMPS

They are connected by a single cable to the sockets on the rear side of the fermentor. Since the **pumps are not integrated into the fermentor** they can be used for other applications elsewhere in the laboratory (e.g. for chromatography etc.). This represents **considerable savings** for the user.
A new connection system provides **double sealing of the tubing** and, therefore, reduces strongly the **contamination** probability during the transfer of solutions into the vessel.

INOCULATION AND SAMPLING PORTS

Inoculation, addition of acid or base and sample removal is made through **four stainless steel capillaries** equipped with **double seal** fittings.

MEASUREMENT AND REGULATION

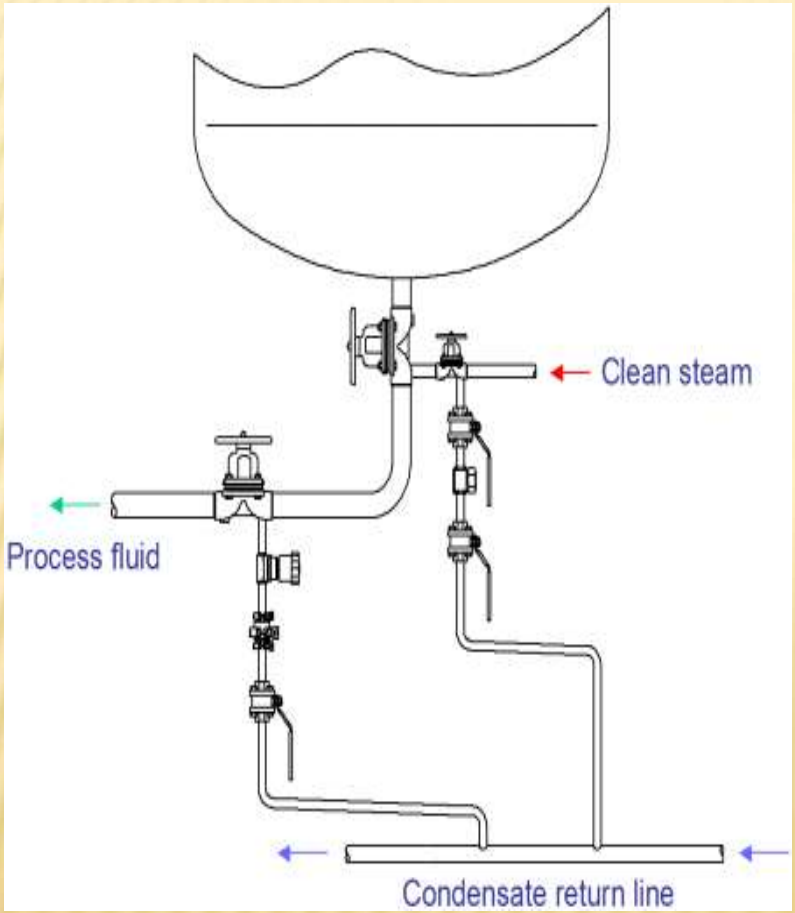
The control panel consists of an LCD display and control buttons.

All parameters (**temperature, pH, pO_2, air flow rate, agitation and one free selectable parameter,** e.g. pCO_2, optical density, antifoam etc.) are visible at a glance on a large LCD back light display **.**

The limits of low or/and high **alarm** can be set.
After alarm activation an acoustic signal is heard, the indication appears on the display and an electric signal is generated on the rear side connector of the fermentor.
Each fermentor can be coupled to a PC thus unlimited possibilities for control and data processing.

STEAM LINE

- Sterilization by Steam



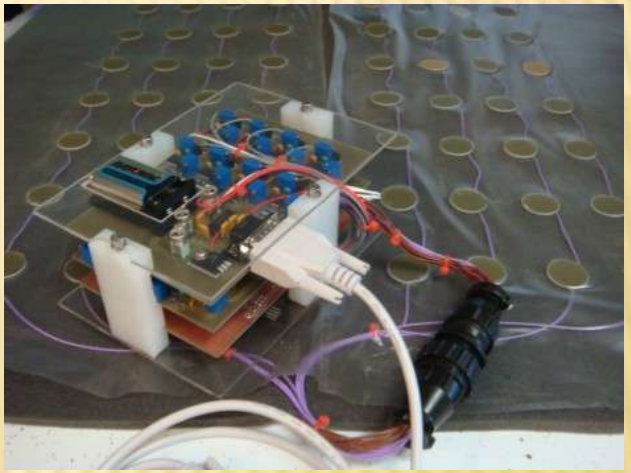
The retriever captures the PIG at the end of the run.
Cleaning is finished when the PIG enters the sight glass.

FOAM SENSORS

- This is the only instrument for foam analysis based on the pressure drop technique . The important feature is that due to the applied partial vacuum the foam in the measuring cells is essentially homogeneous over the whole foam column. measure the most important foam parameter in terms of foam stability and foam lifetime at constant capillary pressure in the liquid phase of the foam

SENSORS

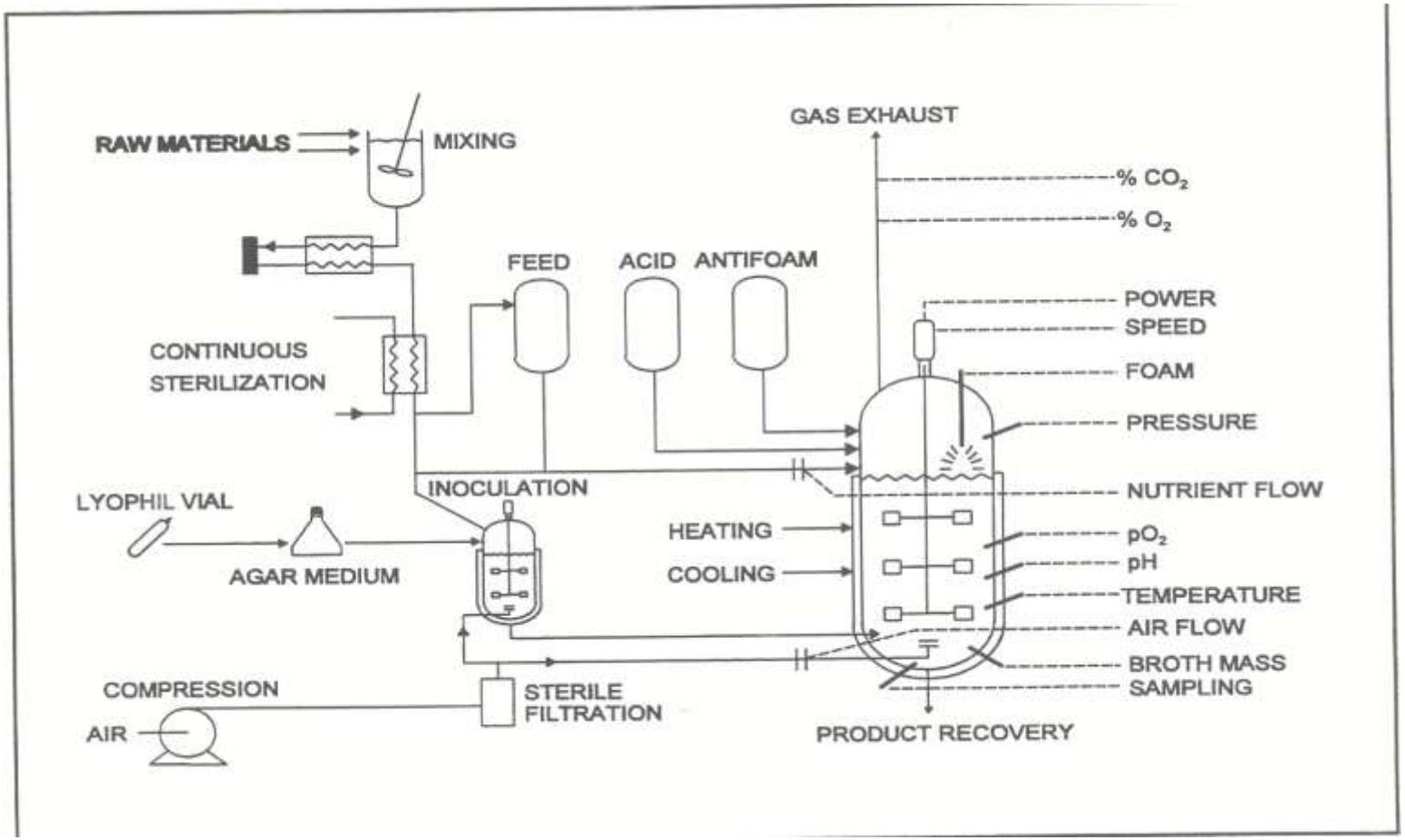
Pressure sensing sensor



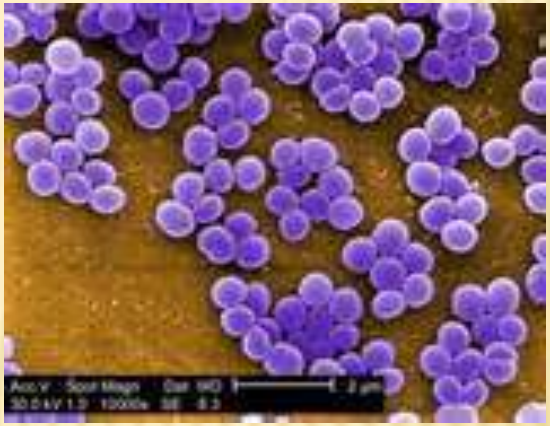
Air Leak Sensor

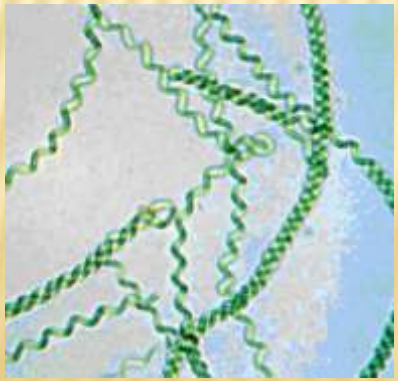
Thermal Sensor

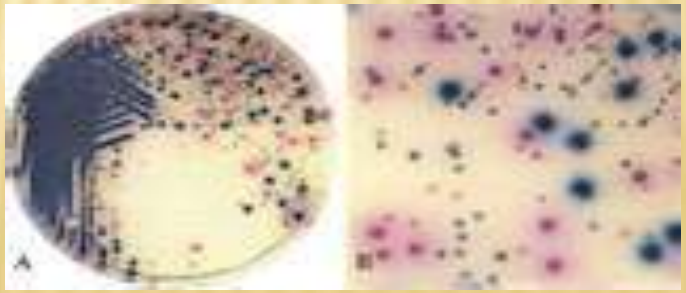
Flow sheet of a multipurpose fermenter and its auxiliary equipment

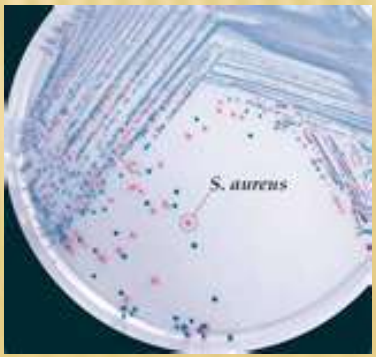


SELECTION OF MICROBIAL STRAIN FOR THE FERMENTOR









Isolation and Screening of Microorganisms

- It should be pure, and free from phage.
- It should be genetically stable, but amenable to genetic modification.
- It should produce both vegetative cells and spores; species producing only mycelium are rarely used.
- It should grow vigorously after inoculation in seed stage vessels.
- Should produce a single valuable product, and no toxic by-products.
- Product should be produced in a short time, e.g., 3 days. It should be amenable to long term conservation.
- The risk of contamination should be minimal under the optimum performance conditions

Strain Improvement

- Product yields can be increased by
  (i) developing a suitable medium for fermentation,
  (ii) refining the fermentation process
  (iii) improving the productivity of the strain.
- Strain improvement is based on the following three approaches:
  (i) mutant selection,
  (ii) recombination, and
  (iii) recombinant DNA technology.

