FEERMENTATION TECHNOLOGY

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Fermentation and Pasteurization

- Pasteur showed that microbes are responsible for fermentation.
- Fermentation is the conversation of sugar to alcohol to make beer and wine.
- Microbial growth is also responsible for spoilage of food.
- Bacteria that use alcohol and produce acetic acid spoil wine by turning it to vinegar (acetic acid).

★ **Pasteur demonstrated that these spoilage bacteria could be killed by heat that was not hot enough to evaporate the alcohol in wine. This application of a high heat for a short time is called pasteurization.**

The Golden Age of Microbiology

- 1857-1914
- Beginning with Pasteur's work, discoveries included the relationship between microbes and disease, immunity, and antimicrobial drugs

FERMENTATION

The term fermentation is derived from the latin verb ‘fevere’ which means ‘to boil’

The use of fermentation is an important process in the industry. Though fermentation can have stricter definitions, when speaking of it in Industrial fermentation, it more loosely refers to the breakdown of organic substances into simpler substances. Somewhat paradoxically, fermenter culture in industrial capacity often refers to highly oxygenated and aerobic growth conditions, whereas fermentation in the biochemical context is a strictly anaerobic process.

MECHANISM:

- Natural environment ⟶ isolates ⟶ laboratory Condition

- Inexpensive raw materials —microbes→ Economically valuable products

Fermentation includes almost any process mediated by microorganisms in which a product of economic value is associated. The product may be in the form of either Primary metabolite or as secondary metabolite.

Pharmaceuticals and Biotech industries

There are 5 major groups of commercially important Fermentation

Microbial cells or Biomass as the product: Eg.

1. Bakers Yeast, Lactic acid bacillus, Bacillus sp.
2. Microbial Enzymes: Catalase, Amylase, Protease, Pectinase, Glucose isomerase, Cellulase, Hemicellulase, Lipase, Lactase, Streptokinase etc.
3. Microbial metabolites :

- Primary metabolites – Ethanol, Citric acid, Glutamic acid, Lysine, Vitamins, Polysaccharides etc.
- Secondary metabolites: All antibiotic fermentation

4. Recombinant products : Insulin, HBV, Interferon, GCSF, Streptokinase
5. Biotransformations: Eg. Phenyl acetyl carbinol,Steroid Biotransformation

