SEMESTER IV

18BTU404A | INDUSTRIAL FERMENTATION | 3H - 3C

Total hours/week: L:3 T:0 P:0 | Marks: Internal: 40 External: 60 Total: 100

Course Objectives:

- This paper provides the knowledge of basic principle of fermentation process, which help students to design, develop and operate industrial level fermentation process.
- This fundamental knowledge is essential for the students to make their career in industry based on bioprocess
- Students will understand the knowledge of production of fermentation process and the ability to produce a fermentation product.

Course Outcomes:

- Describe the characteristics of conditions required in fermentation processes.
- learn role of microorganism in fermentation
- learn production technologies for different types of fermented food products

UNIT-I

Microbial products: Microbial products of pharmacological interest, steriod fermentations and transformations. Over production of microbial metabolite, Secondary metabolism – its significance and products. Metabolic engineering of secondary metabolism for highest productivity. Enzyme and cell immobilization techniques in industrial processing, enzymes in organic synthesis, proteolytic enzymes, hydrolytic enzymes, glucose isomerase, enzymes in food technology/ organic synthesis.

UNIT-II

Purification and characterization: Purification & characterization of proteins, Upstream and downstream processing, solids and liquid handling. Distribution of microbial cells, centrifugation, filtration of fermentation broth, ultra centrifugation, liquid extraction, ion-exchange recovery of biological products. Experimental model for design of fermentation systems, Anaerobic and Aerobic fermentations.

UNIT-III

Enzyme Kinetics: Rate equations for enzyme kinetics, simple and complex reactions. Inhibition kinetics; effect of pH and temperature on rate of enzyme reactions. Mathematical derivation of growth kinetics, mathematical derivations of batch and continuous culture operations.

UNIT-IV

Production of industrial chemicals, biochemicals and chemotherapeutic products: Propionic acid, butyric acid, 2-3 butanediol, gluconic acid, itaconic acid, Biofuels: Biogas, Ethanol, butanol, hydrogen, biodiesel, microbial electricity, starch conversion processes; Microbial polysaccharides; Microbial insecticides; microbial flavours and fragrances, newer antibiotics, anti cancer agents, amino acids.

UNIT-V

Mass Transfer operations: Single stage CSTR; mass transfer in aerobic fermentation; resistances encountered; overall mass transfer co-efficient (Ka) determination, factors depending on scale up principle and different methods of scaling up. Metabolic engineering of antibiotic biosynthetic pathways.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore - 641 021.

LECTURE PLAN
DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Mr. JEYAPRAGASH
SUBJECT NAME: INDUSTRIAL FERMENTATION SUB.CODE:18BTU404A
SEMESTER: IV CLASS: II B.Sc (BT)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Microbial products of pharmacological interest	T1: 266-268
2	1	Overproduction of metabolites	T1:232-241
3	1	Metabolic engineering of secondary metabolism	T1: 22-224
4	1	Enzyme and Cell Immobilization	T2: 443-448
5	1	Industrial Processing of Enzymes	T2:445
6	1	Proteolytic enzymes, Glucose isomerase	T2:297-299
	Total No of Hours Planned For Unit 1= 6		
		UNIT-II	
1	1	Purificationa and Characterization of proteins	T1:298-380

2	1	Solids and Liquids handling	T2:262-263
3	1	Centrifugation, Anaerobic Fermentation	T2:286-290
4	1	Filtration and fermentation Broth	T1:222-244
5	1	Ultracentrifugation, Liquid Centrifugation	T2: 294-305
6	1	Liquid Centrifugation, Ion exchange Chromatography	T2:276-280
	Total No of Hours Planned For Unit II=6hours		
		UNIT-III	
1	1	Rate equations for enzyme kinetics	T1:19-21
2	1	Simple and Complex Reaction	T2:232-235
3	1	Inhibition Kinetics	T1:225-229
4	1	Effect of pH and temperature on rate of reactions	T1:225-229
5	1	Mathematical derivations of batch and Continuous culture	T1:251-262
6	1	Revison	-
	Total No of Hours Planned For Unit III=6hours		

		UNIT-IV	
1	1	Propionic acid, Butyric acid, 2,3, Butanediol, Gluconic acid	T1:317-319
2	1	Biofuels: Biogas, ethanol, butanol, hydrogen, biodiesel	T1:285-286
3	1	Microbial electricity and Starch Conversion	
4	1	Effect of pH and temperature on rate of enzyme reactions	T1:351
5	1	Microbial flavors and fragraces	T1:357-359
6	1	New Antibiotics, Anti-cancer agents	T1:251-260
	Total No of Hours Planned For Unit IV=6hrs		

		UNIT-V	
1	1	Single stage CSTR	T2:180-183
2	1	Mass transfer in aerobic fermentations	T2:5-11 T2:7-11
3	1	Resistances encountered	T2:63-67
4	1	Overall mass transfer co efficient (KA) determination	T2:168-169
5	1	Factors depending on	
6	1	Scale up principle and different methods scaling up	T2:269-272
	Total No of Hours Planned for unit V=6 hrs		
Total Planned Hours = 30			

TEXT BOOK

1. Casida LE. (1991). Industrial Microbiology. Wiley Eastern Limited.
2. Crueger W and Crueger A. (2000). Biotechnology: A textbook of Industrial Microbiology (2nd ed.). Panima Publishing Co. New Delhi.
3. Stanbury PF, Whitaker A and Hall SJ. (2006). Principles of Fermentation Technology (2nd ed.). Elsevier Science Ltd.

UNIT-I

Microbial products and enzymes production: Microbial products of pharmacological interest, steriod fermentations and transformations. Over production of microbial metabolite, Secondary metabolism – its significance and products. Metabolic engineering of secondary metabolism for highest productivity. Enzyme and cell immobilization techniques in industrial processing, enzymes in organic synthesis, proteolytic enzymes, hydrolytic enzymes, glucose isomerase, enzymes in food technology/ organic synthesis.

SYLLABUS

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Microbial Products Of Pharmacological Interest

The term industrial microbiology refers to the use of microorganisms for industrial purposes. Such things as anticoagulants, antidepressants, vasodilators, herbicides, insecticides, plant hormones, enzymes, and vitamins have been isolated from microorganisms or produced in large quantities by genetically engineering the organisms with foreign genes. In commercial industrial plants, microorganisms are widely used to produce numerous organic materials that have far-reaching value and application.

Enzymes. Among the enzymes industrially produced by bacteria are amylases, which break down starches to smaller carbohydrates for commercial use. Amylases are also used in brewing, baking, and textile production. Bacteria have been used to produce proteases, which break down proteins and are used for tenderizing meats, preparing leathers, and making detergents and cheese.

Polysaccharides. The food, petroleum, cosmetic, and pharmaceutical industries use microorganisms to manufacture polysaccharides. For example, the bacteriumXanthomonas campestris, produces a polysaccharide called xanthan, which is used to stabilize and thicken foods and as a base for cosmetics. It is also a binding agent in many pharmaceuticals and is used in textile printing and dyeing. Another polysaccharide of microbial origin is dextran. The bacterium Leuconostoc mesenteroides produces this polysaccharide when it grows on sucrose. Dextran is used to extend blood plasma.

Nutrients. Amino acids, nucleotides, vitamins, and organic acids are produced by the ton by microorganisms. Various types of research and health laboratories use these products, and health-food stores sell them as nutritional supplements. For example, the lysine prescribed by some doctors to treat herpes simplex infections is a product of the bacterium Corynebacterium glutamicum. Vitamin B12(cyanocobalamine) and vitamin B2 (riboflavin) are produced by a bacterium and a mold, respectively.

Chemotherapeutic agents. Another valuable use of microorganisms in industry is in the production of chemotherapeutic agents. Almost two billion dollars worth of drugs are produced in the United States, mainly by the use of microorganisms. Antibiotics are produced by fungi such as Penicllium and Cephalosporium and by species of the bacterium Streptomyces. Many of these drugs are natural, but several are synthetic or semisynthetic drugs that begin with the naturally occurring molecule, which is then modified.

Steroid fermentation and transformation

Biotransformation of Steroids:

All the steroids possess the basic structure namely cyclopentanoperhydrophenanthrene. Steroids as hormones (glucocorticoids, mineralocorticoids, androgens, estrogens) perform a wide range of functions. They are very useful therapeutically. For instance, cortisone, due to its anti-inflammatory action is used in the treatment of rheumatoid arthritis and skin diseases; derivatives of progesterone and estrogens are employed as contraceptives. Certain derivatives of cortisone (e.g. prednisolone) are more effective in their therapeutic action.