MICROBIAL STRAIN SELECTION

Screening-Primary

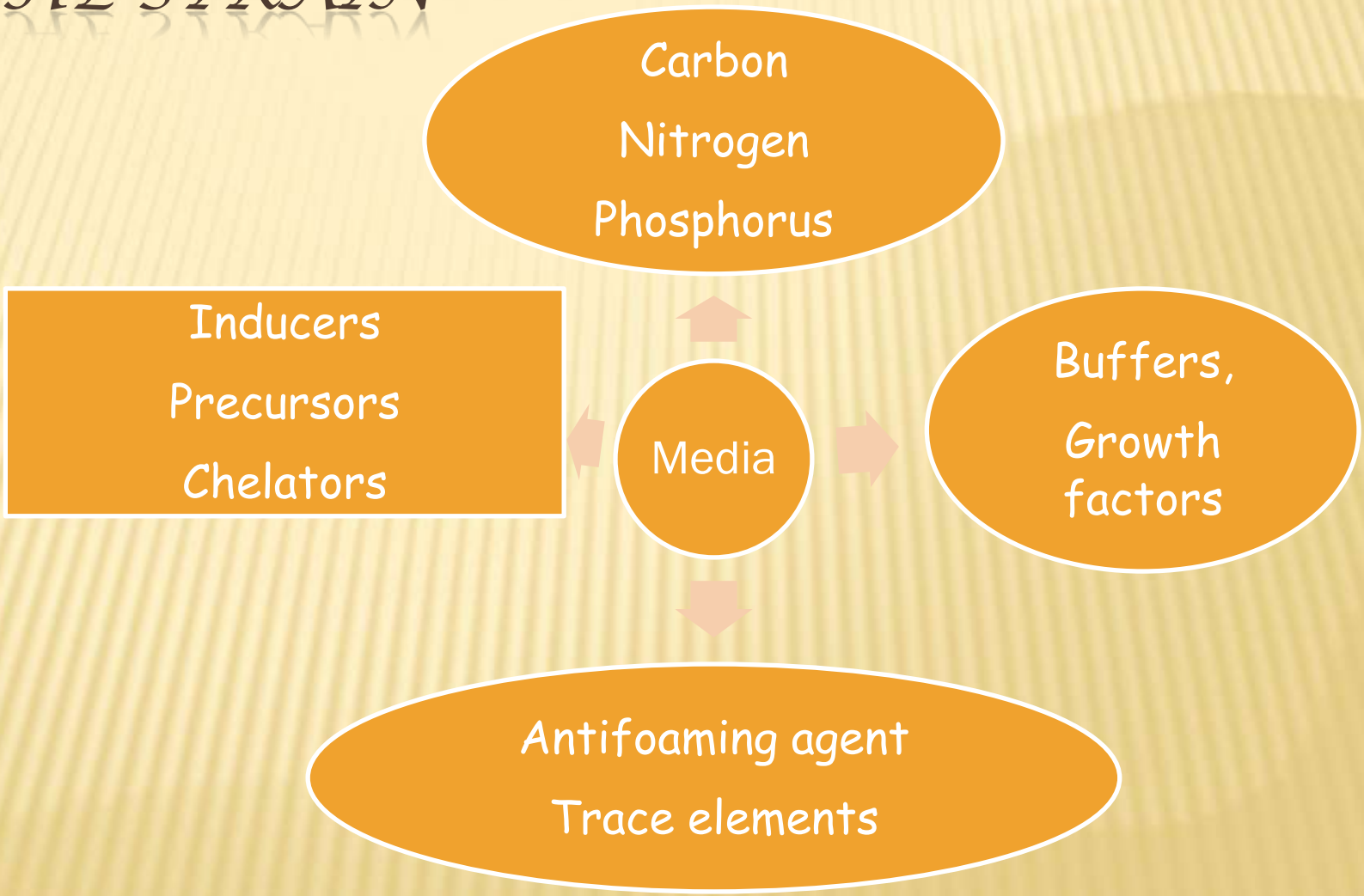
- Primary screening is time consuming and labour intensive since a large number of isolates have to be screened to identify a few potential ones. However this is possibly the most critical step since it eliminates the large bulk of unwanted useless isolates, which are either non producers or producers of known compounds.
- **Crowded plate Technique**
- **Auxanography**
- **Enrichment Culture technique**
- **Use of Indicator dye**

MICROBIAL STRAIN SELECTION

Secondry Screening

- Gene Expression
- Metagenomics
- Random PCR

REQUIREMENTS FOR THE GROWTH & FERMENTATION OF THE STRAIN



Fermentation medium

• Define medium → nutritional, hormonal, and substratum requirement of cells

• In most cases, the medium is independent of the bioreactor design and process parameters

• Even small modifications in the medium could change cell line stability, product quality, yield, operational parameters, and downstream processingFermentation medium consists of:

• Macronutrients (C, H, N, S, P, Mg sources → water, sugars, lipid, amino acids, salt minerals)

• Micronutrients (trace elements/ metals, vitamins)

• Additional factors: growth factors, attachment proteins, transport proteins, etc)

For aerobic culture, oxygen is sparged

FERMENTATION MEDIA

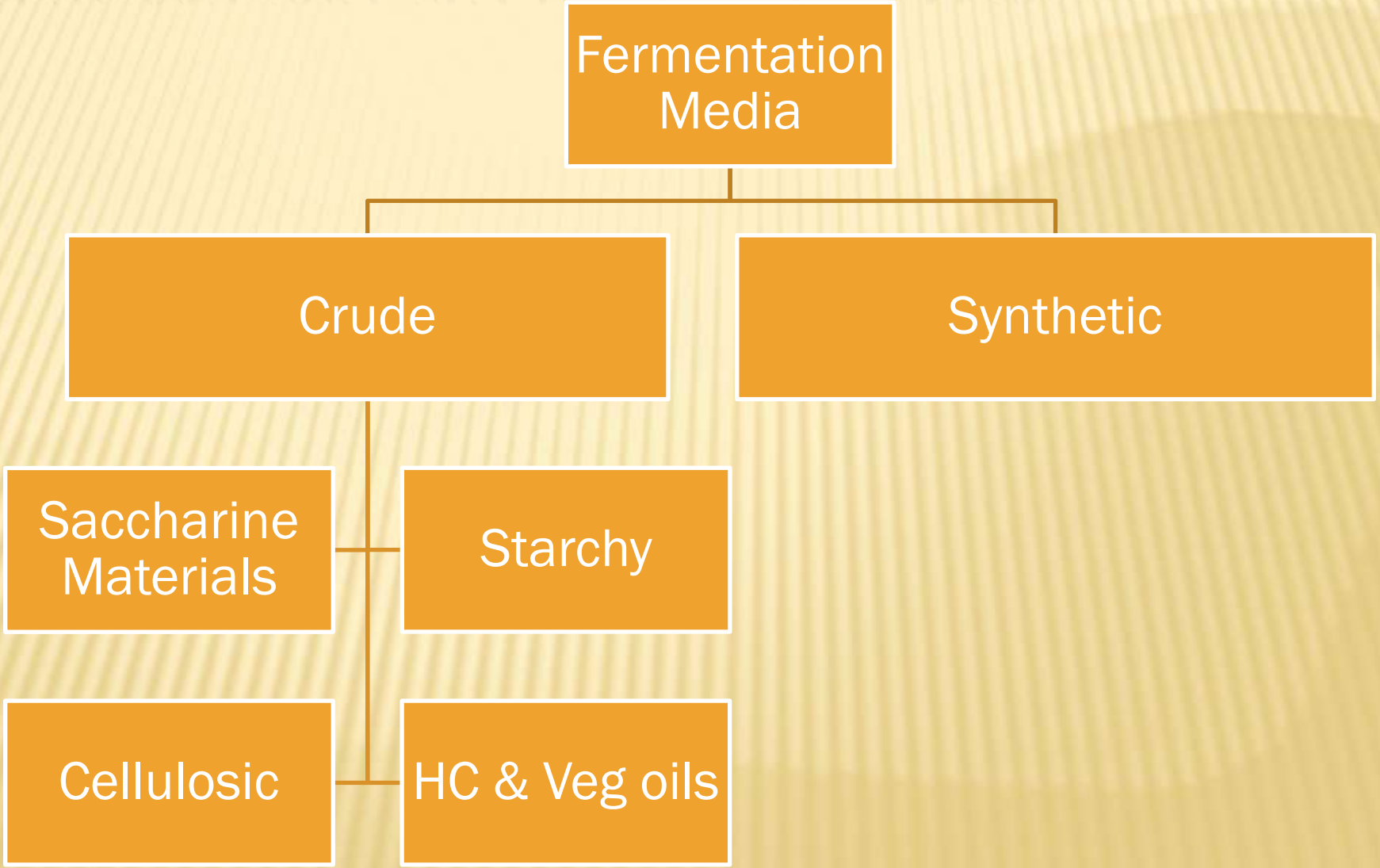
Carbon source

- Glucose Corn sugar, Starch, Cellulose
- Sucrose Sugarcane, Sugar beet molasses
- Lactose Milk whey
- Fats Vegetable oils
- Hydrocarbons Petroleum fractions

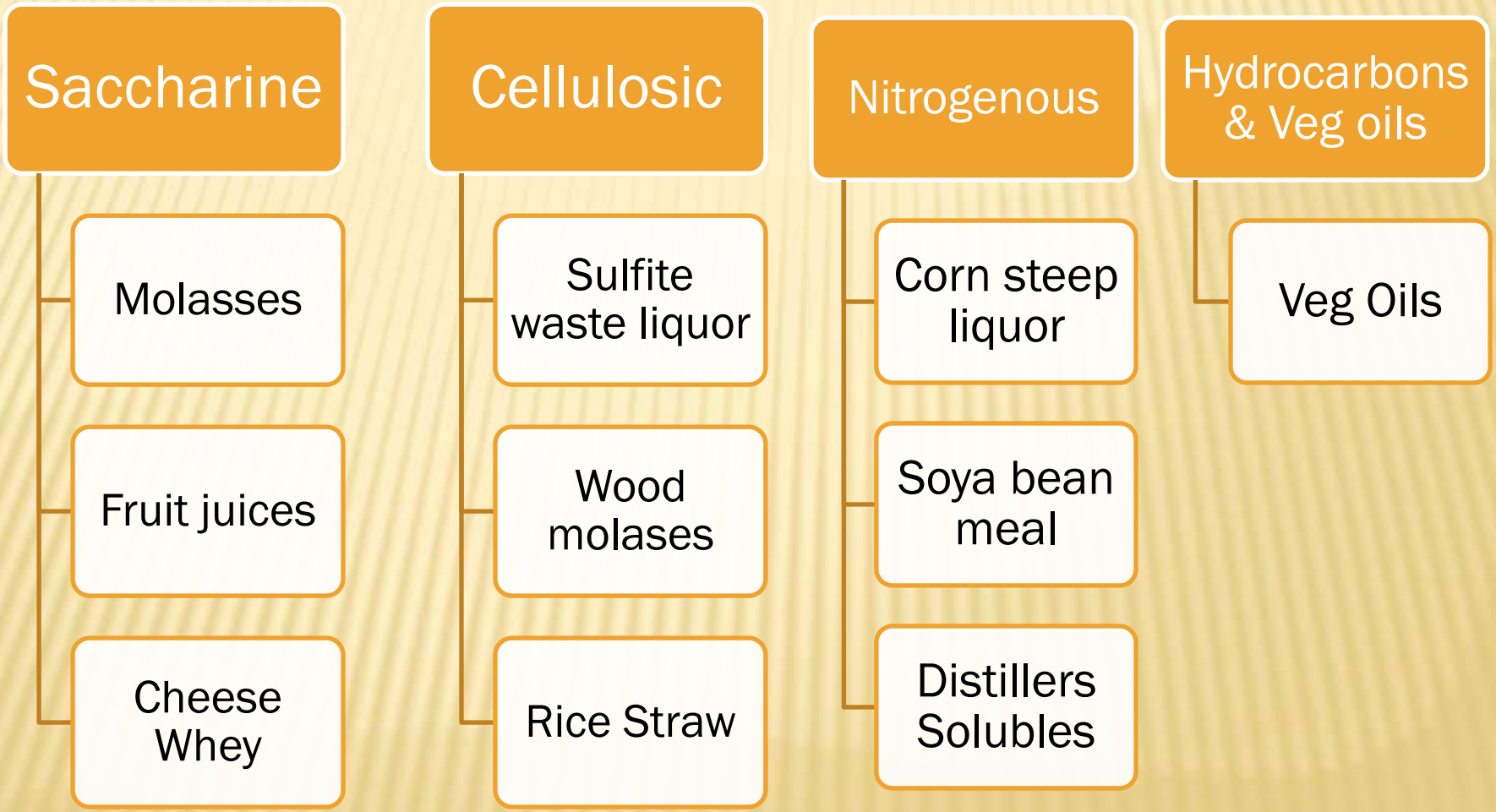
Nitrogen source

- Protein Soybean meal, Cornsteep liquor, Distillers' solubles
- Ammonia Pure ammonia or ammonium salts
- Nitrate Nitrate salts
- Nitrogen Air