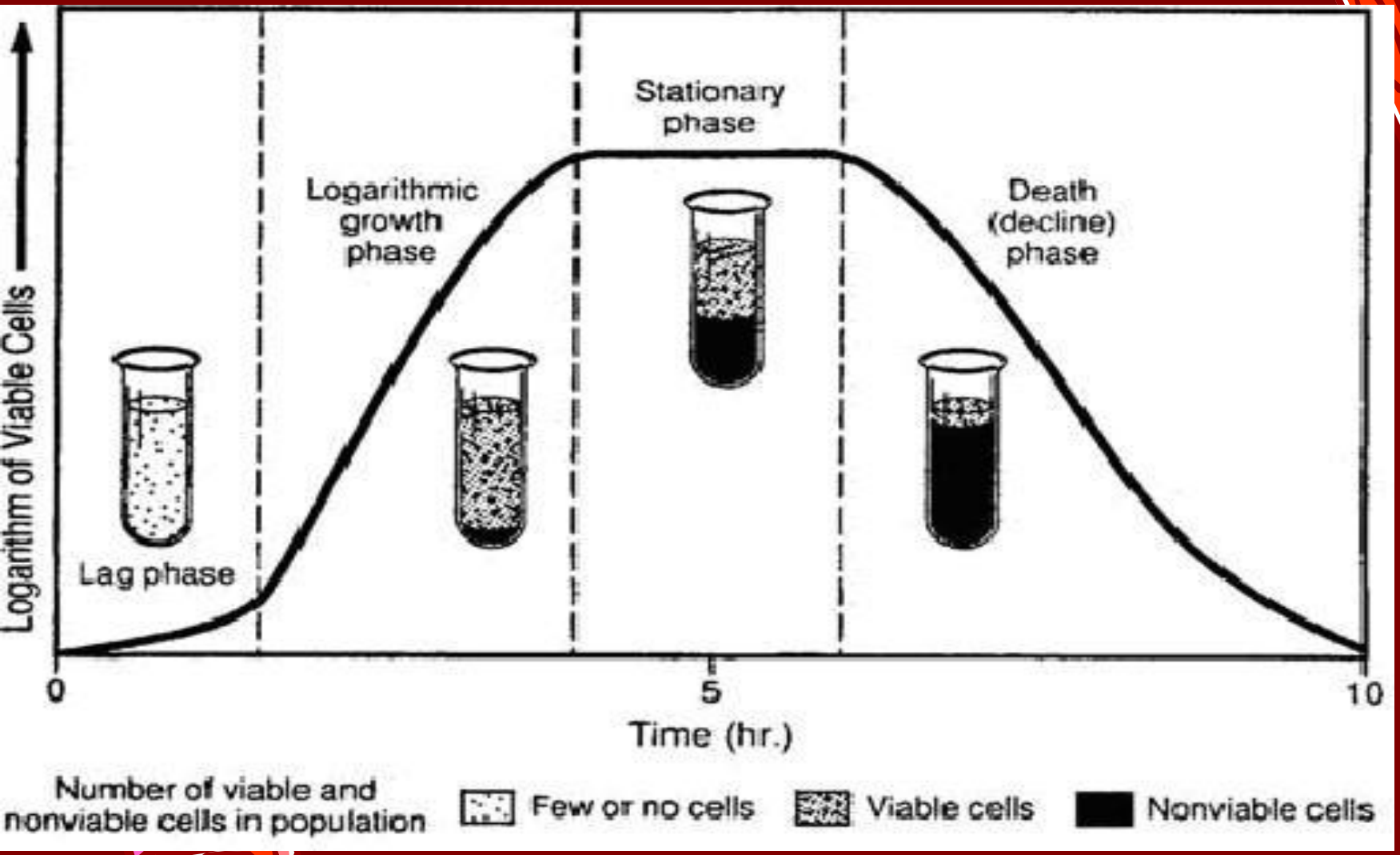
- What are metabolites?
- Microorganisms live in a world of chemical signals. They use small molecular weight compounds, known as metabolites, to regulate their own growth and development, to encourage other organisms beneficial to them and suppress organisms that are harmful. To control competitors, microbes produce antibiotics, such as penicillin, streptomycin and erythromycin, antifungals, such as nystatin, amphotericin and cycloheximide, antiprotozoan metabolites including monensin, salinomycin and trichostatins and herbicides like herbicidin and bialophos.
- Many microbial metabolites are selective, others are broadly active against many species. Microbes use metabolites to regulate the environment in which they live and from this platform they control the function and shape of much of the world's biodiversity.
- Microbial metabolites represent an incredibly diverse array of chemistry. Microbes can make molecules that synthetic chemists cannot access. While over 25,000 microbial metabolites have been reported in the scientific literature, fewer than 2% of these have ever been readily available to the wider research community. Most metabolites have only ever existed in small quantities in the research laboratory in which they were discovered and their biological activity has never been fully investigated.

Primary versus Secondary Metabolites

Primary metabolism requires the cell to use nutrients in its surroundings such as low molecular weight compounds for cellular activity. There are three potential pathways for primary metabolism: the Embden Meyerhof-Parnas Pathway (EMP), the Entner-Dourdorof pathway and the hexose monophosphate (HMP) pathway. The EMP pathway produces two molecules of pyruvate via triose phosphate intermediates. This pathway occurs most widely in animal, plant, fungal, yeast and bacterial cells

Secondary metabolism synthesises new compounds. Secondary metabolites are not vital to the cells survival itself but are more so for that of the entire organism. Relatively few microbial types produce the majority of secondary metabolites. Secondary metabolites are produced when the cell is not operating under optimum conditions e.g. when primary nutrient source is depleted. Secondary metabolites are synthesized for a finite period by
cells that are no longer undergoing balanced growth

The Bacterial Growth Curve



PRODUCTS OF MICROBIAL ACTIVITY OF COMMERCIAL IMPORTANCE:

- Antibiotics
- Organic solvent
- Gases
- Beverages
- Foods
- Flavoring agents
- Organic acids
- Amino acids
- Glycerol
- Steroids
- Bakers' Yeast
- Food and feed yeast
- Bio-fertilizer

CERTAIN SOURCES OF INDUSTRIALLY IMPORTANT CULTURES:

Collection Centre	Type of Microbes
Canada Mold Herbarium & Culture collection University of Alberta Edmonton, Alberta	Fungi
Division of Biol. Sc. National Research council of Canada 100 sussex Drive Ottawa, Ontario	Bacteria Fungi Yeasts
France Institut Pasteur de Lyon; 69365 yon CEDEX2,	Yeasts Bacteria
Japan Dett. of Fermentation Technology Faculty of Engineering Hiroshima University, Hiroshima	Bacteria Fungi Yeasts
Switzerland Mikrobiologisches Institut Eidgs Techn. Hochschule ETH – Zentrum Zurich	Bacteria Fungi Yeasts Actinomycetes