Commercial production of steroids is very important. Cortisone was chemically synthesized, and this process involved as many as 37 reactions. The cost of the so obtained product was around $200/g (in 1950). With the introduction of biotransformation reactions, the number of steps (microbial and chemical put together) was reduced to II, and cost of the product was reduced to just $1/g in 1980! The credit obviously goes to the developments in biotransformation.

Types of reactions in biotransformation of steroids:

The microbial transformation of steroids broadly involves oxidation (introduction of hydroxyl groups, splitting of side chains, production of epoxides etc.) reduction (conversion of aldehydes or ketones to alcohols, hydration of double bonds), hydrolysis and ester formation.

Production process of steroids:

The production of steroids, entirely by bio¬transformation reactions is not practicable. Therefore, microbial transformation along with chemical reactions is carried out. The major steps involved in the biotransformation of steroids are depicted in Fig. 22.1. Stigma sterol extracted from soybeans or diosgenin isolated from the roots of the Mexican barbasco plant can serve as the starting material.

Stigma sterol can be chemically converted to progesterone which is subjected to bio-transformation to form 11 α-hydroxyprogesterone by the microorganism, Rhizopus nigricans. Cortisol (hydrocortisone), produced from 11 α-hydroxy¬progesterone by chemical reactions, undergoes microbial transformation (organism-Corynebacterium simplex) to form prednisolone.

Further, cortisone formed from Cortisol can be subjected to biotransformation by Corynebacterium simplex to produce prednisone. When diosgenin is used as the starting compound, substance S can be produced by chemical reactions which can be converted to Cortisol by biotransformation with the help of the microorganism Curvularia lunata. Biotransformation of steroids is usually carried out by batch fermentation. Use of immobilized cells or immobilized enzymes is gaining importance in recent years.

Since the steroids are not water soluble, the microbial transformation reactions have to be carried out in organic solvent (water-immiscible) system. However, the organic solvents are toxic to micro-organisms or enzymes. It is ideal to use an aqueous two phase system for biotransformation of steroids.

Biotransformation of cholesterol: Certain commercially important steroids (e.g. androstendione, androstadiendione) can be produced directly from cholesterol by biotransformation

2. Biotransformation of Antibiotics:

Production of new antibiotics or modifications in the existing ones for more effective treatment of the diseases is always on the priority of the pharmaceutical industry. Further, antibiotics with wider antimicrobial spectrum, reduced toxicity, low allergic reactions and decreased resistance are highly advantageous. Biotrans¬formation reactions significantly contribute for improving the pharmaceutical products.

ADVERTISEMENTS:

Direct biotransformation:

Acylation and de-acylation, phosphorylation, adenylation and hydrolysis are some of the reactions involved in the microbial transformation of antibiotics.

Biotransformation of penicillin G:

Microbial transformation, in association with chemical synthesis, is routinely used for the commercial production of semisynthetic penicillin's and cephalosporin's. The enzymatic cleavage of penicillin by penicillin acylase into 6-amino- penicillanic acids is a very important reaction (Fig. 22.3). Penicillin G gets inactivated by its conversion to benzylpenicilloic acid by the enzyme penicillinase (β-lactamase).

Biotransformation of narbomycin:

Hydroxylation of narbomycin to picromycin (brought out by Streptomyces sp) is another good example of microbial transformation.

Biotransformation of macrolides:

The macrolide antibiotics on de-acylation will give less active products. These products can be used for the production of more active semisynthetic macrolides.

Indirect biotransformation:

The biosynthetic processes of antibiotics can be controlled by the addition certain inhibitors or modified substrates to the medium. In other words, the biosynthesis of antibiotics occurs in a controlled fashion in the indirect biotransformation.

Biotransformation of actinomycins:

The microorganism Streptomyces parvulus produces new actinomycins in the presence of 4-methyl- proline (proline analog) in the medium. The new antibiotics will have 4-methylproline in place of proline and these actinomycins are more efficient in their function.

Biotransformation of ribostamycin:

In the biosynthesis of neomycin, ribostamycin is an intermediate. By employing mutant strains of Streptomyces fradiae, ribostamycin can be produced in large quantities. Several other mutant strains of microorganisms have been created by recombinant DNA technology for the production of modified antibiotics of aminoglycosides and rifamycins.

3. Biotransformation of Arachidonic Acid to Prostaglandins:

Prostaglandins (PG) have a wide spectrum of biological functions. They are important for pharmaceutical and therapeutic purposes. For instance, PGE1 serves as a contraceptive; PGG1 is used in the treatment of congenital heart failure; PGG2 for relieving labour pains.

The unsaturated fatty acid arachidonic acid is the precursor for the biosynthesis of prostaglandins. Some success has been reported in the biotransformation of arachidonic acid to PGE1, PGE2, PGF1 and PGF2by using fungi. It is expected that in the coming years, prostaglandins with improved efficiency will be produced by bio-transformations.

4. Biotransformation for the Production of Ascorbic Acid:

Ascorbic acid (vitamin C) can be commercially produced by a combination of chemical and microbial transformation processes.

5. Biotransformation of Glycerol to Dihydroxyacetone:

Dihydroxyacetone is used in cosmetics and suntan lotions. Certain acetic acid bacteria can convert glycerol to dihydroxyacetone through the process of biotransformation.Good oxygen supply, temperature 26-28°C and pH 6.0 are ideal for the optimal biotransformation.

Secondary metabolites

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play an important role in plant defense against herbivory and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, and recreational drugs.[1]

Secondary metabolites aid a plant in important functions such as protection, competition, and species interactions, but are not necessary for survival. One important defining quality of secondary metabolites is their specificity. Usually, secondary metabolites are specific to an individual species.[2] Research also shows that secondary metabolic can affect different species in varying ways. In the same forest, four separate species of arboreal marsupial folivores reacted differently to a secondary metabolite in eucalypts.[3] This shows that differing types of secondary metabolites can be the split between two herbivore ecological niches.[3] Additionally, certain species evolve to resist plant secondary metabolites and even use them for their own benefit. For example, monarch butterflies have evolved to be able to eat milkweed (Asclepias) despite the toxic secondary metabolite it contains.[4] This ability additionally allows the butterfly and caterpillar to be toxic to other predators due to the high concentration of secondary metabolites consumed.

Glucose isomerase

Glucose(xylose) isomerase catalyzes the reversible isomerization of glucose to fructose and that of xylose to xylulose. It is an important enzyme used in the industrial production of high-fructose corn syrup (HFCS). Apart from the food industry, this enzyme has recently gained more interest due to its potential applications in the biofuel industry. Currently, ethanol is the major form of biofuel, and numerous technologies have been employed to improve its production. Furthermore, fuel ethanol production from hemicellulosic hydrolysates by Saccharomyces cerevisiae is of great economic interest as an alternative to fossil fuel . Whereas wild-type S. cerevisiae can ferment xylulose to ethanol via the pentose-phosphate pathway , it cannot ferment xylose. Xylose is a major monosaccharide in plant hemicellulosic hydrolysates that can account for up to 30% of total sugars in some plant biomasses such as that of hardwoods and agricultural residues . Therefore, glucose(xylose) isomerase indirectly plays an important role in the ethanol fermentation of plant biomass hydrolysates by S. cerevisiae.

Hydrolase

In biochemistry, a hydrolase or hydrolytic enzyme is an enzyme that catalyzes the hydrolysis of a chemical bond. For example, an enzyme that catalyzed the following reaction is a hydrolase:

$A–B + H2O \rightarrow A–OH + B–H$

Classification

Hydrolases are classified as EC 3 in the EC number classification of enzymes. Hydrolases can be further classified into several subclasses, based upon the bonds they act upon:

- EC 3.1: ester bonds (esterases: nucleases, phosphodiesterases, lipase, phosphatase)
- EC 3.2: sugars (DNA glycosylases, glycoside hydrolase)
- EC 3.3: ether bonds
- EC 3.4: peptide bonds (Proteases/peptidases)
- EC 3.5: carbon-nitrogen bonds, other than peptide bonds

- EC 3.6 acid anhydrides (acid anhydride hydrolases, including helicases and GTPase)
- EC 3.7 carbon-carbon bonds
- EC 3.8 halide bonds
- EC 3.9: phosphorus-nitrogen bonds
- EC 3.10: sulphur-nitrogen bonds
- EC 3.11: carbon-phosphorus bonds
- EC 3.12: sulfur-sulfur bonds
- EC 3.13: carbon-sulfur bonds

Proteolytic enzymes

Proteolysis is the breakdown of proteins into smaller polypeptides or amino acids. Uncatalysed, the hydrolysis of peptide bondsis extremely slow, taking hundreds of years. Proteolysis is typically catalysed by cellular enzymes called proteases, but may also occur by intra-molecular digestion. Low pH or high temperatures can also cause proteolysis non-enzymatically.