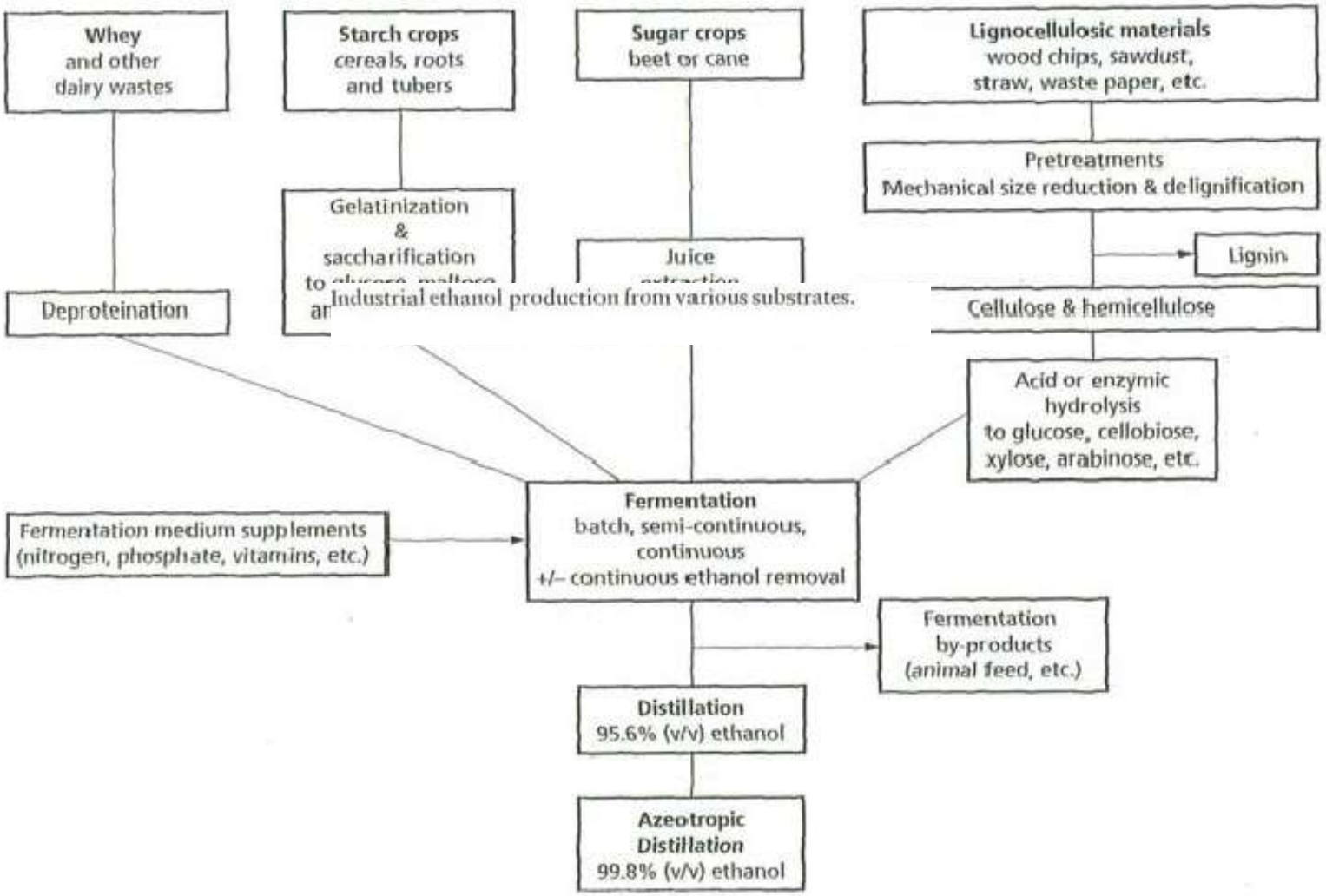
FERMENTATION MEDIA



TYPES OF MEDIA



ETHANOL PRODUCTION FROM SEVERAL SUBSTRATES



Industrial ethanol production from various substrates.

Inoculums

Incoculum is the substance/ cell culture that is introduced to the medium. The cell then grow in the medium, conducting metabolisms.

Inoculum is prepared for the inoculation before the fermentation starts.

It needs to be optimized for better performance:

- Adaptation in the medium
- Mutation (DNA recombinant, radiation, chemical addition)

INOCULUM DEVELOPEMENT

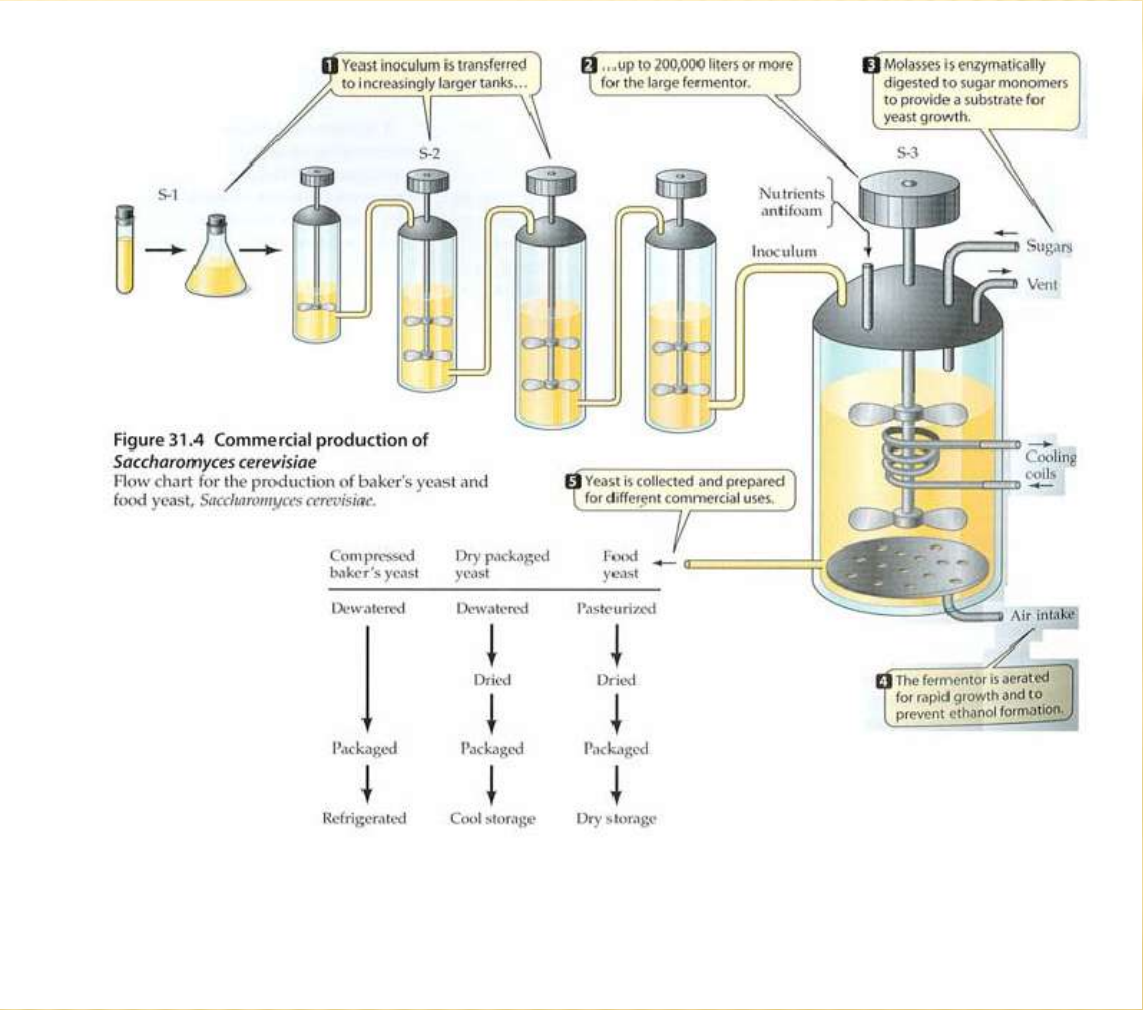


Figure 31.4 Commercial production of *Saccharomyces cerevisiae*
Flow chart for the production of baker's yeast and food yeast, *Saccharomyces cerevisiae*.