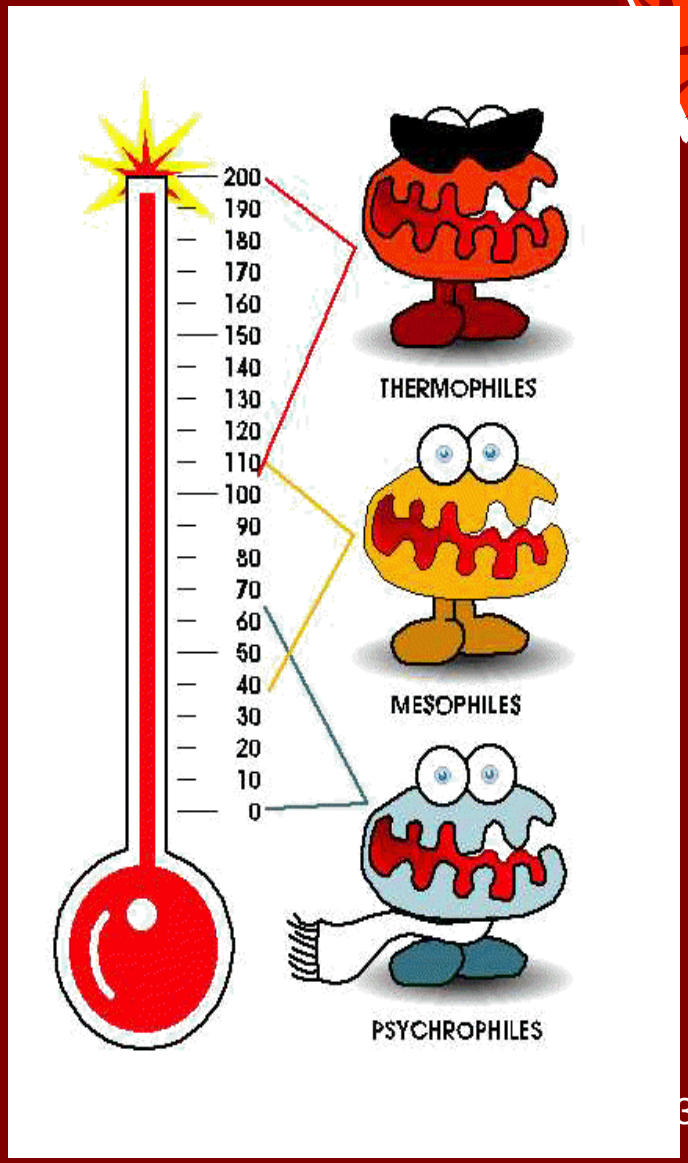
Institute of Microbial Technology
सूक्ष्मजीव प्रौद्योगिकी संस्थान(A Council of Scientific & Industrial Research)
(वैज्ञानिक औद्योगिक अनुसंधान परिषद)

The Microbial Type Culture Collection and Gene Bank (MTCC), a national facility established in 1986 is funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India. The MTCC is a modern facility housed at the Institute of Microbial Technology (IMTECH), Chandigarh. It is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM).The main objectives of this national facility are to act as a depository, to supply authentic microbial cultures

http://www.imtech.res.in/index.php

Industrial microorganisms

- Microbial screening.
  - Wild strains.
- Microbial yield improvement
  - Mutation.
  - Recombinant DNA.
  - Genetically engineered.
- Microbial selection.
- Industrial microorganism



- **Fermentation industry is driven by:**
  - The cost and availability of feed-stocks.
  - The efficiency of industrial microorganism.
  - Fermentation condition and optimization.
  - Down stream process and end-product recovery efficiency.
  - Fermentation by-product utilization.
  - Utility consumption and labor cost.

- The fermentation industry is composed of five major bio-ingredient categories.
- They are:
  - Proteins & amino acids.
  - Organic acids.
  - Antibiotics.
  - Enzymes.
  - Vitamins & hormones.

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
- The type: complex and synthetic medium (mineral medium)
- Even small modifications in the medium could change cell line stability, product quality, yield, operational parameters, and downstream processing.

Fermentation media

- Optimum balance of the media is mandatory for cells propagation and for the maximum production of target metabolite (end-product).
- Media compositions:
  - Carbon source.
  - Nitrogen source.
  - Minerals.
  - Growth factors.
  - Precursors (mutants).

Inoculums

Inoculum 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)

Fermentors

- Small lab / Pilot plant / Large – Scale
- Aerobic / Anaerobic
- Continuous / Batch
- Fabrication material

Types of fermenter

- Simple fermenters (batch and continuous)
- Fed batch fermenter
- Air-lift or bubble fermenter
- Cyclone column fermenter
- Tower fermenter
- Other more advanced systems, etc

The size is few liters (laboratory use) - >500 m^3 (industrial applications)

Introduction

- The function of the fermenter or bioreactor is to provide a suitable environment in which an organism can efficiently produce a target product—the target product might be
- · Cell biomass
- · Metabolite
- · Bioconversion Product
- The sizes of the bioreactor can vary over several orders of magnitudes.
- The microbial cell culture (few mm3), shake flask (100 -1000 ml), laboratory fermenter (1 – 50 L), pilot scale (0.3 – 10 m^3) to plant scale (2 – 500 m^3) are all examples of bioreactors.