Proteolysis in organisms serves many purposes; for example, digestive enzymes break down proteins in food to provide amino acids for the organism, while proteolytic processing of a polypeptide chain after its synthesis may be necessary for the production of an active protein. It is also important in the regulation of some physiological and cellular processes, as well as preventing the accumulation of unwanted or abnormal proteins in cells. Consequently, dis-regulation of proteolysis can cause disease and is used by some venoms.

Proteolysis is important as an analytical tool for studying proteins in the laboratory, as well as industrially, for example in food processing and stain removal.

Biological functions

Post-translational proteolytic processing

Limited proteolysis of a polypeptide during or after translation in protein synthesis often occurs for many proteins. This may involve removal of the N-terminal methionine, signal peptide, and/or the conversion of an inactive or non-functional protein to an active one. The precursor to the final functional form of protein is termed proprotein, and these proproteins may be first synthesized as preproprotein. For example, albumin is first synthesized as preproalbumin and contains an uncleaved signal peptide. This forms the proalbumin after the signal peptide is cleaved, and a further processing to remove the N-terminal 6-residue propeptide yields the mature form of the protein.

Removal of N-terminal methionine

The initiating methionine (and, in prokaryotes, fMet) may be removed during translation of the nascent protein. For E. coli, fMet is efficiently removed if the second residue is small and uncharged, but not if the second residue is bulky and charged.In both prokaryotes and eukaryotes, the exposed N-terminal residue may determine the half-life of the protein according to the N-end rule.

Removal of the signal sequence

Proteins that are to be targeted to a particular organelle or for secretion have an N-terminal signal peptide that directs the protein to its final destination. This signal peptide is removed by proteolysis after their transport through a membrane.

Cleavage of polyproteins

Some proteins and most eukaryotic polypeptide hormones are synthesized as a large precursor polypeptide known as a polyprotein that requires proteolytic cleavage into individual smaller polypeptide chains. The polyprotein pro-opiomelanocortin (POMC) contains many polypeptide hormones. The cleavage pattern of POMC, however, may vary between different tissues, yielding different sets of polypeptide hormones from the same polyprotein.

Many viruses also produce their proteins initially as a single polypeptide chain that were translated from a polycistronic mRNA. This polypeptide is subsequently cleaved into individual polypeptide chains.[1]

Cleavage of precursor proteins

Many proteins and hormones are synthesized in the form of their precursors - zymogens, proenzymes, and prehormones. These proteins are cleaved to form their final active structures. Insulin, for example, is synthesized as preproinsulin, which yields proinsulin after the signal peptide has been cleaved. The proinsulin is then cleaved at two positions to yield two polypeptide chains linked by two disulfide bonds. Removal of two C-terminal residues from the B-chain then yields the mature insulin. Protein folding occurs in the single-chain Proinsulin form which facilitates formation of the ultimately inter-peptide disulfide bonds, and the ultimately intra-peptide disulfide bond, found in the native structure of insulin.

Proteases in particular are synthesized in the inactive form so that they may be safely stored in cells, and ready for release in sufficient quantity when required. This is to ensure that the protease is activated only in the correct location or context, as inappropriate activation of these proteases can be very destructive for an organism. Proteolysis of the zymogen yields an active protein; for example, when trypsinogen is cleaved to form trypsin, a slight rearrangement of the protein structure that completes the active site of the protease occurs, thereby activating the protein.

Proteolysis can, therefore, be a method of regulating biological processes by turning inactive proteins into active ones. A good example is the blood clotting cascade whereby an initial event triggers a cascade of sequential proteolytic activation of many specific proteases, resulting in blood coagulation.

Protein degradation

Protein degradation may take place intracellularly or extracellularly. In digestion of food, digestive enzymes may be released into the environment for extracellular digestion whereby proteolytic cleavage breaks down proteins into smaller peptides and amino acids so that they may be absorbed and used by an organism. In animals the food may be processed extracellularly in specialized digestive organs or guts, but in many bacteria the food may be internalized into the cell via phagocytosis. Microbial degradation of protein in the environment can be regulated by

nutrient availability. For example, limitation for major elements in proteins (carbon, nitrogen, and sulfur) has been shown to induce proteolytic activity in the fungus Neurospora crassa as well as in whole communities of soil organisms.

Proteins in cells are also constantly being broken down into amino acids. This intracellular degradation of protein serves a number of functions: It removes damaged and abnormal protein and prevent their accumulation, and it also serves to regulate cellular processes by removing enzymes and regulatory proteins that are no longer needed. The amino acids may then be reused for protein synthesis.

Enzymes in food technology/ organic synthesis.

Immobilization of enzymes (or cells) refers to the technique of confining/anchoring the enzymes (or cells) in or on an inert support for their stability and functional reuse. By employing this technique, enzymes are made more efficient and cost-effective for their industrial use. Some workers regard immobilization as a goose with a golden egg in enzyme technology. Immobilized enzymes retain their structural conformation necessary for catalysis.

There are several advantages of immobilized enzymes:

a. Stable and more efficient in function.

b. Can be reused again and again.

c. Products are enzyme-free.

d. Ideal for multi-enzyme reaction systems.

e. Control of enzyme function is easy.

f. Suitable for industrial and medical use.

g. Minimize effluent disposal problems.

There are however, certain disadvantages also associated with immobilization.

a. The possibility of loss of biological activity of an enzyme during immobilization or while it is in use.

b. Immobilization is an expensive affair often requiring sophisticated equipment.

Immobilized enzymes are generally preferred over immobilized cells due to specificity to yield the products in pure form. However, there are several advantages of using immobilized multi-enzyme systems such as organelles and whole cells over immobilized enzymes. The immobilized cells possess the natural environment with cofactor availability (and also its regeneration capability) and are particularly suitable for multiple enzymatic reactions.

Methods of Immobilization:

The commonly employed techniques for immobilization of enzymes are—adsorption, entrapment, covalent binding and cross-linking.

Adsorption

Adsorption involves the physical binding of enzymes (or cells) on the surface of an inert support. The support materials may be inorganic (e.g. alumina, silica gel, calcium phosphate gel, glass) or organic (starch, carboxymethyl cellulose, DEAE-cellulose, DEAE-sephadex).

Adsorption of enzyme molecules (on the inert support) involves weak forces such as van der Waals forces and hydrogen bonds (Fig. 21.3). Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature. This is a disadvantage for industrial use of enzymes.

Entrapment:

Enzymes can be immobilized by physical entrapment inside a polymer or a gel matrix. The size of the matrix pores is such that the enzyme is retained while the substrate and product molecules pass through. In this technique, commonly referred to as lattice entrapment, the enzyme (or cell) is not subjected to strong binding forces and structural distortions.

Some deactivation may however, occur during immobilization process due to changes in pH or temperature or addition of solvents. The matrices used for entrapping of enzymes include polyacrylamide gel, collagen, gelatin, starch, cellulose, silicone and rubber. Enzymes can be entrapped by several ways.

1. Enzyme inclusion in gels:

This is an entrapment of enzymes inside the gels (Fig. 21.4A).

2. Enzyme inclusion in fibres:

The enzymes are trapped in a fibre format of the matrix (Fig. 21.4B).

3. Enzyme inclusion in microcapsules:

In this case, the enzymes are trapped inside a microcapsule matrix (Fig. 21.4C). The hydrophobic and hydrophilic forms of the matrix polymerise to form a microcapsule containing enzyme molecules inside. The major limitation for entrapment of enzymes is their leakage from the matrix. Most workers prefer to use the technique of entrapment for immobilization of whole cells. Entrapped cells are in use for industrial production of amino acids (L-isoleucine, L-aspartic acid), L-malic acid and hydroquinone.