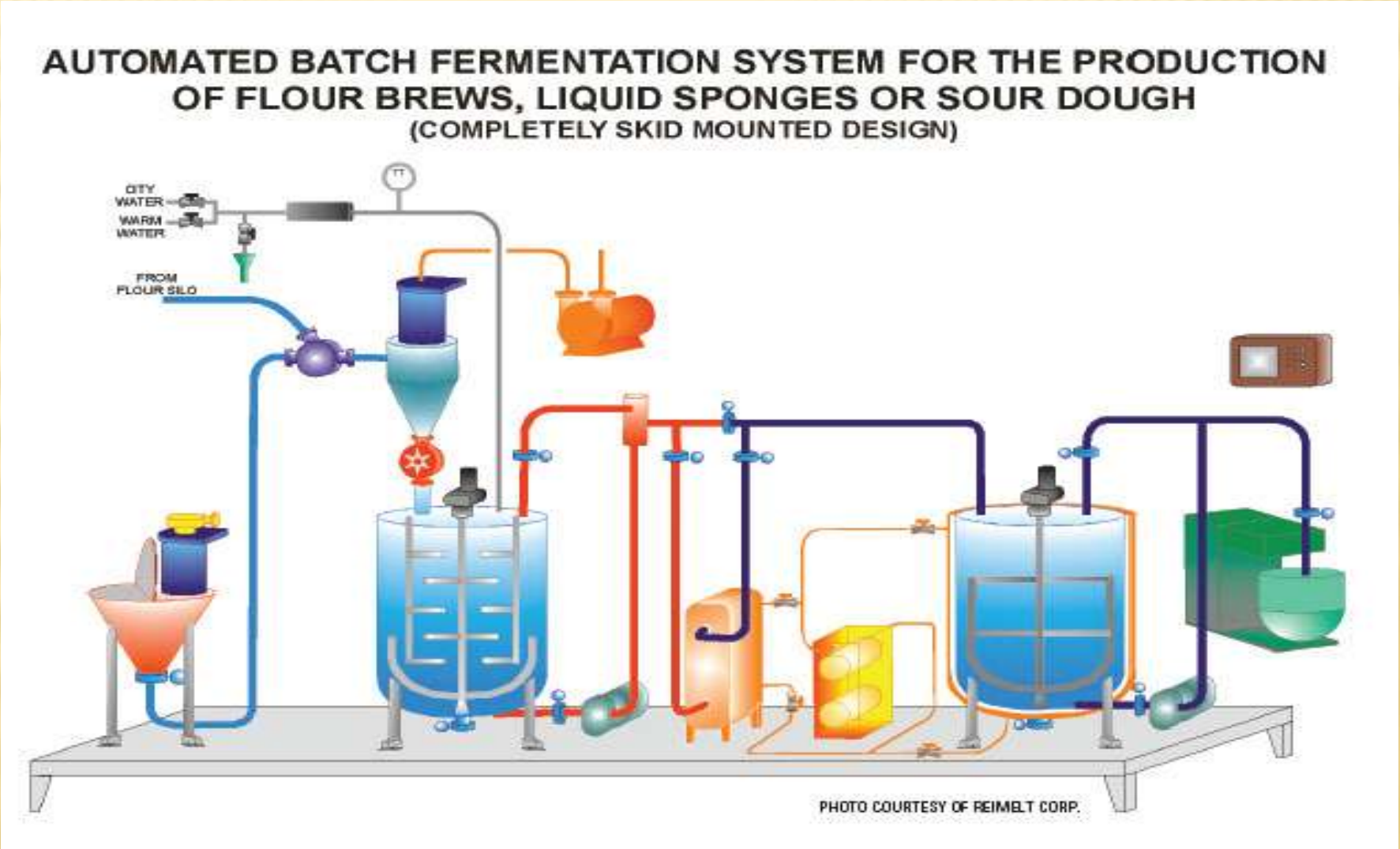
Fermentation techniques

Submerged Fermentation

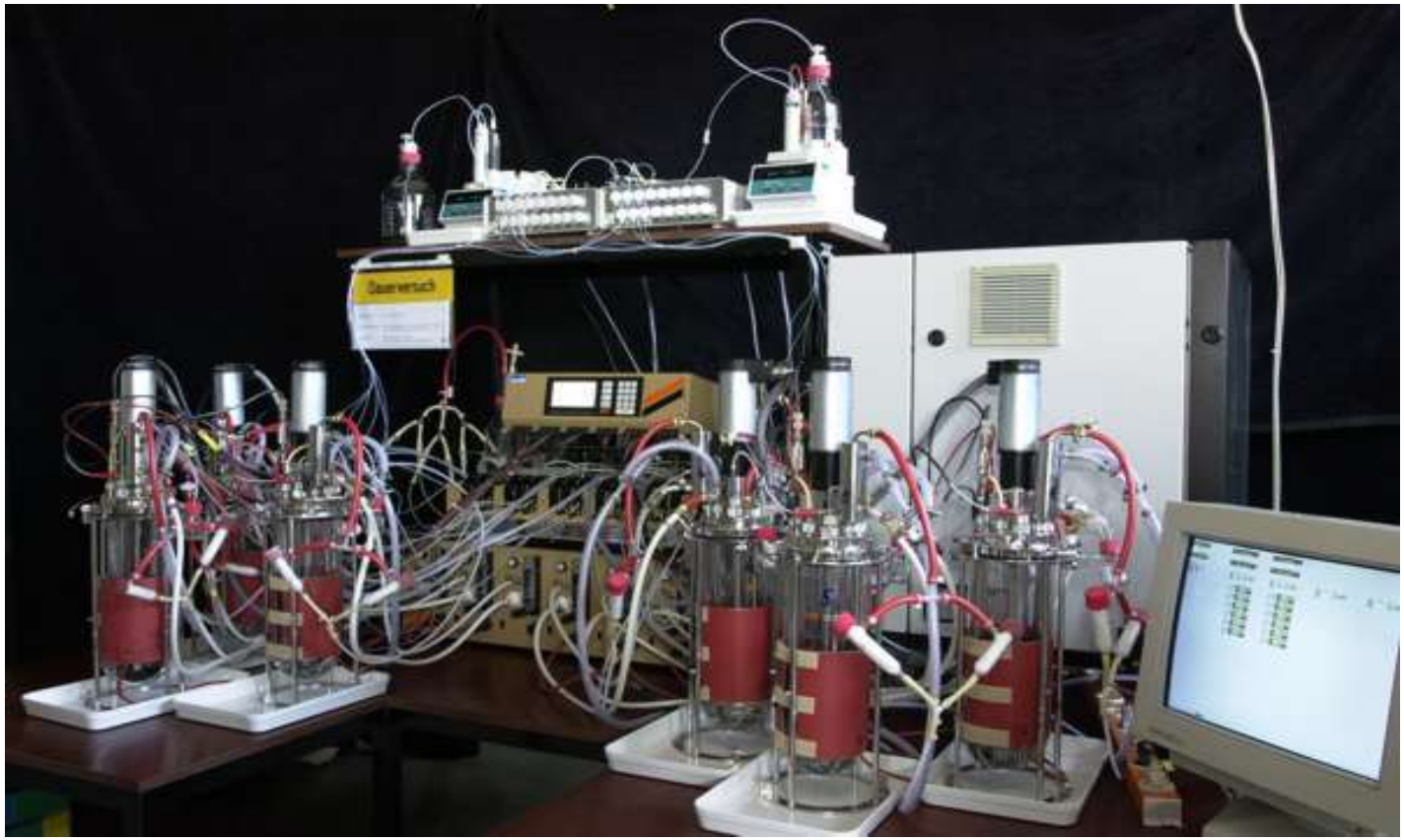
Surface Fermentation

The microorganisms are cultivated on the Surface of a liquid or solid substrate.

BATCH FERMENTATION

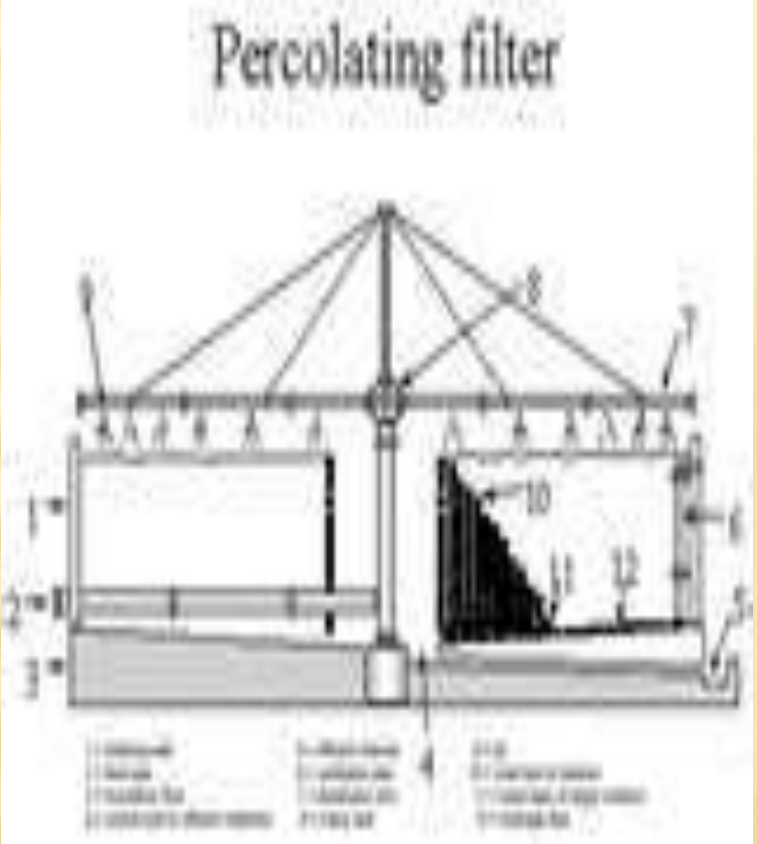


Continuous Fermentation



TYPES OF FERMENTORS- TOWER FERMENTOR

TRICKLING FILTER



BIOREACTOR

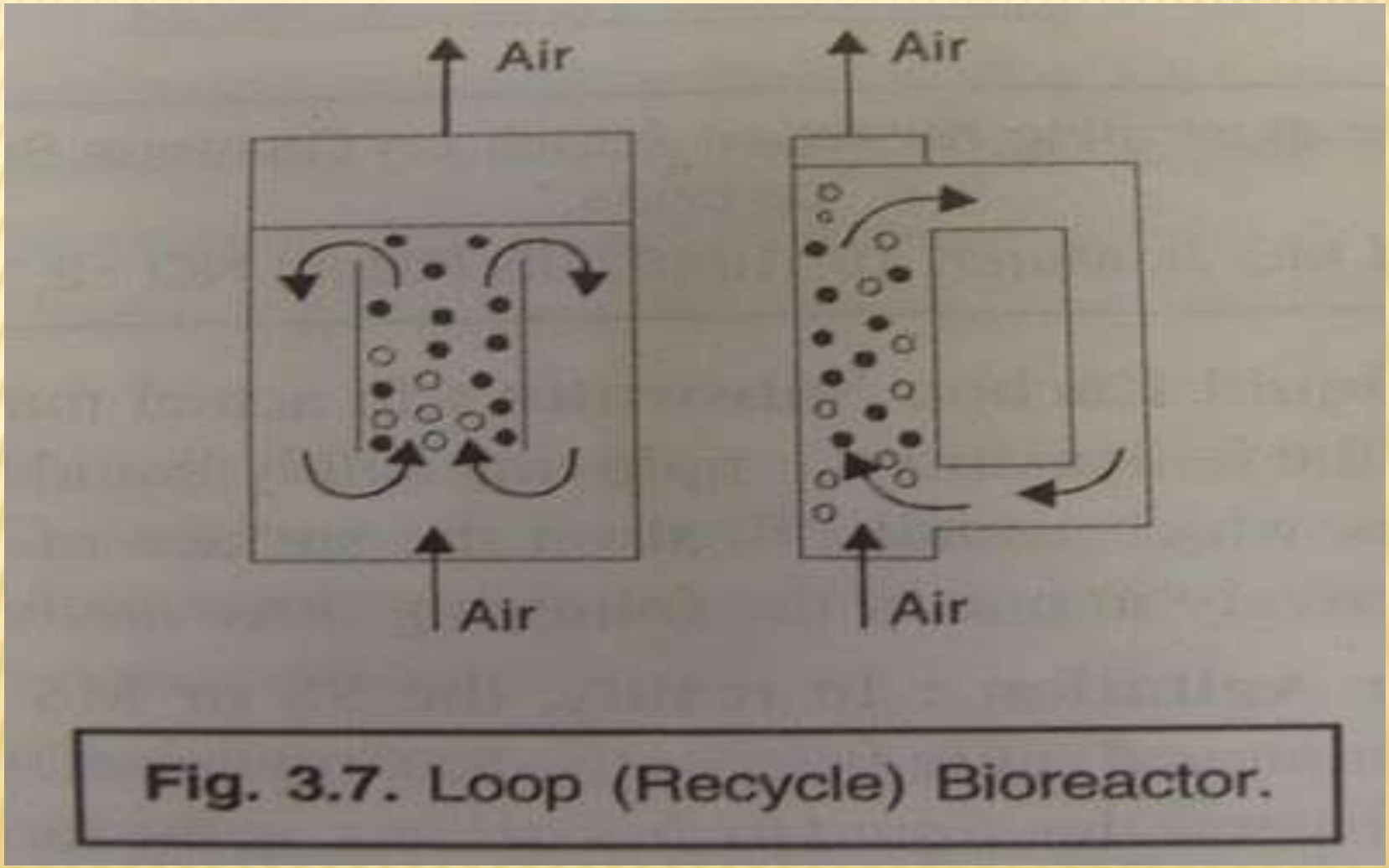


Fig. 3.7. Loop (Recycle) Bioreactor.