Introduction

The performance of any fermenter depends on the following key factors:

- Agitation rate
- Oxygen transfer
- pH
- Temperature
- Foam production
- The design and mode of operation of a fermenter mainly depends on the production organism, the optimal operating condition required for target product formation, product value and scale of production.
- The design also takes into consideration the capital investment and running cost.

Fermenter design

The fermenter should have:

- Heat and oxygen transfer configuration
- Sterilization procedures
- Foam control
- Fast and thorough cleaning system
- Proper monitoring and control system
- Traditional design is open cylindrical or rectangular vessels made from wood or stone.
- Most fermentations are now performed in close system to avoid contamination.
- It should be constructed from non-toxic, corrosion-resistant materials.
- Small fermentation vessels of a few liters capacity are constructed from glass and/or stainless steel.

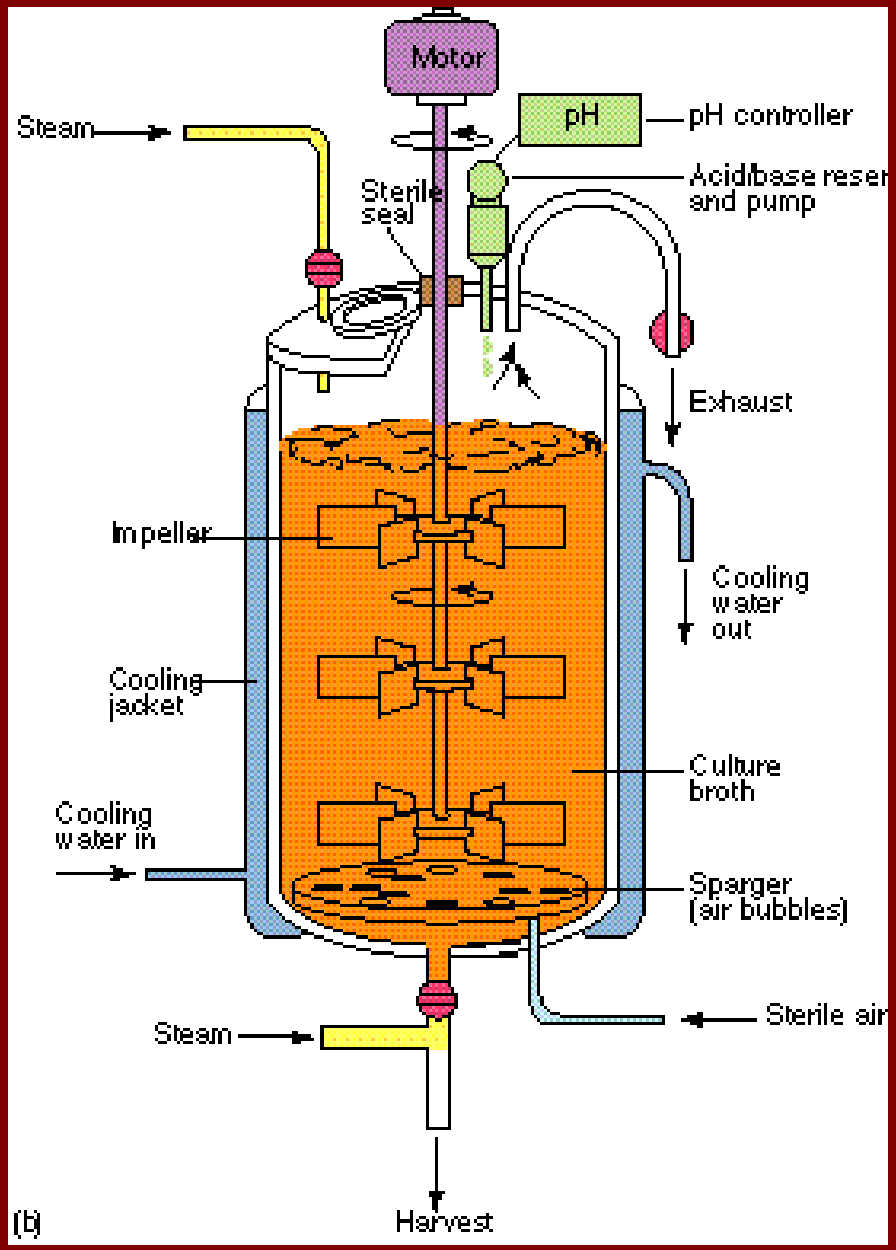
Fermenter design……

- Pilot scale and many production vessels are normally made of stainless steel with polished internal surfaces
- Very large fermenters are often constructed from mild steel lined with glass or plastic, in order to reduce the cost.
- If aseptic operation is required, all associated pipelines transporting air, inoculum and nutrients for the fermentation need to be sterilizable, usually by steam.
- Most vessel cleaning operations are now automated using spray jets, and called cleaning in place CIP and located within the vessel.
- Associated pipe work must also be designed to reduce the risk of microbial contamination.

