SEMESTER 7
C15 APPLIED BIOTECHNOLOGY
Paper Code (BBT 7001 [A])
(Credits: Theory-4, Practicals-2)
THEORY Lectures: 20

Course Objective(s)

- To study microbial growth and its maintenance.
- To develop understanding different bioreactors.
- To provide knowledge biochemistry of different industrial chemicals

Course Outcome(s):

- Learn basics of fermentation technology, bioreactors, production optimization, and downstream processing.
- On successful completion of the course the students should have understood the basics of fermentation technology and learnt the concept of different metabolite production by microbes in industrial setup.

THEORY

Unit-I Microbial Cell Growth and Death Kinetics: (10 Lectures)
Screening and Improvement of industrially important microorganisms, Microbial Growth and Death Kinetics, Media for Industrial Fermentation, Air and Media Sterilization. IKS: Contribution of Indian Scientists.

Unit-II Operation and Control of Bioreactors: (10 Lectures)
Types of Fermentation Processes: Analysis of batch, fed-batch and continuous bioreactors, stability of microbial bioreactors, analysis of mixed populations, specialized bioreactors-pulsed,

fluidized, photo bioreactors, etc. Measurement and Control of bioprocess parameters. Downstream processing, Whole cell immobilization and their industrial applications.

Unit-III Fermentation Technology: (10 Lectures)
Industrial production of chemicals: Ethanol, Acids (citric, acetic and gluconic acid), Solvents (glycerol, acetone, butanol), Antibiotics (penicillin, streptomycin, tetracyclin), Semi-synthetic antibiotics, Amino acids (lysine, glutamic acid), Single cell protein.

Unit-IV Applications of Bioprocess Engineering: (10 Lectures)
Agitation and aeration: requirement in industrial processes, concept of volumetric oxygen transfer coefficient and its determination (Kla), Factors affecting Kla values; Uses of microbes in mineral beneficiation and oil recovery. Introduction to food technology; Elementary idea of canning and packaging, Sterilization and pasteurization of food products.

PRACTICALS

1. To plot Microbial growth curve for shake flask culturing using turbidity method.
2. Prepare a standard curve of reducing sugar by 3,5-Dinitrosalicylic acid method
3. To produce invertase enzyme and find its activity from Baker's Yeast
4. Preparation of standard curve of Ethanol
5. Quantitative estimation of ethanol produced during Yeast fermentation
6. Production of Penicillin and assaying its activity
7. To get familiarized with the lab scale fermenter (bench top fermenter)
8. To determine dissolved oxygen concentration in tap and aerated water.
9. To determine the volumetric transfer coefficient (K la)
10. Estimation of BOD in a given waste water sample.
11. Centrifugation studies during settling of yeast cells.
12. Yeast cell disruption by mechanical methods.

SUGGESTED BOOKS

1. Bioprocess Engineering, Shular M &Kargi F, Prentice Hall
2. Biochemical Engineering Fundamentals, Bailey JE &Ollis DF
3. Bioprocess Engineering Principles, Doran, PM, Academic Press, California

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Unit-II

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52

fluidized, photo bioreactors, etc. Measurement and Control of bioprocess parameters. Downstream processing, Whole cell immobilization and their industrial applications.

Fermentation

- Fermentation is the generation of energy by the catabolism of organic compounds.
- The catabolism of sugar is an oxidative process, which results in the production of reduced pyridine nucleotides, which must be reoxidized for the process to continue.
- Under aerobic conditions, reoxidation of reduced pyridine nucleotide occurs by electron transfer, via the cytochrome system, with oxygen acting as the terminal electron acceptor.
- Under anaerobic condition, reduced pyridine nucleotide oxidation is coupled with the reduction of an organic compound, which is often a subsequent product of the catabolic pathway. In the case of the action of yeast on fruit or grain extracts, NADH is regenerated by the reduction of pyruvic acid to ethanol.

Bacterial Fermentation Products of Pyruvate

Pyruvate formed by the catabolism of glucose is further metabolized by pathways which are characteristic of particular organisms and which serve as a biochemical aid to identification. End products of fermentations are italicized (Dawes & Large, 1982).

A, Lactic acid bacteria (*Streptococcus, Lactobacillus*);

B, *Clostridium propionicum*;

C, Yeast, *Acetobacter, Zymomonas, Sarcina ventriculi, Erwinia amylovora;*

D, Enterobacteriaceae (coli-aerogenes);

E, Clostridia;

F, *Klebsiella*;

G, Yeast;

H, Clostridia (butyric, butylic organisms);

I, Propionic acid bacteria.