FRINGS GENERATOR

ROTATING DRUM BIOREACTOR

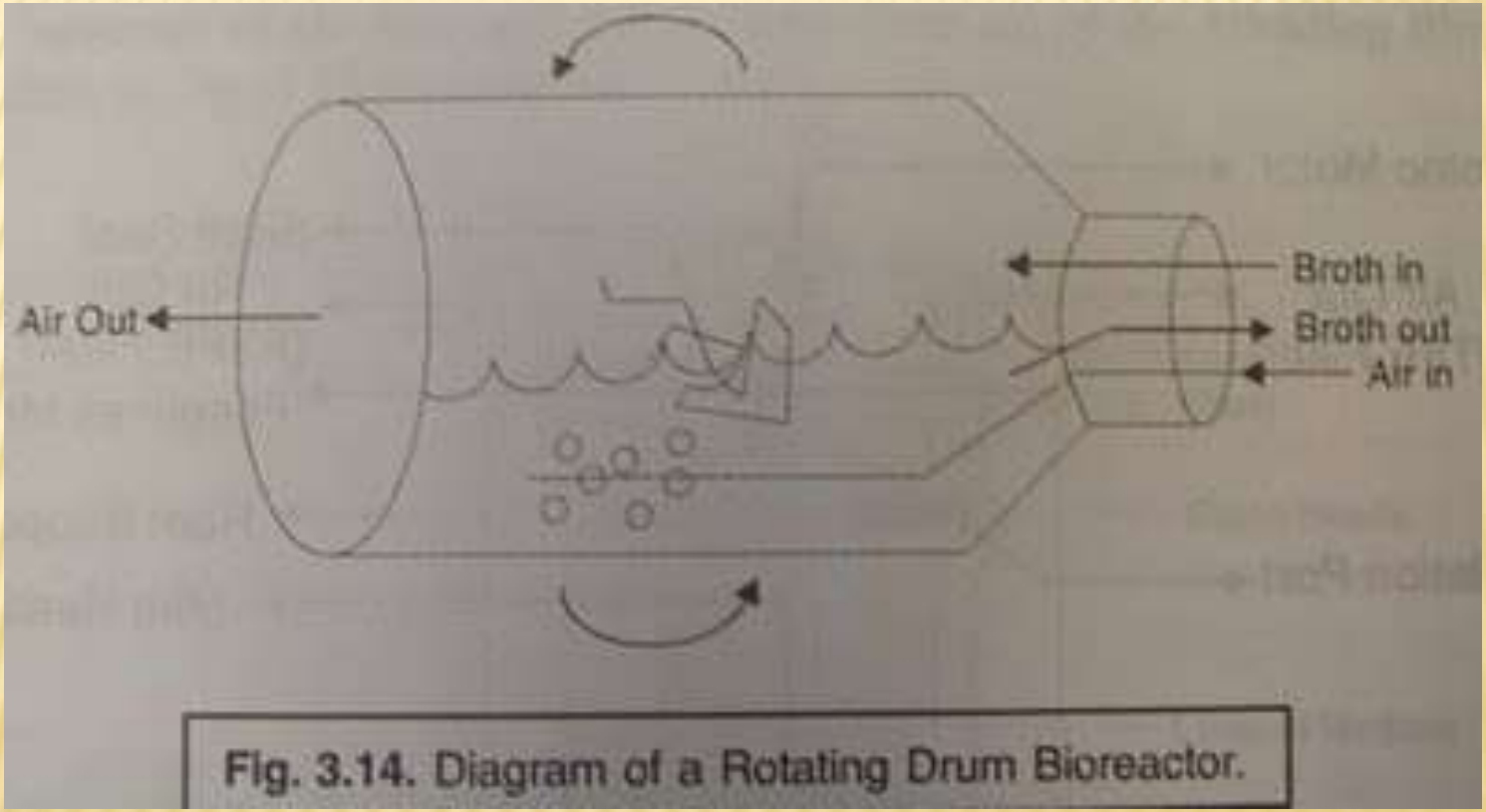
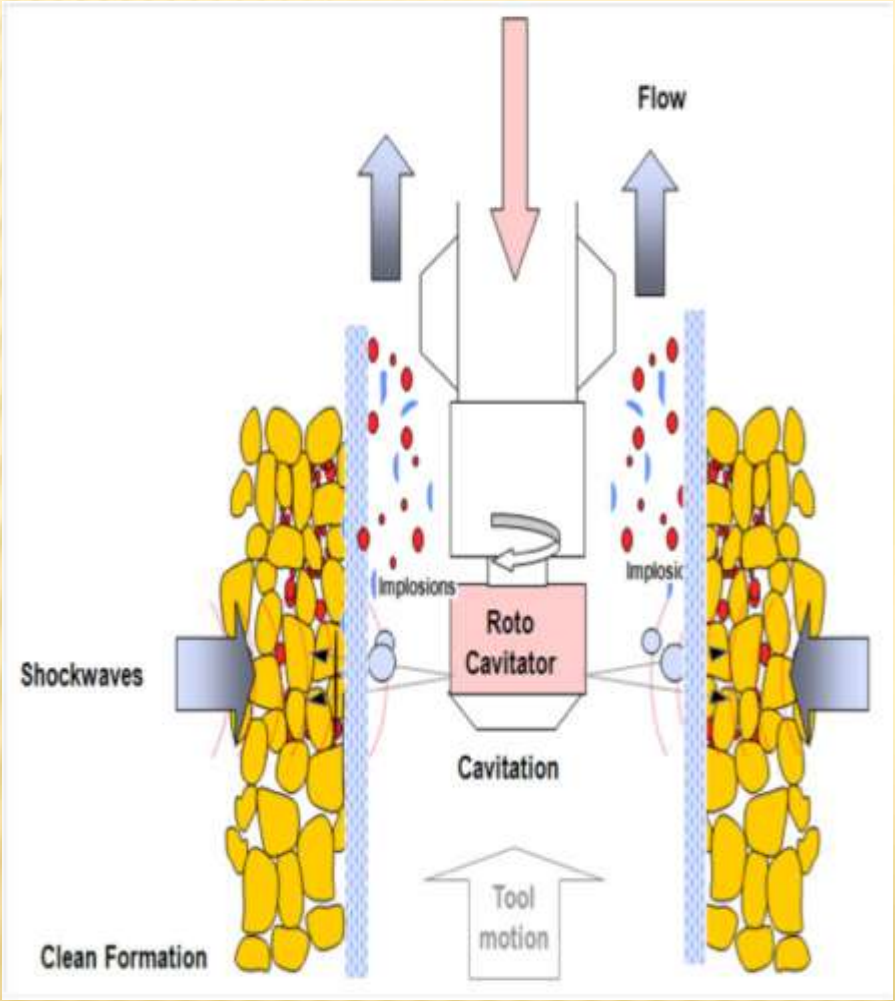
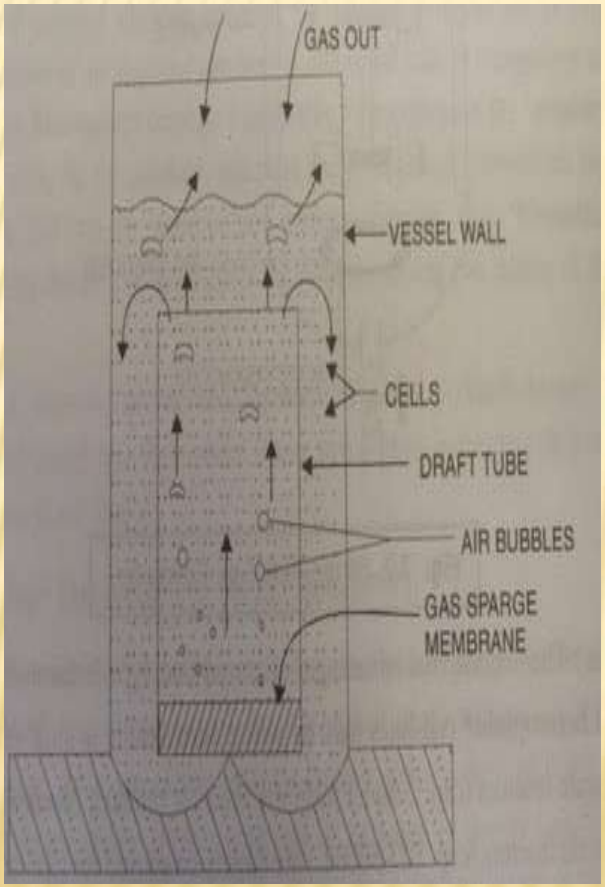


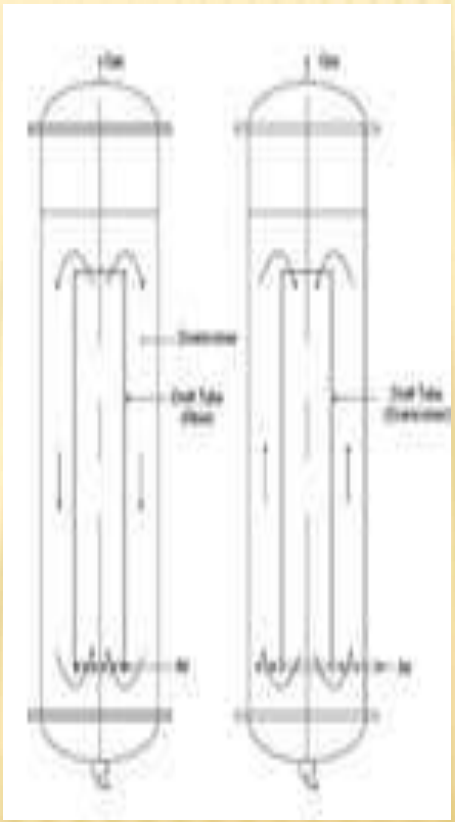
Fig. 3.14. Diagram of a Rotating Drum Bioreactor.