Microencapsulation:

Microencapsulation is a type of entrapment. It refers to the process of spherical particle formation wherein a liquid or suspension is enclosed in a semipermeable membrane. The membrane may be polymeric, lipoidal, lipoprotein-based or non-ionic in nature. There are three distinct ways of microencapsulation.

1. Building of special membrane reactors.

2. Formation of emulsions.

3. Stabilization of emulsions to form microcapsules.

Microencapsulation is recently being used for immobilization of enzymes and mammalian cells. For instance, pancreatic cells grown in cultures can be immobilized by microencapsulation. Hybridoma cells have also been immobilized successfully by this technique.

Covalent Binding:

Immobilization of the enzymes can be achieved by creation of covalent bonds between the chemical groups of enzymes and the chemical groups of the support (Fig. 21.5). This technique is widely used. However, covalent binding is often associated with loss of some enzyme activity. The inert support usually requires pretreatment (to form pre-activated support) before it binds to enzyme. The following are the common methods of covalent binding.

1. Cyanogen bromide activation:

The inert support materials (cellulose, sepharose, sephadex) containing glycol groups are activated by CNBr, which then bind to enzymes and immobilize them (Fig. 21.6A).

2. Diazotation:

Some of the support materials (amino benzyl cellulose, amino derivatives of polystyrene, aminosilanized porous glass) are subjected to diazotation on treatment with NaNO2 and HCI. They, in turn, bind covalently to tyrosyl or histidyl groups of enzymes (Fig. 21.6B).

3. **Peptide bond formation**:

Enzyme immobi¬lization can also be achieved by the formation of peptide bonds between the amino (or carboxyl) groups of the support and the carboxyl (or amino) groups of enzymes (Fig. 21.6C). The support material is first chemically treated to form active functional groups.

4. **Activation by bi- or poly-functional reagents**:

Some of the reagents such as glutaraldehyde can be used to create bonds between amino groups of enzymes and amino groups of support (e.g. aminoethylcellulose, albumin, amino alkylated porous glass). This is depicted in Figure.

Alkaloid Biosynthesis:

Metabolic engineering of Alkaloid, belongs to the broad category of secondary metabolism. These nitrogenous com¬pounds are used as active principle to combat various diseases. More than 10,000 alkaloids have been isolated and their structures elucidated.

They have very important functions in plants and also in animals. Historically, the use of alkaloids containing plants extract used as medici¬nal and poisonous. Several new drug discoveries have been made based on alkaloids.

With the introduction of biotechnology into the plant alkaloid field, alkaloid biosynthesis can be manipulated and also potential to alter the pattern of alkaloid accumulation in plants (Fig. 17.11).

Thus, alkaloid field is now an exciting area for metabolic engineering of tailor- made plants that accumulate increased quantities of desirable pharmaceutical or to produce food stuff plants with lower alkaloids content as in case of coffee without caffeine. Following are some of the examples of transgenic work were carried out in alkaloid biosynthesis based on their classification. The most common indole alkaloids are antimalarial quinine from cinchona officinalis, strychnine, and anticancerous vincristine, vinblastin from catharanthus roseus.

Therefore, alkaloid biosynthetic pathway was too long to be engineered in microorganism could be modified in the parent plant using antisense or co-suppression technology. Implication of their novel technology provides accumulation of desired alkaloids by blocking side pathways or catabolic steps (Fig. 17.12).

One of the most drawbacks with these approaches is the requirement of complete knowledge of the pathway and involvement of enzymes. Thus, progress towards identifying enzymes of indole alkaloid biosynthesis has been characterized. The first successful cDNA cloning experiment into alkaloid was achieved with two cDNA encoding enzymes: tryptophan decarboxylase and strictoside synthase.

Tryptophan decarboxylase catalyses the decarboxylation of L-tryptophan to protoalkaloid tryptomine. Tryptomine can then serve as substrate for an¬other enzyme strictosidine synthase, which catalyses the stereospecific condensation of the tryptamine and aldehyde moiety, secaloganin to form the first monoterpenoid indole alkaloid.

The tryptophan decarboxylase cDNA from Catharanthus roses has been heterologously expressed in tobacco plants. Introduction of transgene increases levels of tryptomine and tyrasimine. A fine example of metabolic engineering could be seen by the transformation of Brassica napus with the C. rosens tryptophan decarboxylase cDNA. Brassica seed has limited use as animal feed due to the presence of indole glucosinolates.

Expression of transgene for tryptophan decarboxylase redirects tryptophan pools away from indole glucosinolate produc¬tions and accumulates more of tryptomine. As a result the mature seeds of the transgenic B napus plants contain reduced level of glucosinolates and achieve a potentially and economi¬cally useful product.

In another transgenic approach, strictoside synthase from Rawalfia serpentina has been functionally expressed in microorganisms like E. coli and yeast. The same enzyme from C. rosens has been expressed in tobacco.

Tropane alkaloids are derived via arginine metabolism. In an attempt to produce tropane alkaloid 6β-4 hydroxylase and tropinove reductase, both of which are enzymes of scopalamine biosynthesis in Hyocyamus niger, have been cloned. One of the most medically important alka¬loid is the scopalamine.

Currently, Duboisia is the commercial source of scopalmine. Certain tropane alkaloid producing plants such as Atropa accumulates hyocyamine instead of scopalamine as major alkaloid. Expression of transgene in medicinal plant could alter the alka¬loid pattern such that pharmaceutically useful alkaloids, scopalamine, could be produced.

In order to achieve this, the cDNA encoding hyocyamin 6β-hydroxylase from H. niger was intro- duced into Atropa belladona as a consequence, resulted transgenic plant and hairy roots accu-

mulated enhanced level of scopalamine. These successful transformation experiments clearly show that it has a distinct implication for the future of metabolic engineering of medicinal plants.

Later efforts to increase the tropane alkaloid content of deadly night shade by 35S driven over- expression of putrescine N-methyl transferase was found to be unsatisfactory. In the extended study, transformed Nicotiana sylvestris showed increased levels of nicotine, aris¬ing from increased supply of the tropane moiety.

Phenylpropanoids:Metabolic engineering of phenylpropanoid path way received considerable attention. Phe¬nyl propropanoids are generally a aromatic metabolities containing one or more phenolic hydroxyl functions. Many of these phenolics produced by plant can easily undergoes oxidation.

Many other phenylpropanoid metabolities acts as defensive role, lignin formation in woods. The flavanols found in grape and so in wine, tomatoes comprises of important antioxidantcompo¬nent. It also acts as anticancerous agent.

In the core pathway of phenylpropanoid, a key enzyme phenyl alanine ammonia lyase (PAL), play a central role, which catalyses the initial conversion of phenyl alanine to cinnamate. Further reaction was depicted in the (Fig. 17.13).

Secondary metabolities such as stibenes. Coumarins and flavanoids are resultant prod¬uct of this pathway.

One of the earliest attempts made in this pathway is directed towards the anthocyanins involved in flower and coloration. The enzyme CHS was targeted in this concern by over ex¬pression under Cam35 promoter. However, flavonoids level was found to be decreased probably due to gene silencing.

In the later studies, over expression of chalcone isomerase (CHI), driven by Cam35 in tomato ehanced significant level of antioxidant flavanols in the fruit peel.

In another study, expression of cytocrome P450 isoflavone synthase (IFS) in Arabidopsis, result¬ing in the production of low levels of the isoflavone genistein. Manipulatism of enzymes for lignin production in this pathway have been successfully attempted.

Possible questions

1. Describe steroid fermentation.
2. Write in detail about enzymes biosynthesis
3. Make short note on Microbial products of pharmacological interest
4. Elaborate Over production of microbial metabolite,
5. Write in detail about Secondary metabolism – its significance and products
6. Discuss Enzyme immobilization techniques in industrial processing
7. Describe cholesterol fermentation

UNIT-II

Purification and characterization techniques: Purification & characterization of proteins, Upstream and downstream processing, solids and liquid handling. Distribution of microbial cells, centrifugation, filtration of fermentation broth, ultra centrifugation, liquid extraction, ion-exchange recovery of biological products. Experimental model for design of fermentation systems, Anaerobic fermentations.

SYLLABUS

Purification and characterization techniques: Purification & characterization of proteins, Upstream and downstream processing, solids and liquid handling. Distribution of microbial cells, centrifugation, filtration of fermentation broth, ultra centrifugation, liquid extraction, ion-exchange recovery of biological products. Experimental model for design of fermentation systems, Anaerobic fermentations.