Acetate
ACETYL CoA
CO_2 2H
PYRUVATE
2H A *Lactate* B *Acrylate* H_2O
CO_2 Oxaloacetate
2H Malate
H_2O Fumarate
2H *Succinate*
CO_2 *Propionate*
B 2H
CO_2 2H
CO_2 C Acetaldehyde 2H *Ethanol*
F Acetolactate
F CO_2 Acetoin
F, G 2H *2,3-Butanediol*
G
D E
Formate + Acetyl CoA + H_2 + CO_2
H_2 CO_2
H
4H D *Ethanol*
D *Acetate*
Acetoacetyl CoA
CO_2 *Acetone*
2H *Iso-Propanol*
H_2O 4H Butyryl CoA *Butyrate*
4H *Butanol*

THE RANGE OF FERMENTATION PROCESSES

There are five major groups of commercially important fermentations:

1. Those that produce microbial cells (or biomass) as the product.

2. Those that produce microbial enzymes.

3. Those that produce microbial metabolites.

4. Those that produce recombinant products.

5. Those that modify a compound that is added to the fermentation—the transformation process.

1. MICROBIAL BIOMASS

The commercial production of microbial biomass may be divided into two major processes: the production of yeast to be used in the baking industry and the production of microbial cells to be used as human food or animal feed (single-cell protein).

2. MICROBIAL ENZYMES

Enzymes have been produced commercially from plant, animal, and microbial sources. However, microbial enzymes have the enormous advantage of being able to be produced in large quantities by established fermentation techniques. Also, it is infinitely easier to improve the productivity of a microbial system compared with a plant or an animal one. Furthermore, the advent of recombinant DNA technology has enabled enzymes of animal origin to be synthesized by microorganisms.

Table 1.1 Commercial Applications of Enzymes

Industry	Application	Enzyme	Source
Baking and milling	Reduction of dough viscosity, acceleration of fermentation, increase in loaf volume, improvement of crumb softness, and maintenance of freshness	Amylase	Fungal
	Improvement of dough texture, reduction of mixing time, increase in loaf volume	Protease	Fungal/bacterial
Brewing	Mashing	Amylase	Fungal/bacterial
	Chill proofing	Protease	Fungal/bacterial
	Improvement of fine filtration	β-Glucanase	Fungal/bacterial
Cereals	Precooked baby foods, breakfast foods	Amylase	Fungal
Chocolate and cocoa	Manufacture of syrups	Amylase	Fungal/bacterial
Coffee	Coffee bean fermentation	Pectinase	Fungal
	Preparation of coffee concentrates	Pectinase, hemicellulase	Fungal
Confectionery	Manufacture of soft center candies	Invertase, pectinase	Fungal/bacterial
Cotton	Low temperature processing	Pectate lyase	Fungal
Corn syrup	Manufacture of high-maltose syrups	Amylase	Fungal
	Production of low D.E. syrups	Amylase	Bacterial
	Production of glucose from corn syrup	Amyloglycosidase	Fungal
	Manufacture of fructose syrups	Glucose isomerase	Bacterial

Table 1.1 Commercial Applications of Enzymes

Industry	Application	Enzyme	Source
Dairy	Manufacture of protein hydrolysates	Protease	Fungal/bacterial
	Stabilization of evaporated milk	Protease	Fungal
	Production of whole milk concentrates, ice cream, and frozen desserts	Lactase	Yeast
	Curdling milk	Protease	Fungal/bacterial
Eggs, dried	Glucose removal	Glucose oxidase	Fungal
Fruit juices	Clarification	Pectinases	Fungal
	Oxygen removal	Glucose oxidase	Fungal
Laundry	Detergents	Protease, lipase	Bacterial
Leather	Dehairing, baiting	Protease	Fungal/bacterial
Meat	Tenderization	Protease	Fungal
Paper	Removal of wood waxes	Lipase	Fungal
Pharmaceutical	Digestive aids	Amylase, protease	Fungal

3. MICROBIAL METABOLITES

The growth of a microbial culture can be divided into a number of stages, the behavior of a culture may also be described according to the products that it produces during the various stages of the growth curve. During the log phase of growth, the products produced are either anabolites (products of biosynthesis) essential to the growth of the organism and include amino acids, nucleotides, proteins, nucleic acids, lipids, carbohydrates, etc. or are catabolites (products of catabolism) such as ethanol and lactic acid. These products are referred as the primary products of metabolism and the phase in which they are produced (equivalent to the log, or exponential phase) as the **trophophase** (Bu'Lock et al., 1965).