CAVITATOR

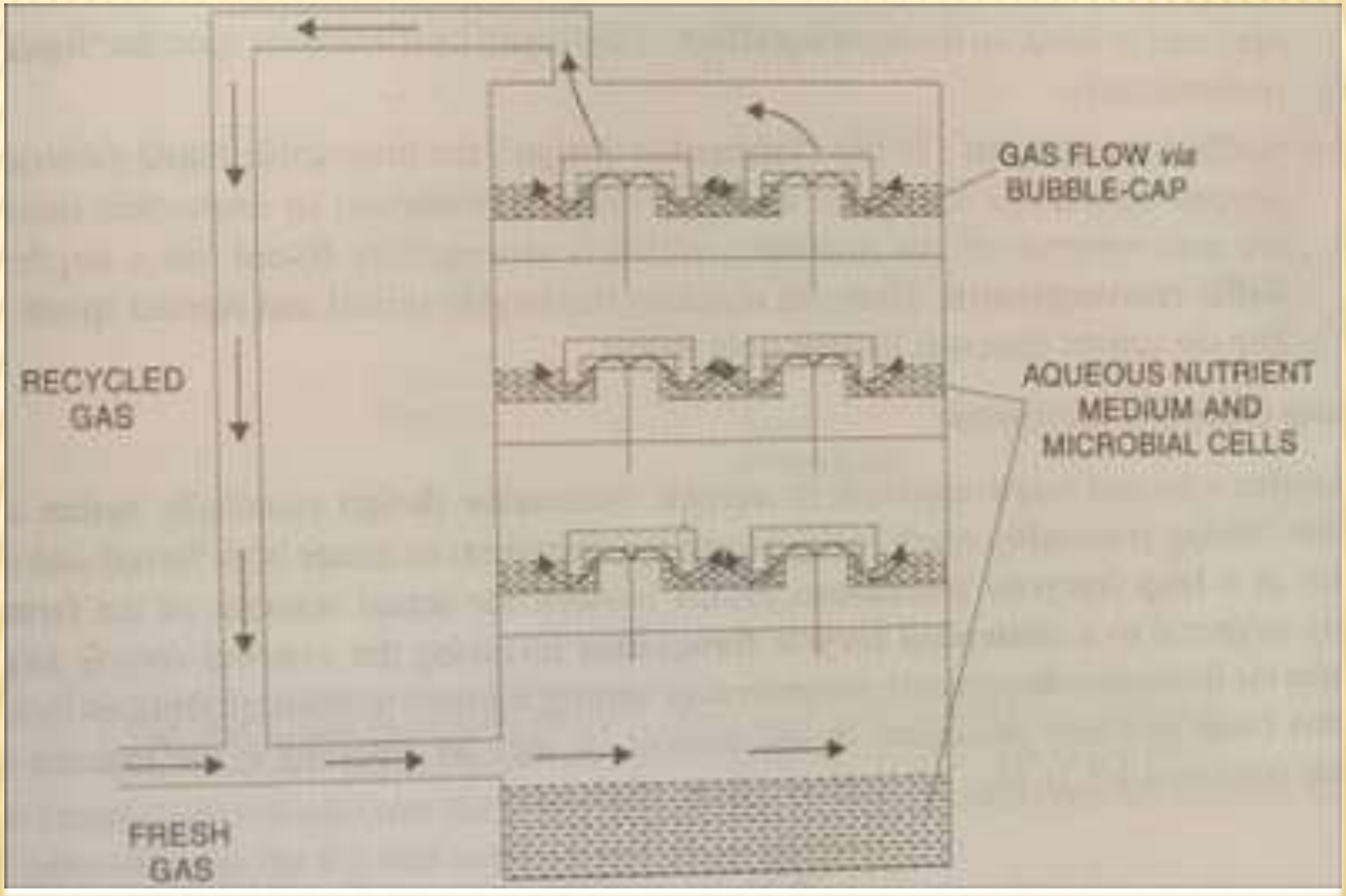

Flow
Implosions
Implosic
Roto
Cavitator
Shockwaves
Cavitation
Tool
motion
Clean Formation

AIR LIFT FERMENTOR





BUBBLE CAP FERMENTOR



HORTON SPHERES

ACTIVATED SLUDGE FERMENTOR

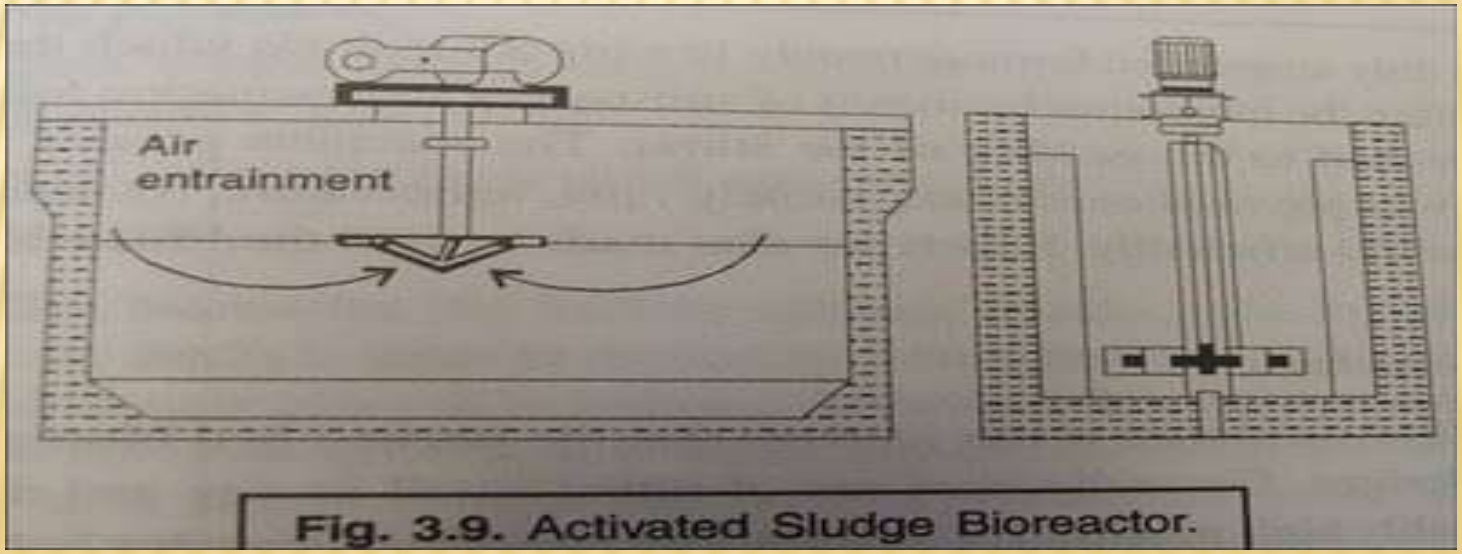


Fig. 3.9. Activated Sludge Bioreactor.

Major commercial products obtained from microbes

I. Foods, flavoring agents and food supplements, and beverages
- Foods
  - Fermented meat
  - Cheeses and milk products
  - Edible mushrooms
  - Leavened bread-baker's yeast
  - Coffee
  - Pickles, olives, sauerkraut
  - Single-cell protein
- Flavoring agents and food supplements
  - Vinegar
  - Nucleosides
  - Amino acids
  - Vitamins
- Beverages
  - Wines
  - Beer, ale
  - Whiskey
- Vitamins
  - B_{12}
  - Riboflavin

II. Organic acids
- Citric acid
- Itaconic acid

III. Enzymes and microbial transformations
- Commercial enzymes
- Sterol conversions

IV. Inhibitors
- Biocides
- Antibiotics

V. Products of genetically engineered microbes
- Insulin
- Human growth factor

Primary vs. Secondary metabolism

Primary metabolites:

- produced during active growth
- generally a consequence of energy metabolism and

necessary for the continued
growth of the microorganism

Substrate A → Product

Substrate A → B → C → Product

- ethanol, lactic acid,…

Secondary metabolites:

- synthesized after the growth phase nears completion
- a result of complex reactions that occur during the latter stages of primary growth

Substrate A → B → C → Primary metabolism (no product)
↘
D → E → Product of secondary metabolism

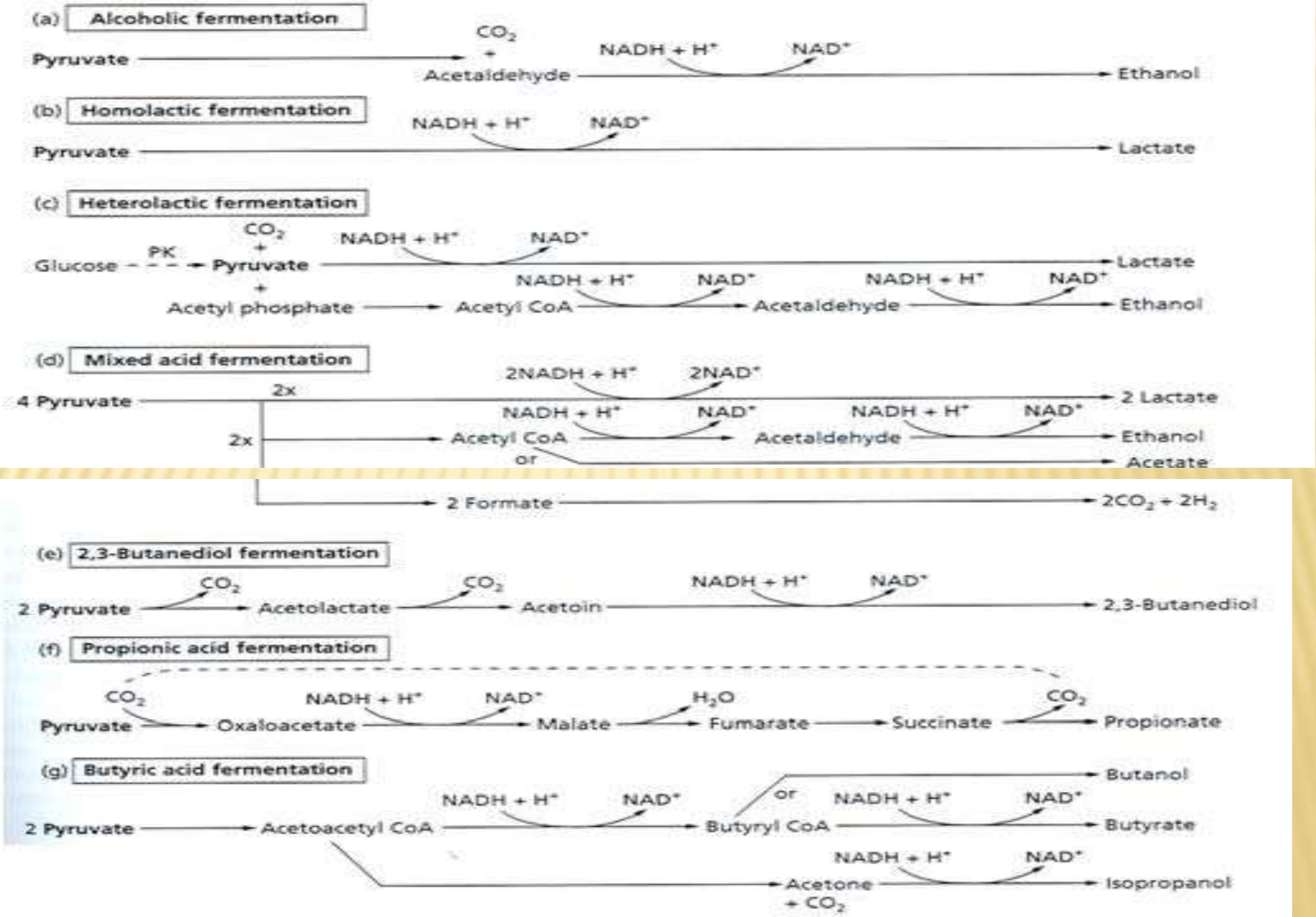
Substrate A → B → C → Primary metabolism (no product)

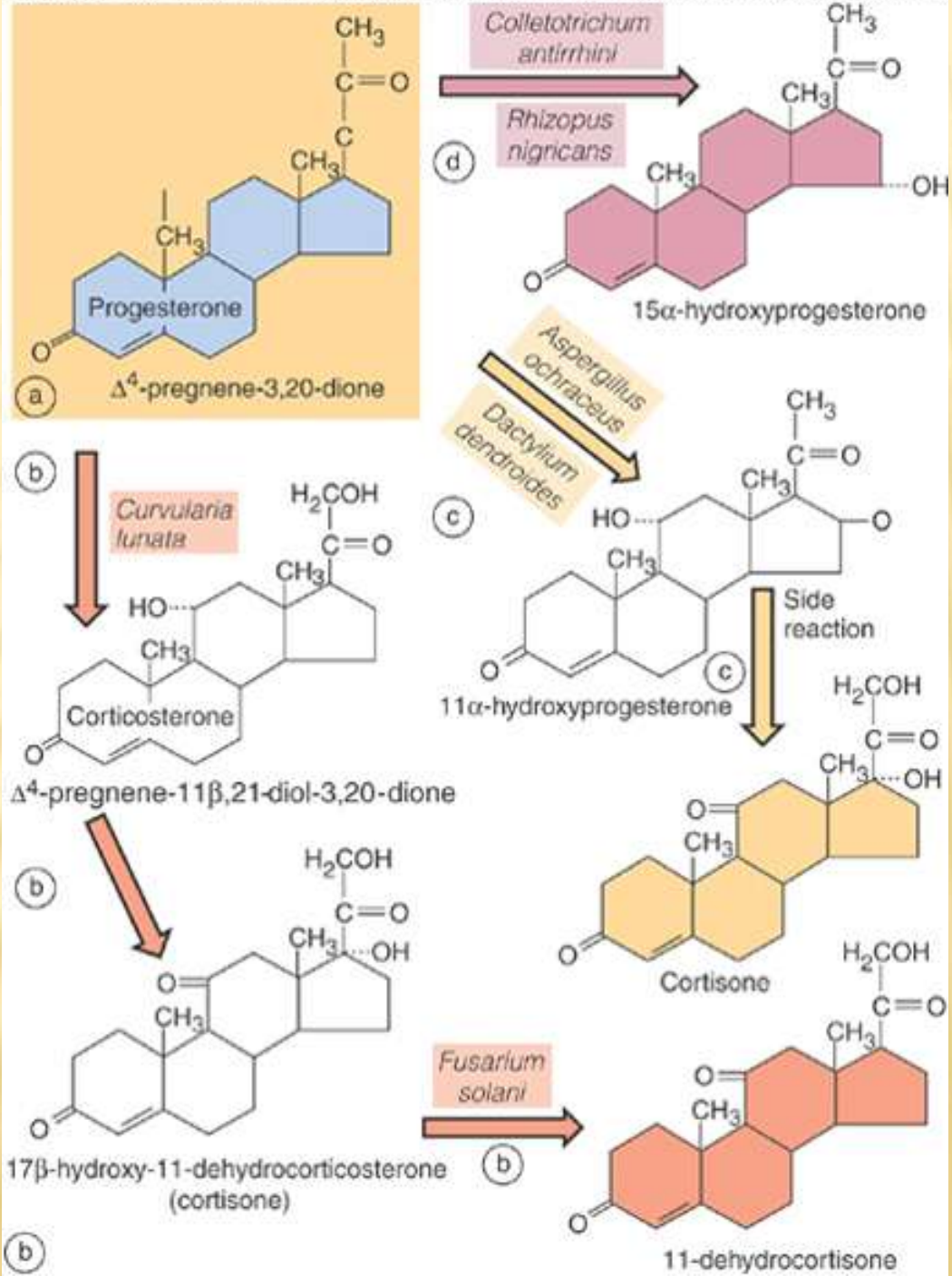
afterwards, the product is formed by metabolism of an intermediate