Purification and characterization techniques: purification and characterization of protein, upstream and downstream processing, solid and liquid handling. Distribution of microbial cells, centrifugation, filtration of fermentation broth, ultra centrifugation, liquid extraction, ion exchange recovery of biological products. Experimental model for design of fermentation system, anaerobic fermentation.

Purification of proteins

Protein purification is a series of processes intended to isolate one or a few proteins from a complex mixture, usually cells, tissues or whole organisms. Protein purification is vital for the characterization of the function, structure and interactions of the protein of interest. The purification process may separate the protein and non-protein parts of the mixture, and finally separate the desired protein from all other proteins. Separation of one protein from all others is typically the most laborious aspect of protein purification. Separation steps usually exploit differences in protein size, physico-chemical properties, binding affinity and biological activity. The pure result may be termed **protein isolate**.

Protein purification is either *preparative* or *analytical*. **Preparative purifications** aim to produce a relatively large quantity of purified proteins for subsequent use. Examples include the preparation of commercial products such as enzymes (e.g. lactase), nutritional proteins (e.g. soy protein isolate), and certain biopharmaceuticals (e.g. insulin). **Analytical purification** produces

a relatively small amount of a protein for a variety of research or analytical purposes, including identification, quantification, and studies of the protein's structure, post-translational modifications and function. Pepsin and urease were the first proteins purified to the point that they could be crystallized.

Size exclusion chromatography

Chromatography can be used to separate protein in solution or denaturing conditions by using porous gels. This technique is known as size exclusion chromatography. The principle is that smaller molecules have to traverse a larger volume in a porous matrix. Consequentially, proteins of a certain range in size will require a variable volume of eluent (solvent) before being collected at the other end of the column of gel.

In the context of protein purification, the eluent is usually pooled in different test tubes. All test tubes containing no measurable trace of the protein to purify are discarded. The remaining solution is thus made of the protein to purify and any other similarly-sized proteins.

Separation based on charge or hydrophobicity]

Hydrophobic interaction chromatography

HIC media is amphiphilic, with both hydrophobic and hydrophilic regions, allowing for separation of proteins based on their surface hydrophobicity. In pure water, the interactions between the resin and the hydrophobic regions of protein would be very weak, but this interaction is enhanced by applying a protein sample to HIC resin in high ionic strength buffer. The ionic strength of the buffer is then reduced to elute proteins in order of decreasing hydrophobicity.

Ion exchange chromatographyIon exchange chromatography separates compounds according to the nature and degree of their ionic charge. The column to be used is selected according to its type and strength of charge. Anion exchange resins have a positive charge and are used to retain and separate negatively charged compounds (anions), while cation exchange resins have a negative charge and are used to separate positively charged molecules (cations).

Before the separation begins a buffer is pumped through the column to equilibrate the opposing charged ions. Upon injection of the sample, solute molecules will exchange with the buffer ions as each competes for the binding sites on the resin. The length of retention for each solute depends upon the strength of its charge. The most weakly charged compounds will elute first, followed by those with successively stronger charges. Because of the nature of the separating mechanism, pH, buffer type, buffer concentration, and temperature all play important roles in controlling the separation.

Ion exchange chromatography is a very powerful tool for use in protein purification and is frequently used in both analytical and preparative separations.

Free-flow-electrophoresis

Free-flow electrophoresis (FFE) is a carrier-free electrophoresis technique that allows preparative protein separation in a laminar buffer stream by using an orthogonal electric field. By making use of a pH-gradient, that can for example be induced by ampholytes, this technique allows to separate protein isoforms up to a resolution of < 0.02 delta-pI.

Affinity chromatography

Affinity Chromatography is a separation technique based upon molecular conformation, which frequently utilizes application specific resins. These resins have ligands attached to their surfaces which are specific for the compounds to be separated. Most frequently, these ligands function in a fashion similar to that of antibody-antigen interactions. This "lock and key" fit between the ligand and its target compound makes it highly specific, frequently generating a single peak, while all else in the sample is unretained.

Many membrane proteins are glycoproteins and can be purified by lectin affinity chromatography. Detergent-solubilized proteins can be allowed to bind to a chromatography resin that has been modified to have a covalently attached lectin. Proteins that do not bind to the lectin are washed away and then specifically bound glycoproteins can be eluted by adding a high concentration of a sugar that competes with the bound glycoproteins at the lectin binding site. Some lectins have high affinity binding to oligosaccharides of glycoproteins that is hard to compete with sugars, and bound glycoproteins need to be released by denaturing the lectin.

Metal binding

A common technique involves engineering a sequence of 6 to 8 histidines into the N- or C-terminal of the protein. The polyhistidine binds strongly to divalent metal ions such as nickel and cobalt. The protein can be passed through a column containing immobilized nickel ions, which binds the polyhistidine tag. All untagged proteins pass through the column. The protein can be eluted with imidazole, which competes with the polyhistidine tag for binding to the column, or by a decrease in pH (typically to 4.5), which decreases the affinity of the tag for the resin. While this procedure is generally used for the purification of recombinant proteins with an engineered affinity tag (such as a 6xHis tag or Clontech's HAT tag), it can also be used for natural proteins with an inherent affinity for divalent cations.

Immunoaffinity chromatography

Immunoaffinity chromatography uses the specific binding of an antibody-antigen to selectively purify the target protein. The procedure involves immobilizing a protein to a solid substrate (e.g. a porous bead or a membrane), which then selectively binds the target, while everything else flows through. The target protein can be eluted by changing the pH or the salinity. The immobilized ligand can be an antibody (such as Immunoglobulin G) or it can be a protein (such as Protein A). Because this method does not involve engineering in a tag, it can be used for proteins from natural sources.

Purification of a tagged protein

Another way to tag proteins is to engineer an antigen peptide tag onto the protein, and then purify the protein on a column or by incubating with a loose resin that is coated with an immobilized antibody. This particular procedure is known as immunoprecipitation. Immunoprecipitation is quite capable of generating an extremely specific interaction which usually results in binding only the desired protein. The purified tagged proteins can then easily be separated from the other proteins in solution and later eluted back into clean solution.

When the tags are not needed anymore, they can be cleaved off by a protease. This often involves engineering a protease cleavage site between the tag and the protein.

HPLC

High performance liquid chromatography or high pressure liquid chromatography is a form of chromatography applying high pressure to drive the solutes through the column faster. This means that the diffusion is limited and the resolution is improved. The most common form is "reversed phase" HPLC, where the column material is hydrophobic. The proteins are eluted by a gradient of increasing amounts of an organic solvent, such as acetonitrile. The proteins elute according to their hydrophobicity. After purification by HPLC the protein is in a solution that only contains volatile compounds, and can easily be lyophilized. HPLC purification frequently results in denaturation of the purified proteins and is thus not applicable to proteins that do not spontaneously refold.

Characterization of proteins

Protein characterisation is an essential part of recombinant protein production, enabling quality control check on a purified or partially purified protein. Modern, sensitive techniques enable physical properties such as mass, size, concentration, amino acid sequence, stability and structure of a protein to be determined. Other methodologies, often protein-specific, can be used to elucidate biological functioning.

Some of the more commonly encountered characterisation techniques are described below.

Protein Quantification

The simplest method for protein quantification is measuring absorbance at 280 nm. This method does not require a dye-based kit or the preparation of a standard curve. However, the absorbance is sequence-dependent, so measurements of proteins with low tryptophan or tyrosine content may be inaccurate.

Two non-specific colorimetric assays are commonly used to obtain accurate protein concentration measurements. Both methods have different strengths and weaknesses, and the

most appropriate assay will depend on the individual protein, buffer conditions and the intended application.

Bicinchoninic acid (BCA) – Proteins reduce copper ions in solution, resulting in the formation of a complex that absorbs strongly at 562 nm. Assay is linear over a broad range and is incompatible with reducing agents and chelating agents

Bradford assay – Proteins bind to Coomassie Brilliant Blue dye, resulting in a colour change in solution that can be measured at 595 nm. Assay has a relatively narrow linear range and is incompatible with detergents

Mass Spectrometry (MS)

MS is a powerful technique that can be used to determine various protein physical characteristics, including native molecular weight, sequencing information and post-translational modifications. Proteins are ionised, accelerated and separated according to their mass-to-charge ratio (m/z) under the influence of a magnetic field. As this technique is very sensitive it is possible to detect very small changes in mass e.g. a change in amino acid or chemical modification.