Many products of primary metabolism are of considerable economic importance and are being produced by fermentation.

The productivity of anabolic primary metabolites can be improve by the selection of induced mutants, the use of recombinant DNA technology, and the control of the process environment of the producing organism.

This is exemplified by the production of amino acids where productivity has been increased by several orders of magnitude.

The advances in metabolic engineering arising from genomics, proteomics, and metabolomics have provided new powerful techniques to further understand the physiology of “over production” and to reengineer microorganisms to “over-produce” end products and intermediates of primary metabolism.

During the deceleration and stationary phases, some microbial cultures synthesize compounds which are not produced during the trophophase and which do not appear to have any obvious function in cell metabolism. These compounds are referred to as the secondary compounds of metabolism and the phase in which they are produced (equivalent to the stationary phase) as **the idiophase** (Bu’Lock et al., 1965).

Secondary metabolism may occur in continuous cultures at low growth rates and is a property of slow-growing, as well as non growing cells.

It is **the idiophase** state that prevails in nature rather than the **trophophase.**

Glucose (C_6)
Kojic acid
Saccharides
Glycosides
Pentose (C_5)
Tetrose (C_4)
Aromatic secondary metabolites
Aromatic amino-acids
Shikimate (C_7)
Triose (C_3)
Serine (C_3N)
Glycine (C_2N)
C_1-pool
Polyketides (nC_2)
CO_2
Alanine (C_3N)
Pyruvate (C_3)
Valine (C_5N)
CO_2
CO_2
Malonate (C_3)
Acetate (C_2)
Mevalonate (C_6)
Isopentenyl pyrophosphate (C_5)
CO_2
Fatty acids (nC_2)
Terpenes/Steroids (nC_5)
Aspartic acid (C_4N)
TCA cycle
Secondary metabolites
Oxaloacetate (C_4)
Citrate (C_6)
CO_2
CO_2
Secondary metabolites
α-Oxoglutarate (C_5)
Glutamic acid (C_5N)

The Interrelationships Between Primary and Secondary Metabolism

Some Secondary Products of Microbial Metabolism and Their Commercial Significance

Secondary Metabolite	Commercial Significance
Penicillin, cephalosporin, streptomycin	Antibiotics
Bleomycin, mitomycin	Anticancer agents
Lovastatin	Cholesterol-lowering agent
Cyclosporine A	Immunosuppressant
Avermectins	Antiparasitic agents

4. RECOMBINANT PRODUCTS

The advent of recombinant DNA technology has extended the range of potential fermentation products. Genes from higher organisms may be introduced into microbial cells such that the recipients are capable of synthesizing “foreign” proteins.

These proteins are described as “heterologous” meaning “derived from a different organism.” A wide range of microbial cells has been used as hosts for such systems including *Escherichia coli, Saccharomyces cerevisiae,* and filamentous fungi. Animal cells cultured in fermentation systems are also widely used for the production of heterologous proteins. Although the animal cell processes were based on microbial fermentation technology, a number of novel problems had to be solved—animal cells were considered extremely fragile compared with microbial cells, the achievable cell density is very much less than in a microbial process and the media are very complex.

TRANSFORMATION PROCESSES

Microbial cells may be used to convert a compound into a structurally related, financially more valuable, compound. Because microorganisms can behave as chiral catalysts with high positional specificity and stereo specificity, microbial processes are more specific than purely chemical ones and enable the addition, removal, or modification of functional groups at specific sites on a complex molecule without the use of chemical protection. The reactions, which may be catalyzed include dehydrogenation, oxidation, hydroxylation, dehydration and condensation, decarboxylation, animation, deamination, and isomerization. Microbial processes have the additional advantage over chemical reagents of operating at relatively low temperatures and pressures without the requirement for potentially polluting heavy-metal catalysts. Although the production of vinegar is the oldest established microbial transformation process (conversion of ethanol to acetic acid), the majority of these processes involve the production of high-value compounds including steroids, antibiotics, and prostaglandins.