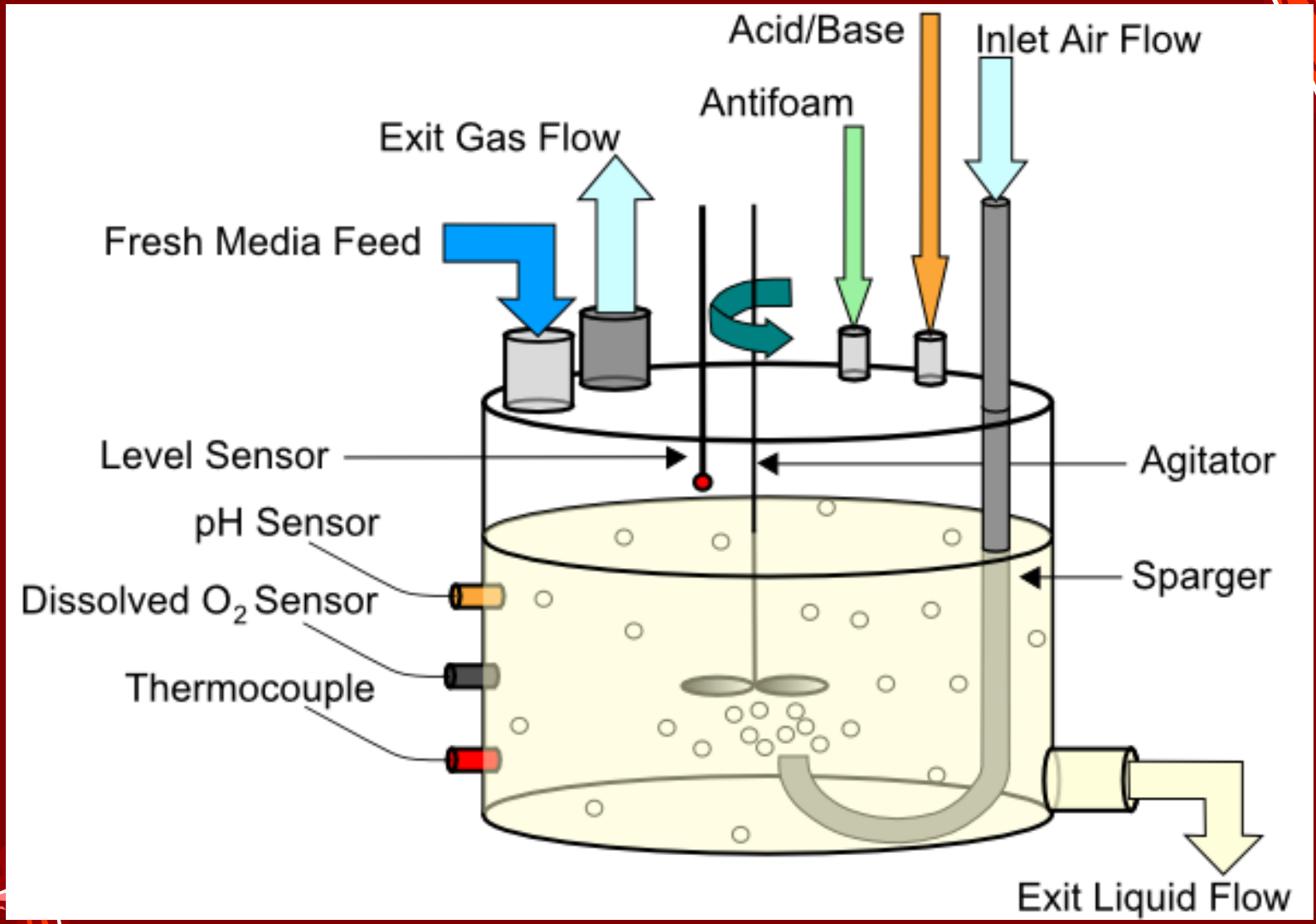
Fermenter design…..