Analytical Size Exclusion Chromatography (SEC)

Analytical SEC can be used to elucidate protein quaternary structure. The solid phase consists of porous particles that retard the flow of smaller molecules, thereby enabling separation of proteins based on their size. The native protein molecular weight can be inferred by comparison to a reference protein, such as bovine serum albumin (BSA).

Circular dichroism (CD)

CD generates a unique spectrum based on protein secondary structure (alpha helices and beta sheets) that is used to verify correct protein folding. It is particularly useful for studying protein structure and thermodynamic stability under various environmental conditions.

Dynamic Light Scattering (DLS)

DLS is an analytical technique used to determine protein size distribution profile, and is amenable to high throughput applications. The Brownian motion of proteins in solution causes light to be scattered, with the resultant scattered intensity fluctuations dependent on particle size. Thus, average radius and the width of the distribution in terms of polydispersity can be determined. DLS can be used to quantify protein stability as a function of temperature, pH, concentration, and excipient profile.

Asymmetrical Flow Field-Flow Fractionation with Multi-angle Light Scattering (AF4-MALS)

The AF4-MALS technique offers high-resolution biophysical characterisation of large protein complexes and virus like-particles (VLPs). Complexes are separated based on their size, followed by MALS detection of each component's dynamic properties. AF4-MALS is commonly applied to detect batch-to-batch variation, degradation or aggregation during VLP formulation and storage.

Transmission electron microscopy (TEM)

TEM is used for morphological characterisation of protein complexes or VLPs. A beam of electrons is transmitted through a fixed protein sample, enabling large-scale protein structures to be imaged. This technique is often used in conjunction with AF4-MALS for VLP characterisation.

Bioprocessing

A **bioprocess** is a specific process that uses complete living cells or their components (e.g., bacteria, enzymes, chloroplasts) to obtain desired products.

Transport of energy and mass is fundamental to many biological and environmental processes. Areas, from food processing to thermal design of building to biomedical devices to pollution control and global warming, require knowledge of how energy and mass can be transported through materials (mass, momentum, heat transfer).

Cell bioprocessing

Cell therapy bioprocessing is a discipline that bridges the fields of cell therapy and bioprocessing (i.e., biopharmaceutical manufacturing), and is a sub-field of bioprocess engineering. The goals of cell therapy bioprocessing are to establish reproducible and robust manufacturing processes for the production of therapeutic cells.[1][2] Commercially relevant bioprocesses will:

1. Produce products that maintain all of the quality standards of biopharmaceutical drugs[3]
2. Supply both clinical and commercial quantities of therapeutic cells throughout the various stages of development. The processes and production technologies must be scalable,[2] and
3. Control the cost of goods (CoGs) of the final drug product. This aspect is critical to building the foundation for a commercially viable industry.

Upstream bioprocessing

Therapeutic cell manufacturing processes can be separated into upstream processes and downstream processes. The upstream process is defined as the entire process from early cell isolation and cultivation, to cell banking and culture expansion of the cells until final harvest (termination of the culture and collection of the live cell batch).

Aside from technology challenges, concerning the scalability of culture apparatus, a number of raw material supply risks have emerged in recent years[*when?*], including the availability of GMP grade fetal bovine serum.

The upstream part of a bioprocess refers to the first step in which microbes/cells are grown, e.g. bacterial or mammalian cell lines (see cell culture), in bioreactors. Upstream processing involves all the steps related with inoculum development, media development, improvement of inoculum by genetic engineering process, optimization of growth kinetics so that product development can improve tremendously. Fermentation has two parts: upstream and downstream. After product development, the next step is purification of product for desired quality. When they reach the

desired density (for batch and fed batch cultures) they are harvested and moved to the downstream section of the bioprocess.

Downstream bioprocessing[edit]

The downstream part of a bioprocess refers to the part where the cell mass from the upstream are processed to meet purity and quality requirements. Downstream processing is usually divided into three main sections: cell disruption, a purification section and a polishing section. The volatile products can be separated by distillation of the harvested culture without pre-treatment. Distillation is done at reduced pressure at continuous stills. At reduced pressure distillation of product directly from fermentor may be possible. The steps of downstream processing are:

1. Separation of biomass: separating the biomass (microbial cells) generally carried out by centrifugation or ultra-centrifugation. If the product is biomass, then it is recovered for processing and spent medium is discarded. If the product is extra cellular the biomass will be discarded. Ultra filtration is an alternative to the centrifugation.
2. Cell disruption: If the desired product is intra cellular the cell biomass can be disrupted so that the product should be released. The solid-liquid is separated by centrifugation or filtration and cell debris is discarded.
3. Concentration of broth: The spent medium is concentrated if the product is extracellular.
4. Initial purification of metabolites: According to the physico-chemical nature of the product molecule several methods for recovery of product from the clarified fermented broth were used (precipitation, etc.)
5. De-watering: If low amount of product is found in very large volume of spent medium, the volume is reduced by removing water to concentrate the product. It is done by vacuum drying or reverse osmosis.
6. Polishing of metabolites: this is the final step of making the product 98 to 100% pure. The purified product is mixed with several inert ingredients called excipients. The formulated product is packed and sent to the market for the consumers

Centrifugation

Centrifugation is a process which involves the application of the centrifugal force for the sedimentation of heterogeneous mixtures with a centrifuge, and is used in industrial and laboratory settings. This process is used to separate two miscible substances, but also to analyze the hydrodynamic properties of macromolecules.[1] More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate (pellet) to gather on the bottom of the tube. The remaining solution (supernatant) may be discarded with a pipette.[*citation needed*]

There is a correlation between the size and density of a particle and the rate that the particle separates from a heterogeneous mixture, when the only force applied is that of gravity. The larger the size and the larger the density of the particles, the faster they separate from the mixture. By applying a larger effective gravitational force to the mixture, like a centrifuge does, the separation of the particles is accelerated. This is ideal in industrial and lab settings because particles that would naturally separate over a long period of time can be separated in much less time.[2]

The rate of centrifugation is specified by the angular velocity usually expressed as revolutions per minute (RPM), or acceleration expressed as *g*. The conversion factor between RPM and *g* depends on the radius of the centrifuge rotor. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity. The most common application is the separation of solid from highly concentrated suspensions, which is used in the treatment of sewage sludges for dewatering where less consistent sediment is produced.

In the chemical and food industries, special centrifuges can process a continuous stream of particle-laden liquid.

Centrifugation is the most common method used for uranium enrichment, relying on the slight mass difference between atoms of U238 and U235 in uranium hexafluoridegas

Types of centrifugation machine

Microcentrifuges

Microcentrifuges are used to process small volumes of biological molecules, cells, or nuclei. Microcentrifuge tubes generally hold 0.5 - 2.0 mL of liquid, and are spun at maximum angular speeds of 12,000–13,000 rpm. Microcentrifuges are small enough to fit on a table-top and have rotors that can quickly change speeds. They may or may not have a refrigeration function.

High-speed centrifuges

High-speed or superspeed centrifuges can handle larger sample volumes, from a few tens of millilitres to several litres. Additionally, larger centrifuges can also reach higher angular velocities (around 30,000 rpm). The rotors may come with different adapters to hold various sizes of test tubes, bottles, or microtiter plates.

Fractionation process

General method of fractionation: Cell sample is stored in a suspension which is:

1. Buffered - neutral pH, preventing damage to the structure of proteins including enzymes (which could affect ionic bonds)
2. Isotonic (of equal water potential) - this prevents water gain or loss by the organelles
3. Cool - reducing the overall activity of enzyme released later in the procedure

- Cells are homogenised in a blender and filtered to remove debris
- The homogenised sample is placed in an ultracentrifuge and spun in low speed - nuclei settle out, forming a pellet
- The supernatant (suspension containing remaining organelles) is spun at a higher speed - chloroplasts settle out
- The supernatant is spun at a higher speed still - mitochondria and lysosomes settle out
- The supernatant is spun at an even higher speed - ribosomes, membranes settle out

The ribosomes, membranes and Golgi complexes can be separated by another technique called density gradient centrifugation.