C → D → Product

- growth phase = trophophase
- idiophase = phase involved in production of metabolites
- citric acid, antibiotics,…

Fermentation products from pyruvate





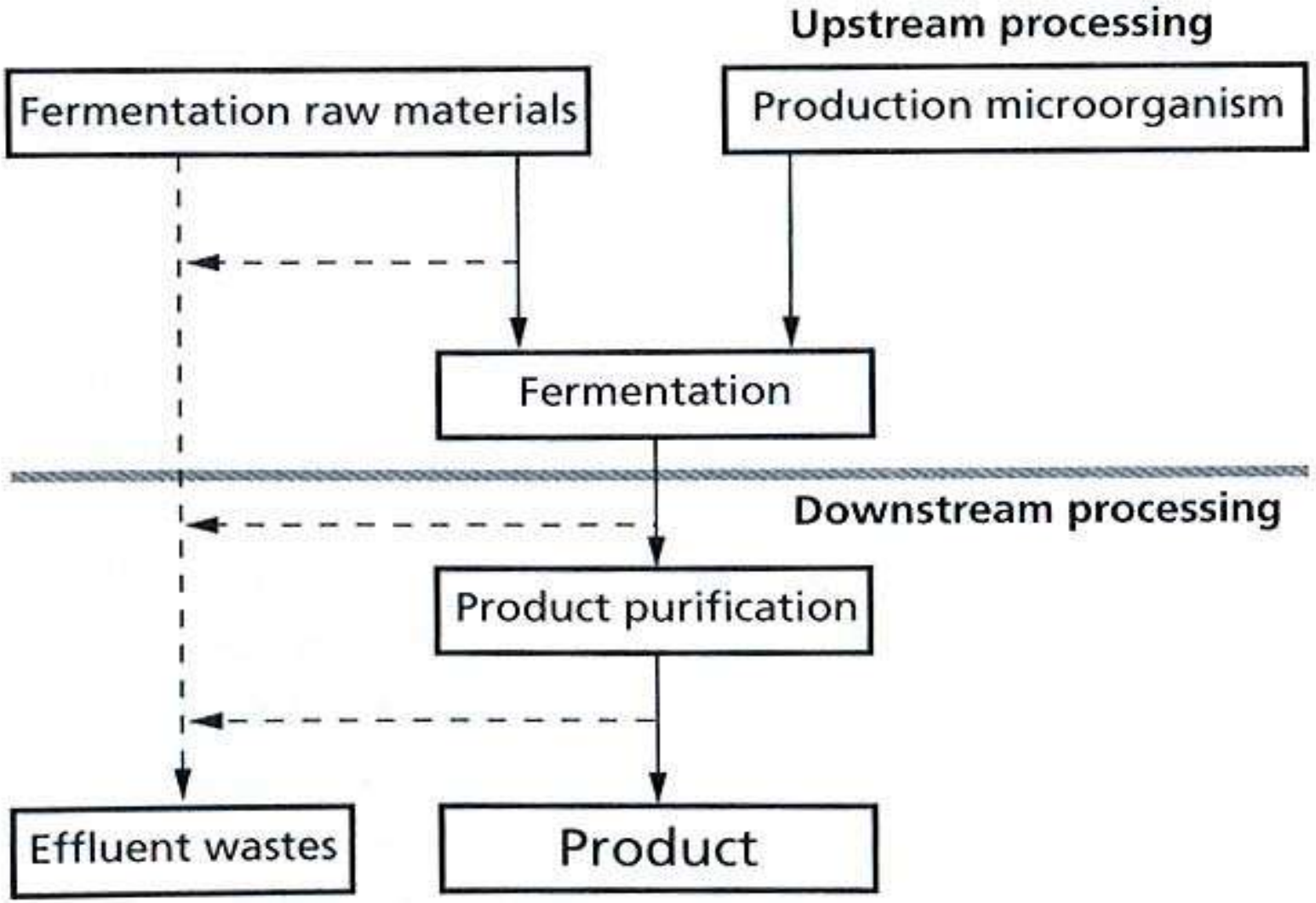
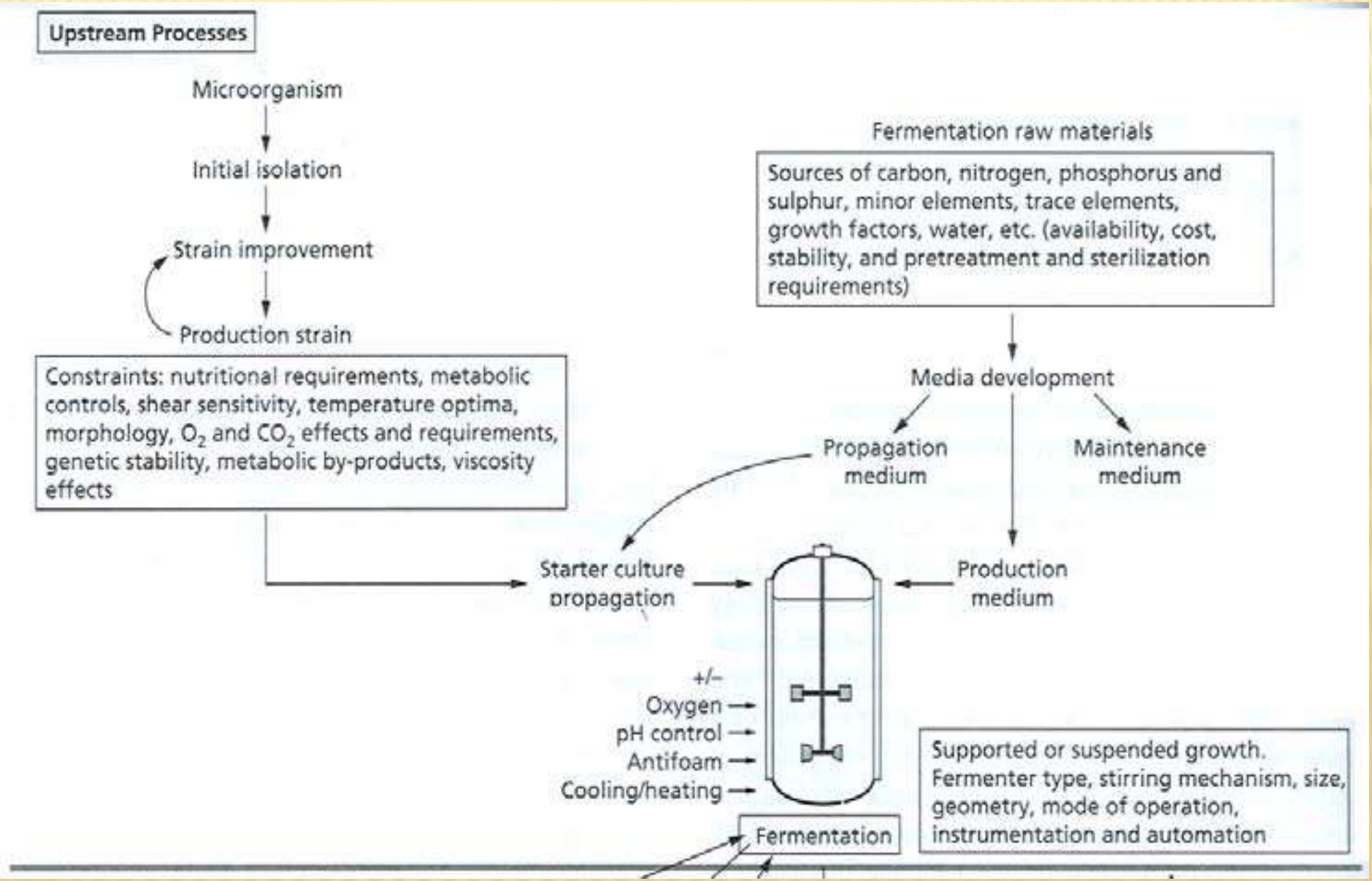


Fig. i Outline of a fermentation process.