Table 1.4 The Stages in the Chronological Development of the Fermentation Industry

Stage	Main Products	Vessels	Process Control	Culture Method	Quality Control	Pilot Plant Facilities	Strain Selection
1 Pre-1900	Alcohol	Wooden, up to 1500 barrels capacity Copper used in later breweries	Use of thermometer, hydrometer and heat exchangers	Batch	Virtually nil	Nil	Pure yeast cultures used at the Carlsberg brewery (1886)
	Vinegar	Barrels, shallow trays, trickle filters		Batch	Virtually nil	Nil	Fermentations inoculated with 'good' vinegar
2 1900–1940	Bakers' yeast glycerol, citric acid, lactic acid and acetone/butanol	Steel vessels of up to 200 m^3 for acetone/butanol Air spargers used for bakers' yeast Mechanical stirring used in small vessels	pH electrodes with off-line control Temperature control	Batch and fed-batch systems	Virtually nil	Virtually nil	Pure cultures used
3 1940–date	Penicillin, streptomycin, other antibiotics, gibberellin, amino acids, nucleotides, transformations, enzymes	Mechanically aerated vessels, operated aseptically—true fermenters	Sterilizable pH and oxygen electrodes. Use of control loops which were later computerized	Batch and fed-batch common Continuous culture introduced for brewing and some primary metabolites	Very important	Becomes common	Mutation and selection programmes essential

4 1964–date	Single-cell protein using hydrocarbon and other feedstocks	Pressure cycle and pressure jet vessels developed to overcome gas and heat exchange problems	Use of computer linked control loops	Continuous culture with medium recycle	Very important	Very important	Genetic engineering of producer strains attempted
5 1982–date	Production of heterologous proteins by microbial and animal cells Monoclonal antibodies produced by animal cells	Fermenters developed in stages 3 and 4. Animal cell reactors developed	Control and sensors developed in stages 3 and 4	Batch, fed-batch or continuous Continuous perfusion developed for animal cell processes	Very important	Very important	Introduction of foreign genes into microbial and animal cell hosts. In vitro recombinant DNA techniques used in the improvement of stage 3 products
6 2000–date	Use of “synthetic biology” to improve established fermentations and develop new bulk chemical processes	Fermenters developed in stages 3 and 4	Control and sensors developed in stages 3 and 4	Batch, fed-batch or continuous	Very important	Very important	Synthetic biology used to develop existing and novel fermentations

THE COMPONENT PARTS OF A FERMENTATION PROCESS

Regardless of the type of fermentation (with the possible exception of some transformation processes) an established process may be divided into six basic component parts:

1. The formulation of media to be used in culturing the process organism during the development of the inoculum and in the production fermenter.

2. The sterilization of the medium, fermenters, and ancillary equipment.

3. The production of an active, pure culture in sufficient quantity to inoculate the production vessel.

4. The growth of the organism in the production fermenter under optimum conditions for product formation.

5. The extraction of the product and its purification.

6. The disposal of effluents produced by the process.

A Generalized Schematic Representation of a Typical Fermentation Process

Inoculum development
Stock culture
Shake flask
Seed fermenter
Production fermenter
Culture fluid
Cell separation
Biomass
Cell-free supernatant
Product extraction
Product purification
Effluent treatment
Product packaging
Medium sterilization
Medium formulation
Medium raw materials

BASIC FUNCTIONS OF A FERMENTER

The main function of a fermenter is to provide a controlled environment for the growth of microorganisms or animal cells, to obtain a desired product. In designing and constructing a fermenter, a number of points must be considered:

1. The vessel should be capable of being operated aseptically for a number of days and should be reliable in long-term operation and meet the requirements of containment regulations.

2. Adequate aeration and agitation should be provided to meet the metabolic requirements of the microorganism. However, mixing should not cause damage to the organism nor cause excessive foam generation.

3. Power consumption should be as low as possible.

4. A system of temperature control, both during sterilization and fermentation, should be provided.

5. A system of pH monitoring and control should be provided together with the monitoring and control of other parameters (eg, dissolved oxygen, redox, etc.) as appropriate.

6. Sampling facilities should be provided.

7. Evaporation losses from the fermenter should not be excessive.

8. The vessel should be designed to require the minimal use of labor in operation, harvesting, cleaning, and maintenance.

9. Ideally the vessel should be suitable for a range of processes, but this may be restricted because of containment regulations.

10. The vessel should be constructed to ensure smooth internal surfaces, using welds instead of flange joints whenever possible.

11. The vessel should be of similar geometry to both smaller and larger vessels in the pilot plant or plant to facilitate scale-up .