Ultracentrifugations

Ultracentrifugation makes use of high centrifugal force for studying properties of biological particles. Compared to microcentrifuges or high-speed centrifuges, ultracentrifuges can isolate much smaller particles, including ribosomes, proteins, and viruses. Ultracentrifuges can also be used in the study of membrane fractionation. This occurs because ultracentrifuges can reach maximum angular velocities in excess of 70,000 rpm. Additionally, while microcentrifuges and supercentrifuges separate particles in batches (limited volumes of samples must be handled manually in test tubes or bottles), ultracentrifuges can separate molecules in batch or continuous flow systems.

In addition to purification, analytical ultracentrifugation (AUC) can be used for determination of the properties of macromolecules such as shape, mass, composition, and conformation. Samples are centrifuged with a high-density solution such as

sucrose, caesium chloride, or iodixanol. The high-density solution may be at a uniform concentration throughout the test tube ("cushion") or a varying concentration ("gradient"). Molecular properties can be modeled through sedimentation velocity analysis or sedimentation equilibrium analysis. During the run, the particle or molecules will migrate through the test tube at different speeds depending on their physical properties and the properties of the solution, and eventually form a pellet at the bottom of the tube, or bands at various heights.

Density Gradient Centrifugation

Density gradient centrifugation Is considered one of the more efficient methods of separating suspended particles. Density gradient centrifugation can be used both as a separation technique and as a method of measuring the densities of particles or molecules in a mixture.[6] A tube, after being centrifuged by this method, has particles in order of density based on height. The object or particle of interest will reside in the position within the tube corresponding to its density.

Linderstorm-Lang, in 1937, discovered that density gradient tubes could be used for density measurements. He discovered this when working with potato yellow-dwarf virus.[6]

This method was also used in Meselson and Stahl's famous experiment in which they proved that DNA replication is semi-conservative by using different isotopes of nitrogen. They used

density gradient centrifugation to determine which isotope or isotopes of nitrogen were present in the DNA after cycles of replication.

Nevertheless, some non-ideal sedimentations are still possible when using this method. The first potential issue is the unwanted aggregation of particles, but this can occur in any centrifugation. The second possibility occurs when droplets of solution that contain particles sediment. This is more likely to occur when working with a solution that has a layer of suspension floating on a dense liquid, which in fact have little to no density gradient.

Differential Centrifugation

Differential Centrifugation is a type of centrifugation in which one selectively spins down components of a mixture by a series of increasing centrifugation forces. This method is commonly used to separate organelles and membranes found in cells. Organelles generally differ from each other in density in size, making the use of differential centrifugation, and centrifugation in general, possible. The organelles can then be identified by testing for indicators that are unique to the specific organelles.[8]

Other applications

- Separating chalk powder from water
- Removing fat from milk to produce skimmed milk
- Separating particles from an air-flow using cyclonic separation
- The clarification and stabilization of wine
- Separation of urine components and blood components in forensic and research laboratories
- Aids in separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation.

Filtration of fermentation broth

The hydraulic resistance of cakes formed during the ultrafiltration of Streptomyces pristinaespiralis broths has been investigated for different harvesting conditions. S. pristinaespiralis broth was harvested after the point of microorganism activity declines (0-h aged broth) and afterwards held for different durations of up to 16 h (16 aged broths). Aging behavior

occurring between the end of microorganism activity and harvest was compared for different acidification procedures (pH) and the mechanisms for which the hydraulic resistance of the cake is affected by aging have been investigated. For broths harvested under conditions where the acidification is fixed at pH 2 or 3, hydraulic resistance associated with cake build-up is directly determined by the interactions between the cells. Holding broths beyond 5 h contributes to a release of a soluble component from the cell surface. Enhanced cell surface interactions then turn the cake structure into a more open one and reduce the specific hydraulic resistance. For broths harvested under conditions where the acidification is fixed at pH 4, hydraulic resistance associated with cake build-up is both determined by cell interactions and cell morphology. The cause of the increase in specific hydraulic resistance with aging is due to the binding of a soluble component released by the microorganisms, which decreases the cell surface interactions.

Liquid extraction

Liquid–liquid extraction (LLE), also known as **solvent extraction** and **partitioning**, is a method to separate compounds or metal complexes, based on their relative solubilities in two different immiscible liquids, usually water (polar) and an organic solvent (non-polar). There is a net transfer of one or more soluble species from one liquid into another liquid phase, generally from aqueous to organic, and the drive to make that happen comes from chemical potential .i.e. on doing the transfer, the overall system of protons and electrons that make up the solutes and the two solvents, find themselves in more stable configuration (Lesser free energy). The solvent that is enriched in solute(s) is called extract. The feed solution that is depleted in solute(s) is called the raffinate. LLE is a basic technique in chemical laboratories, where it is performed using a variety of apparatus, from separatory funnels to countercurrent distribution equipment called as mixer settlers.[*not verified in body*] This type of process is commonly performed after a chemical reaction as part of the work-up, often including an acidic work up.

The term *partitioning* is commonly used to refer to the underlying chemical and physical processes involved in *liquid–liquid extraction*, but on another reading may be fully synonymous with it. The term *solvent extraction* can also refer to the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. In that case, a soluble compound is separated from an insoluble compound or a complex matrix.[*not verified in body*]

From a hydrometallurgical perspective, solvent extraction is exclusively used in separation and purification of uranium and plutonium, zirconium and hafnium, separation of cobalt and nickel, separation and purification of rare earth elements etc., it's greatest advandage being it's ability to selectively separate out even very similar metals. We obtain high purity single metal streams on 'stripping' out the metal value from the 'loaded' organic. wherein we can precipitate or deposit the metal value. Stripping is the opposite of extraction, transfer of mass from organic to aqueous phase.

LLE is also widely used in the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries.[*not verified in body*] It is among the most common initial separation techniques, though some difficulties result in extracting out closely related functional groups.

Liquid–liquid extraction is possible in non-aqueous systems: In a system consisting of a molten metal in contact with molten salts, metals can be extracted from one phase to the other. This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam that modifies its electrochemistry greatly. For example, it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations are not reduced. Instead, water is reduced to hydrogen. A detergent or fine solid can be used to stabilize an emulsion, or third phase.[*not verified in body*]

Anaerobic respiration

Anaerobic fermentation is a method cells use to extract energy from carbohydrates when oxygen or other electron acceptors are not available in the surrounding environment. This differentiates it from anaerobic respiration, which doesn’t use oxygen but does use electron-accepting molecules

that come from outside of the cell. The process can follow glycolysis as the next step in the breakdown of glucose and other sugars to produce molecules of adenosine triphosphate (ATP) that create an energy source for the cell.

Through this method, a cell is able to regenerate nicotinamide adenine dinucleotide (NAD+) from the reduced form of nicotinamide adenine dinucleotide (NADH), a molecule necessary to continue glycolysis. Anaerobic fermentation relies on enzymes to add a phosphate group to an individual adenosine diphosphate (ADP) molecule to produce ATP, which means it is a form of substrate-level phosphorylation. This contrasts with oxidative phosphorylation, which uses energy from an established proton gradient to produce ATP.

There are two major types of anaerobic fermentation: ethanol fermentation and lactic acid fermentation. Both restore NAD+ to allow a cell to continue generating ATP through glycolysis. Ethanol fermentation converts two pyruvate molecules, the products of glycolysis, to two molecules of ethanol and two molecules of carbon dioxide. The reaction is a two-step process in which pyruvate is converted to acetaldehyde and carbon dioxide first, by the enzyme pyruvate decarboxylase.

In the second step, alcohol dehydrogenase converts acetaldehyde to ethanol. This metabolic process occurs in certain types of bacteria cells and in yeast cells. This makes yeast popular for making bread, beer, and wine, by using either the carbon dioxide or the ethanol from fermentation.

Lactic acid fermentation is another form of anaerobic fermentation, and is commonly used by muscle cells during times of stress when not enough oxygen is available. These cells convert the two molecules of pyruvate from glycolysis into two molecules of L-lactate using the enzyme lactate dehydrogenase. This process is known as homolactic fermentation, because two molecules of pyruvate undergo the same chemical reactions, and this form of lactic acid fermentation occurs in animal muscle cells and red blood cells.

In heterolactic fermentation, the pyruvate molecules undergo different chemical reactions. One is converted to lactate, while the other is converted to ethanol and carbon dioxide. This process occurs in some species of anaerobic organisms.

In animals, the lactate byproduct from anaerobic fermentation is pumped into the bloodstream, where it is transported to the liver. In a process called the Cori cycle, the liver uses its own set of enzymes to convert the lactate back to glucose, where it can be recycled by the body. The glucose is usually transported back to the muscles, where it can be stored as glycogen for future energy needs.