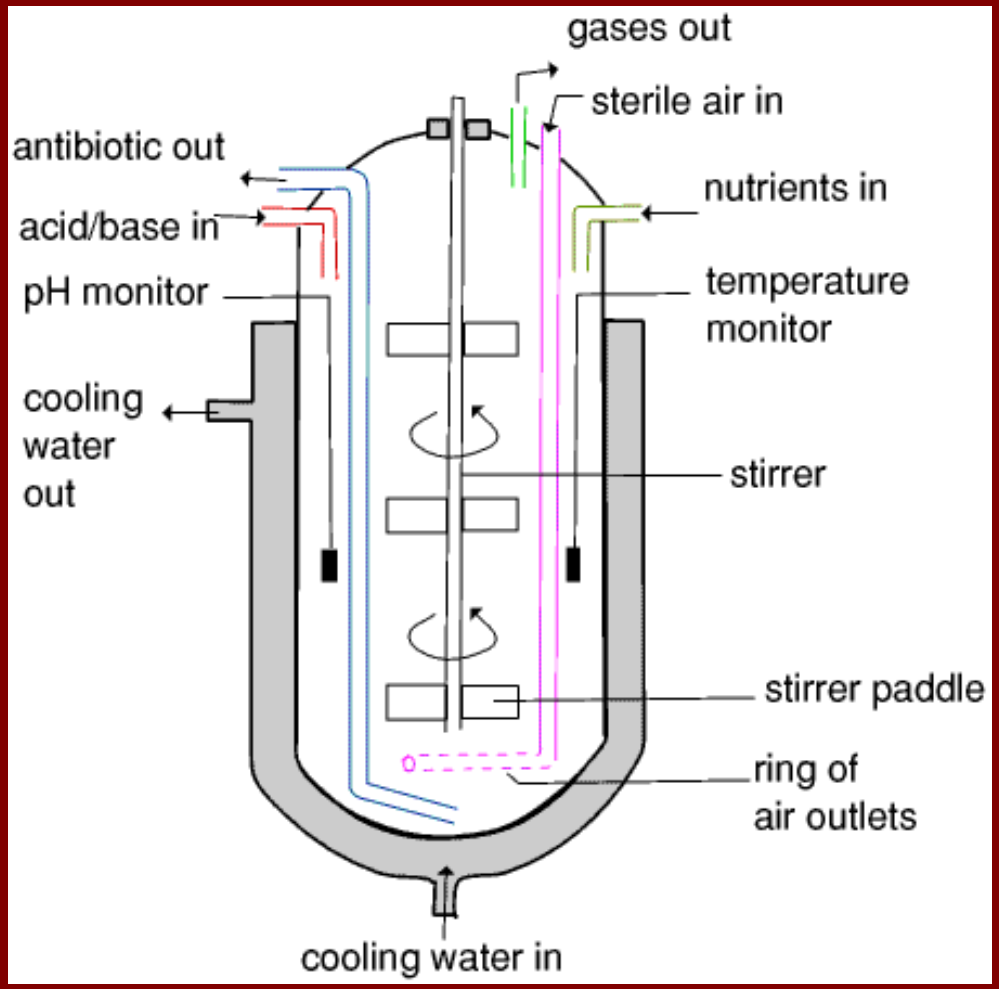
- Normally, fermenters up to 1000 L capacity have an external jacket, and larger vessels have internal coils.
- Pressure gauges and safety pressure valves must be incorporated, (required during sterilization and operation).
- For transfer of media pumps are used. Centrifugal pumps (generate high shear forces and path for easy contaminations), magnetically coupled, jet and peristaltic pumps.
- Alternate methods of liquid transfer are gravity feeding or vessel pressurization.
- In fermentations operating at high temperatures or containing volatile compounds, a sterilizable condenser may be required to prevent evaporation loss.