12. The cheapest materials, which enable satisfactory results to be achieved should be used.

13. There should be adequate service provisions for individual plants

Table 7.1 Service Provisions for a Fermentation Plant

Compressed air
Sterile compressed air (at 1.5–3.0 atm)
Chilled water (12–15°C)
Cold water (4°C)
Hot water
Steam (high pressure)
Steam condensate
Electricity
Stand-by generator
Drainage of effluents
Motors
Storage facilities for media components
Control and monitoring equipment for fermenters
Maintenance facilities
Extraction and recovery equipment
Accessibility for delivery of materials
Appropriate containment facilities

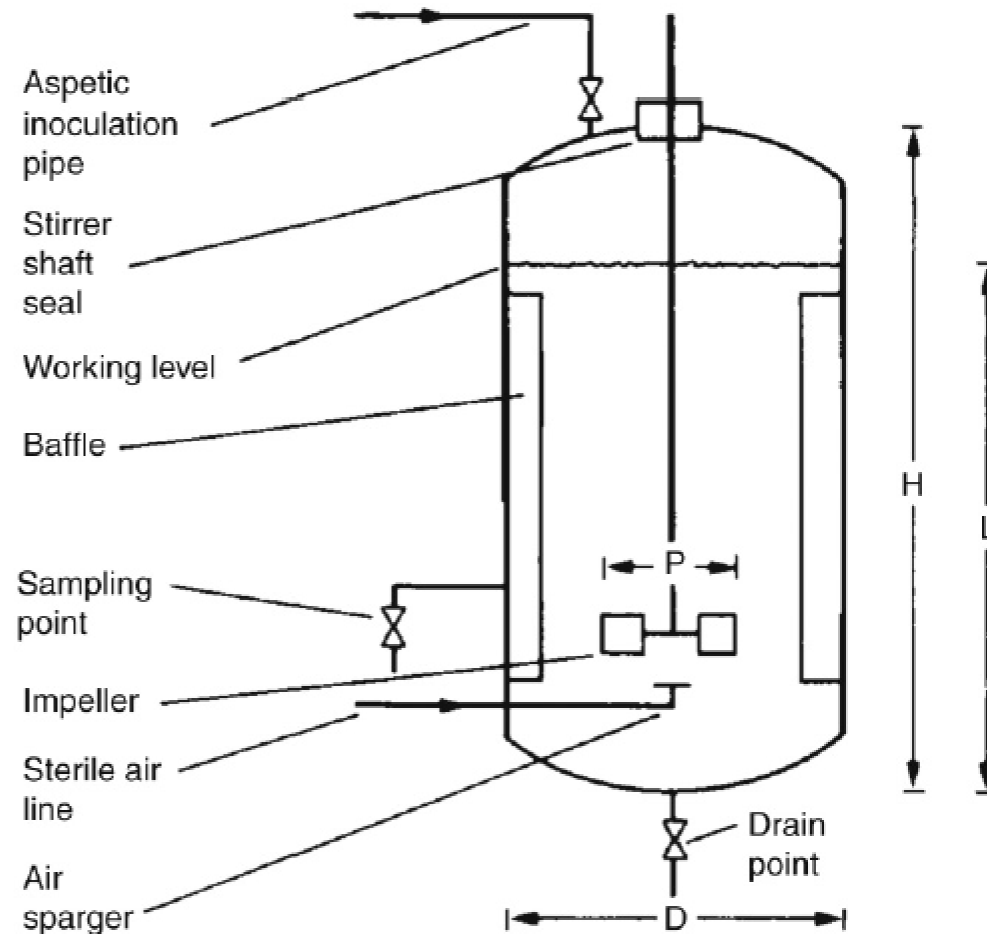

FIGURE 7.1 Diagram of a Fermenter With One Multibladed Impeller

Table 7.2 Details of Geometrical Ratios of Fermenters With Single Multiblade Impellers (Fig. 7.1)

Dimension	Steel and Maxon (1961)	Wegrich and Shurter (1953)	Blakeborough (1967)
Operating volume	250 dm^3	12 dm^3	—
Liquid height (L)	55 cm	27 cm	—
L/D (tank diameter)	0.72	1.1	1.0–1.5
Impeller diameter (P/D)	0.4	0.5	0.33
Baffle width/D	0.10	0.08	0.08–0.10
Impeller height/D	—	—	0.33

Table 7.3 Details of Geometrical Ratios of Fermenters With Three Multibladed Impellers (Fig. 7.2)

Dimension	Jackson (1958)	Aiba, Humphrey, & Millis (1973)	Paca, Ettler, & Gregr (1976)
Operating volume	—	100,000 dm^3 (total)	170 dm^3
Liquid height (L)	—	—	150 cm
L/D (tank diameter)	—	—	1.7
Impeller diameter	0.34–0.5	0.4	0.33
Baffle width/D	0.08–10	0.095	0.098
Impeller height/D	0.5	0.24	0.37
P/V	0.5–1.0	—	0.74
P/W	0.5–1.0	0.85	0.77
P/Y	0.5–1.0	0.85	0.77
P/Z	—	2.1	0.91
H/D	1.0–1.6	2.2	2.95