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

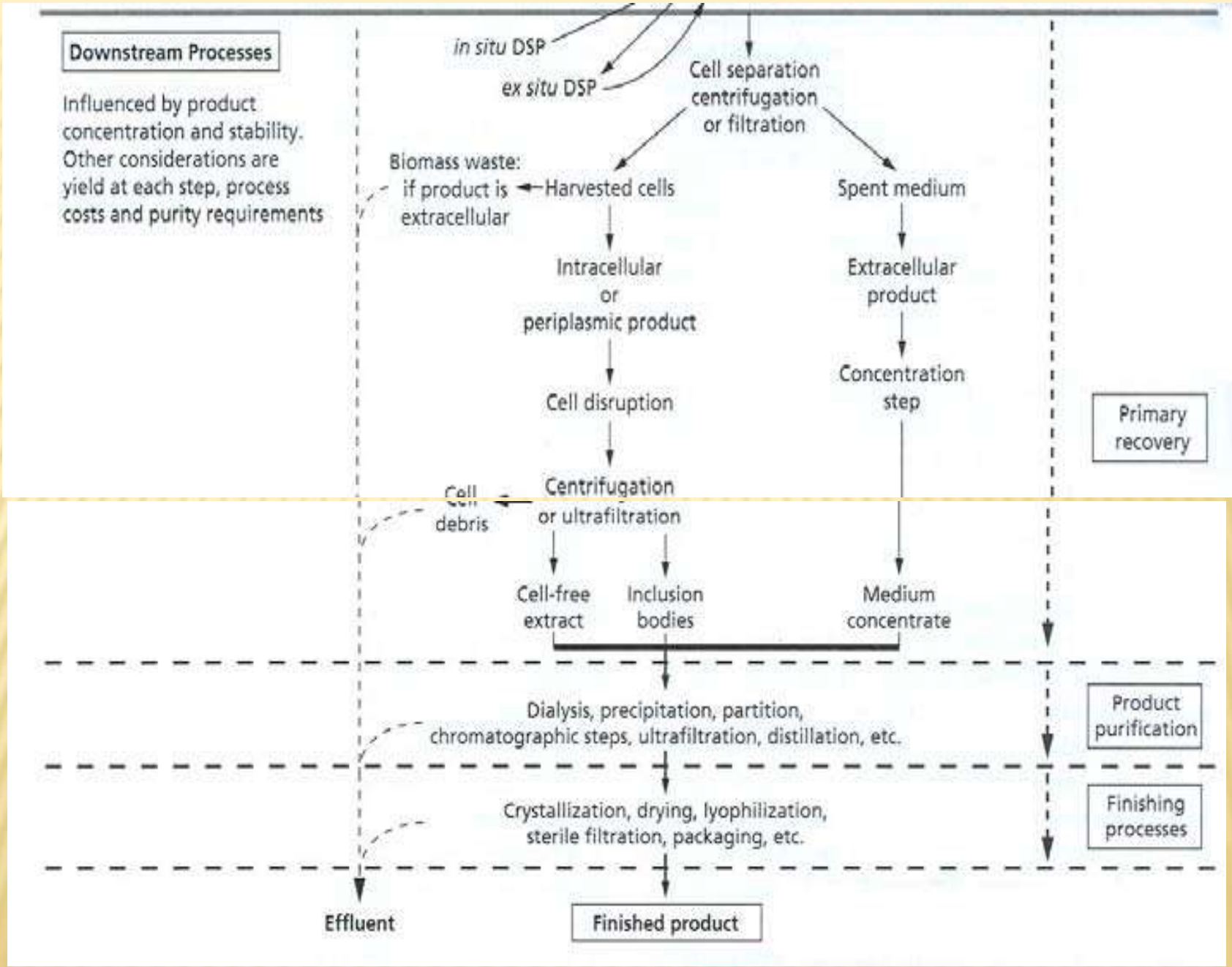

Downstream Processes
Influenced by product concentration and stability. Other considerations are yield at each step, process costs and purity requirements
in situ DSP
ex situ DSP
Cell separation centrifugation or filtration
Biomass waste: if product is extracellular
Harvested cells
Spent medium
Intracellular or periplasmic product
Extracellular product
Cell disruption
Concentration step
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

Table 4.2 Examples of microorganisms classified as GRAS (generally regarded as safe)

Bacteria
Bacillus subtilis
Lactobacillus bulgaricus
Lactococcus lactis
Leuconostoc oenos
Yeasts
Candida utilis
Kluyveromyces marxianus
Kluyveromyces lactis
Saccharomyces cerevisiae
Filamentous fungi
Aspergillus niger
Aspergillus oryzae
Mucor javanicus (Mucor circinelloides f. *circinelloides)*
Penicillium roqueforti

Note: Normally, these microorganisms require no further testing if used under acceptable cultivation conditions.

GENERAL CONCEPTS IN INDUSTRIAL MICROBIOLOGY

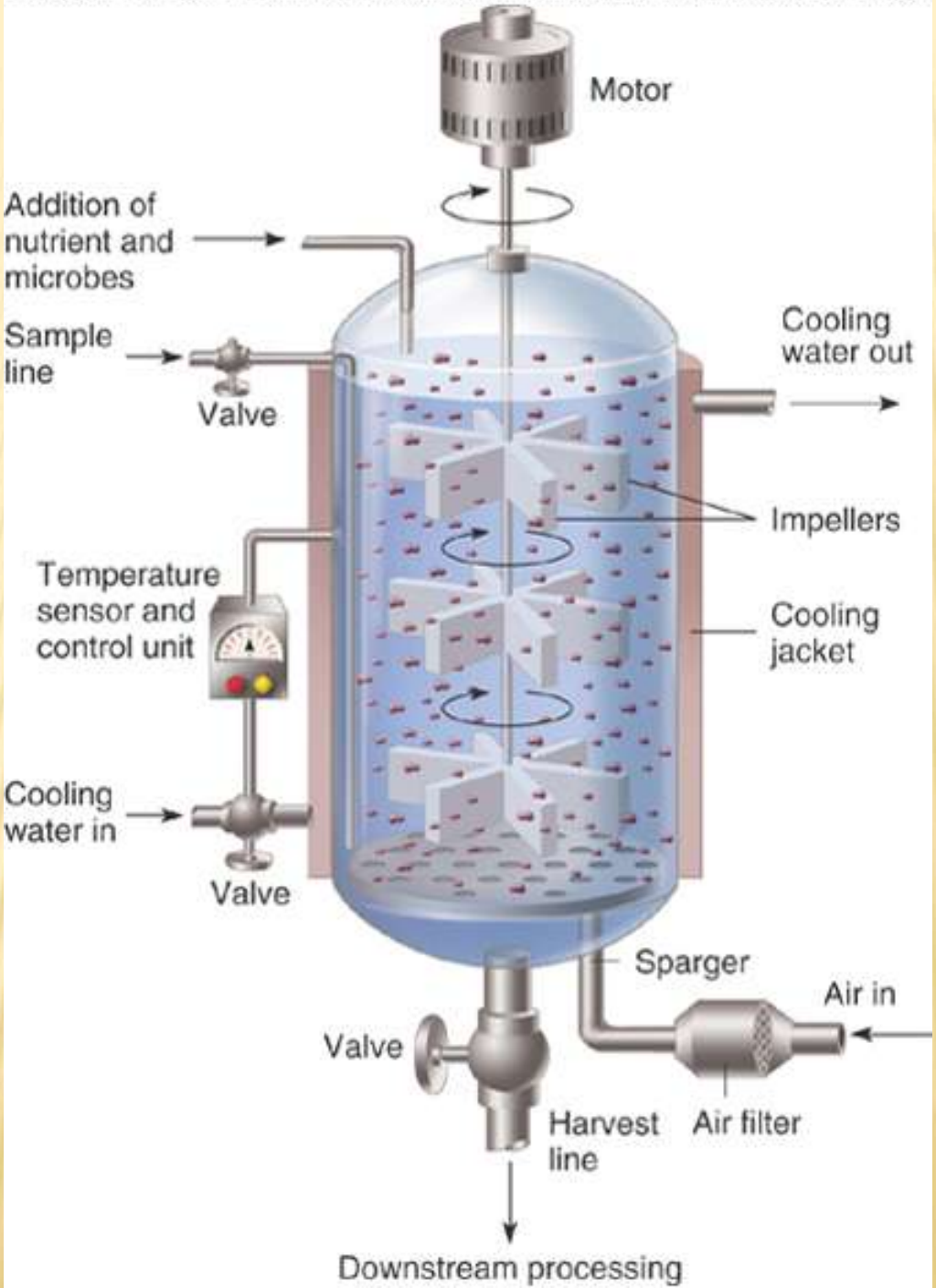
- Bulk production of organic compounds such as antibiotics, hormones, vitamins, acids, solvents, and enzymes
- Any processes involving fermentation

- Mutant strains of bacteria and fungi that synthesize large amounts of metabolites
- **Primary metabolites** - produced during major metabolic pathways and are essential to microbe's function – amino acids, organic acids synthesized during logarithmic growth
- **Secondary metabolites** – by-products of metabolism that may not be critical to microbe's function – vitamins, antibiotics, and steroids synthesized during stationary phase

- Many syntheses occur in sequential fashion involving more than one organism.
- **Biotransformation** – waste product of one organism becomes the building block of the next

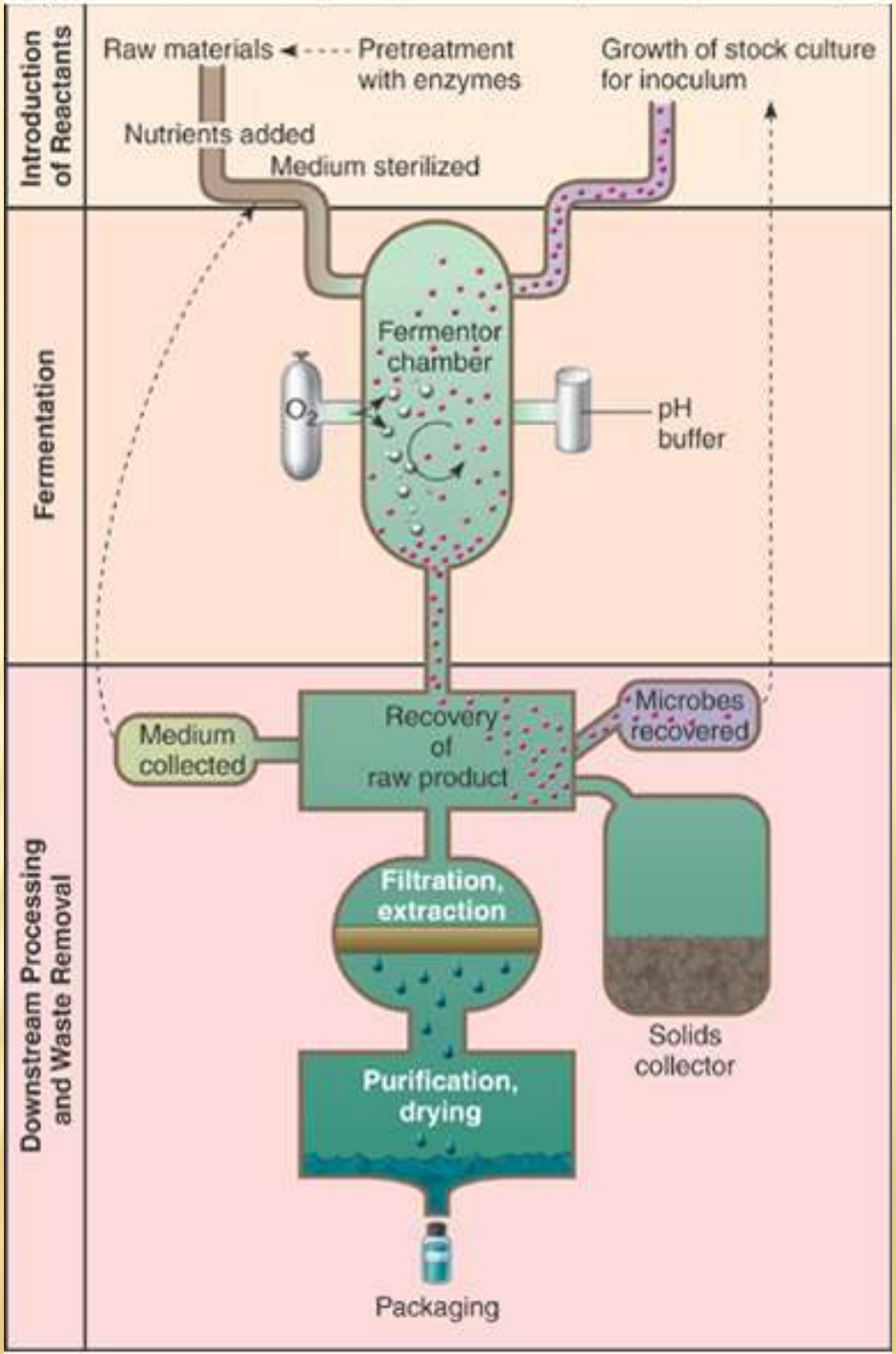
FROM MICROBIAL FACTORIES TO INDUSTRIAL FACTORIES

- Produce appropriate levels of growth and fermentation in a carefully controlled environment
- Commercial fermentation carried out in **fermentors** – large culture devices with mechanisms for controlling environment



SUBSTANCE PRODUCTION

- Steps in mass production:
- Introduction of microbes and sterile media into reaction chamber
- Fermentation
- Downstream processing (recovery, purification, packaging)
- Removal of waste
- Carried out aseptically and monitored for rate of flow and quality of product



- **Batch fermentations** – substrate added to system all at once and taken through a limited run until product is harvested
- **Continuous feed** systems – nutrients are continuously fed into the reactor and product is siphoned off throughout run

- Pharmaceutical products
  - antibiotics
  - vitamins
  - vaccines
- Miscellaneous products
  - biopesticides
  - enzymes
  - amino acids
  - organic acids
  - solvents
  - natural flavor compounds

THANKS

For the Patient hearing