Design of Batch Fermenter

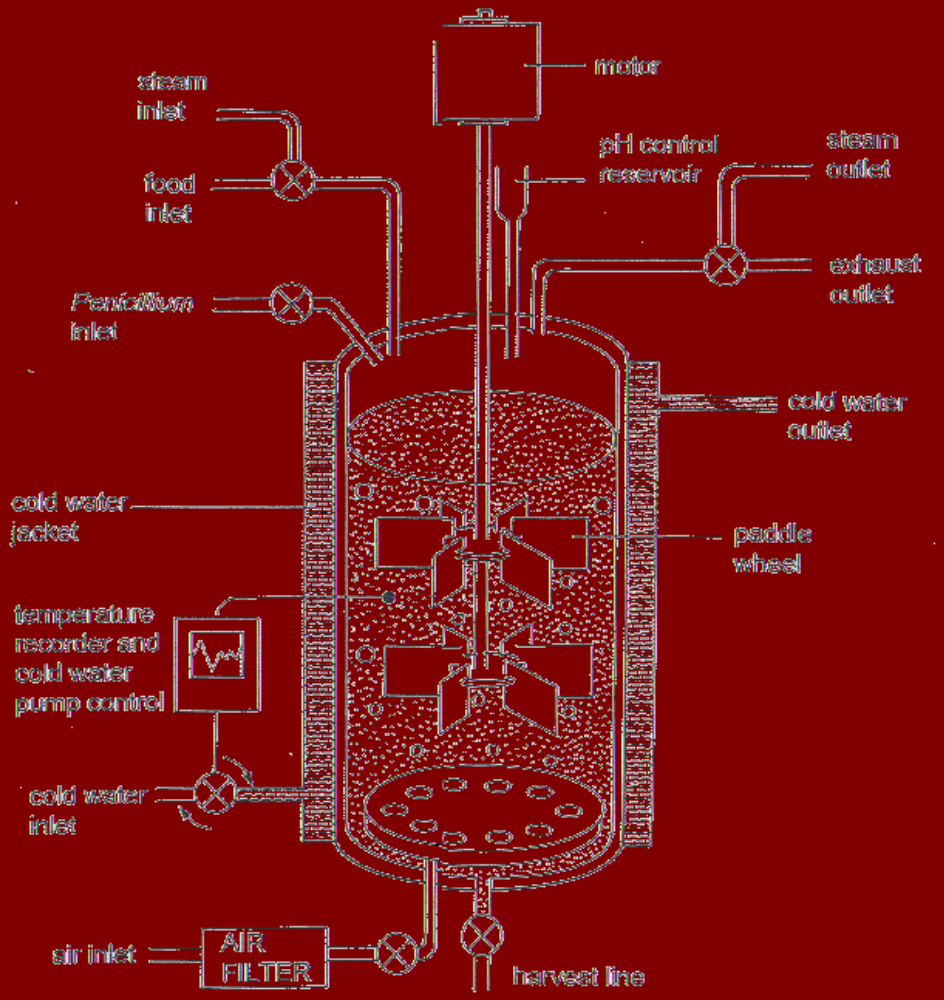


Design of Continuous Fermenter





Cross section of a fermenter for Penicillin production (Copyright: http://web.ukonline.co.uk/webwise/spinneret/microbes/penici.htm)



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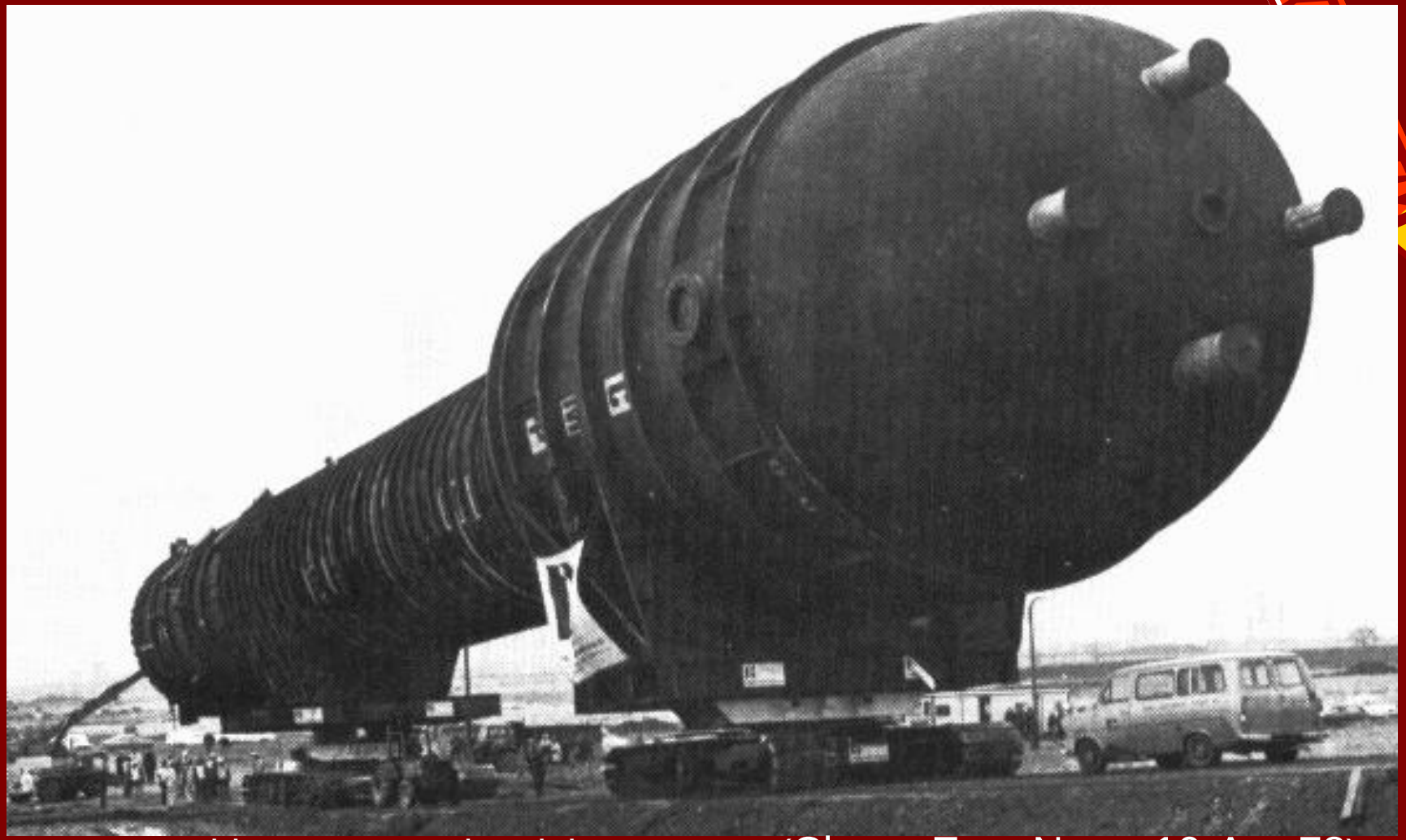
Industrial Bioreactor

Glacial Lakes Energy in Watertown, South Dakota 47+ million gallon per year ethanol production .



World's Largest Industrial Fermenter (Chem. Eng. News,10-Apr-78)
The fermenter is 200' high and 25 ft diam.

Wine making fermenter

Product Handling

- Product harvesting and Recovery
- Purification and Packaging
- Marketing






Some important fermentation products

Product	Organism	Use
Ethanol	*Saccharomyces cerevisiae*	Industrial solvents, beverages
Glycerol	*Saccharomyces cerevisiae*	Production of explosives
Lactic acid	*Lactobacillus bulgaricus*	Food and pharmaceutical
Acetone and butanol	*Clostridium acetobutylicum*	Solvents
α-amylase	*Bacillus subtilis*	Starch hydrolysis

Some important fermentation products

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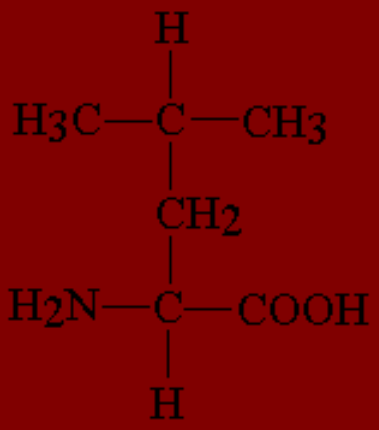
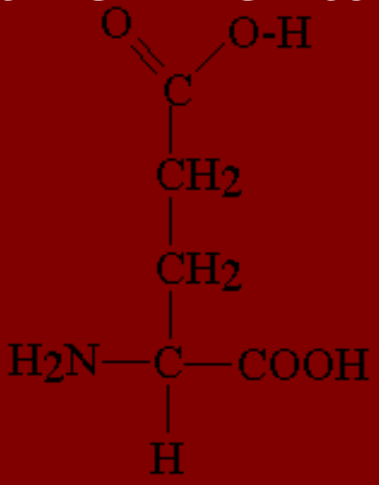
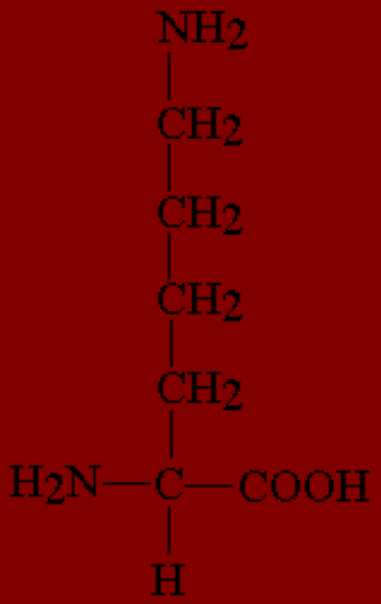


leucine



glutamic acid



lysine

Some important fermentation products

Products of Enzyme Technology

Micro-organisms have been used for thousands of years for making products such as wine, beer, vinegar, soy sauce, bread and cheese

The micro-organisms (such as yeast) are really used as a source of enzymes during the manufacture of these products of biotechnology

Many industrial processes now make use of pure sources of enzymes, i.e. the enzymes have been ISOLATED from the micro-organisms before use


THANKS