A **bioreactor** may refer to any manufactured or engineered device or system that supports a biologically active environment.[1] In one case, a bioreactor is a vessel in which a chemical process is carried out which involves organisms or biochemically active substances derived from such organisms. This process can either be aerobic or anaerobic. These bioreactors are commonly cylindrical, ranging in size from litres to cubic metres, and are often made of stainless steel.

A bioreactor may also refer to a device or system meant to grow cells or tissues in the context of cell culture. These devices are being developed for use in tissue engineering or biochemical engineering.

On the basis of **mode of operation**, a bioreactor may be classified as batch, fed batch or continuous (e.g. a continuous stirred-tank reactor model). An example of a continuous bioreactor is the chemostat.

Organisms growing in bioreactors may be submerged in liquid medium or may be attached to the surface of a solid medium. Submerged cultures may be suspended or immobilized. Suspension bioreactors can use a wider variety of organisms, since special attachment surfaces are not needed, and can operate at much larger scale than immobilized cultures. However, in a continuously operated process the organisms will be removed from the reactor with the effluent. Immobilization is a general term describing a wide variety of cell or particle attachment or entrapment. It can be applied to basically all types of biocatalysis including enzymes, cellular organelles, animal and plant cells.[3]Immobilization is useful for continuously operated processes, since the organisms will not be removed with the reactor effluent, but is limited in scale because the microbes are only present on the surfaces of the vessel.

Large scale immobilized cell bioreactors are:

- moving media, also known as moving bed biofilm reactor (MBBR)
- packed bed
- fibrous bed
- membrane

Bioreactor design

Bioreactor design is a relatively complex engineering task, which is studied in the discipline of biochemical engineering. Under optimum conditions, the microorganisms or cells are able to perform their desired function with limited production of impurities. The environmental conditions inside the bioreactor, such as temperature, nutrient concentrations, pH, and dissolved gases (especially oxygen for aerobic fermentations) affect the growth and productivity of the organisms. The temperature of the fermentation medium is maintained by a cooling jacket, coils, or both. Particularly exothermic fermentations may require the use of external heat exchangers. Nutrients may be continuously added to the fermenter, as in a fed-batch system, or may be charged into the reactor at the beginning of fermentation. The pH of the medium is measured and adjusted with small amounts of acid or base, depending upon the fermentation. For aerobic (and some anaerobic) fermentations, reactant gases (especially oxygen) must be added to the fermentation. Since oxygen is relatively insoluble in water (the basis of nearly all fermentation media), air (or purified oxygen) must be added continuously. The action of the rising bubbles helps mix the fermentation medium and also "strips" out waste gases, such as carbon dioxide. In practice, bioreactors are often pressurized; this increases the solubility of oxygen in water. In an aerobic process, optimal oxygen transfer is sometimes the rate limiting step. Oxygen is poorly soluble in water—even less in warm fermentation broths—and is relatively scarce in air (20.95%). Oxygen transfer is usually helped by agitation, which is also needed to mix nutrients and to keep the fermentation homogeneous. Gas dispersing agitators are used to break up air bubbles and circulate them throughout the vessel.

Fouling can harm the overall efficiency of the bioreactor, especially the heat exchangers. To avoid it, the bioreactor must be easily cleaned. Interior surfaces are typically made of stainless steel for easy cleaning and sanitation. Typically bioreactors are cleaned between batches, or are designed to reduce fouling as much as possible when operated continuously. Heat transfer is an

important part of bioreactor design; small vessels can be cooled with a cooling jacket, but larger vessels may require coils or an external heat exchanger.

Purification and characterization techniques: purification and characterization of protein, upstream and downstream processing, solid and liquid handling. Distribution of microbial cells, centrifugation, filtration of fermentation broth, ultra centrifugation, liquid extraction, ion exchange recovery of biological products. Experimental model for design of fermentation system, anaerobic fermentation.

UNIT III-Possible questions

1. Describe purification and characterization of protein.
2. Write in detail about upstream and downstream processing in fermentation
3. Make short note on handling of solid and liquid during fermentation
4. Elaborate distribution of microbial cells
5. Make short note on different types of centrifugation
6. Write in detail about filtration of fermentation broth
7. Make short note on different methods of recovery of biological products
8. Describe in detail about design of fermentors
9. Discuss anaerobic fermentation techniques in detail

UNIT-III

Kinetics: Rate equations for enzyme kinetics, simple and complex reactions. Inhibition kinetics; effect of pH and temperature on rate of enzyme reactions. Mathematical derIIIation of growth kinetics, mathematical derIIIations of batch and continuous culture operations.

SYLLABUS

Kinetics: Rate equations for enzyme kinetics, simple and complex reactions. Inhibition kinetics; effect of pH and temperature on rate of enzyme reactions. Mathematical derivation of growth kinetics, mathematical derivations of batch and continuous culture operations.

Kinetics:

Kinetics (Greek jimesijor, forcing to move) is a branch of natural science that deals with the rates and mechanisms of any processes - physical, chemical, or biological. Kinetic studies in microbiology cover all dynamic manifestations of microbial life: growth itself, survIIIal and death, product formation, adaptations, mutations, cell cycles, environmental effects, and biological interactions. Kinetics provides a theoretical framework for optimal design in biotechnologies based on fermentation and enzyme catalysis, as well as on employment of outdoor actIIIity of natural microbial populations (wastewater treatment, soil bioremediation, etc.)

Rate equations for enzyme kinetics

Enzymes are the catalysts of biological systems and are extremely efficient and specific as catalysts. In fact, typically, an enzyme accelerates the rate of a reaction by factors of at least a million compared to the rate of the same reaction in the absence of the enzyme. Most biological reactions do not occur at perceptible rates in the absence of enzymes. One of the simplest biological reactions catalyzed by an enzyme is the hydration of CO2. The catalyst in this reaction is carbonic anhydrase. This reaction is part of the respiration cycle which expels CO2 from the

body. Carbonic anhydrase is a highly efficient enzyme – each enzyme molecule can catalyze the hydration of 105 CO2 molecules per second.

Enzymes are highly specific. Typically a particular enzyme catalyzes only a single chemical reaction or a set of closely related chemical reactions. As is true of any catalyst, enzymes do not alter the equilibrium point of the reaction. This means that the enzyme accelerates the forward and reverse reaction by precisely the same factor. For example, consider the interconversion of A and B. A ↔ B (1) Suppose that in the absence of the enzyme the forward rate constant (kf) is 10-4 s-1 and the reverse rate constant (kr) is 10-6 s-1. The equilibrium constant (Keq) is gIIIen by the ratio of the two rate constants. Keq = [B] [A] = kf kr = 10-4 10- 6 = 100 (2) The equilibrium concentration of B is 100 times that of A whether or not an enzyme is present. However, in the absence of an enzyme the reaction could take more than an hour to approach this equilibrium, whereas in the presence of an enzyme, equilibrium could be attained within a 2 second. The enzyme lowers the height of the energy barrier to the reaction thereby increasing the rate of the reaction, but since the rate of both the forward and reverse reaction are affected by the same amount, the equilibrium constant is not affected by the presence of the enzyme. the same amount (see Figure 1) Figure 1 where, EAf is the actIIIation energy for the forward reaction (AÆB) in the absence of a catalyst and E'Af is the actIIIation energy for the forward reaction (AÆB) in the presence of a catalyst, and ΔGo is the change in free energy for the reaction. The equilibrium constant is related to ΔGo as follows: Keq = e −ΔGo / RT Since ΔGo is the same for the catalyzed and uncatalyzed reaction, Keq is the same for both reactions. One reason for the efficiency and specificity of an enzyme is the way the enzyme interacts with the reactant molecule, more commonly known as the substrate, in enzyme catalyzed reactions. The enzyme and substrate interact to form an enzyme-substrate complex. The interactions between the substrate and actIIIe site are weak, noncovalent interactions (i.e. the substrate does not covalently bind to the actIIIe site but weakly interacts with it through interactions like hydrogen-bonding, van der Waals interactions, etc). The orientation in which the two interact 3 is highly favorable for facilitating conversion of the substrate to product. In the enzymesubstrate complex, the substrate molecule binds to a very specific region of the enzyme molecule called the actIIIe site. These actIIIe sites are highly selectIIIe for a specific substrate molecule with which the enzyme binds. This is why enzymes are such highly specific catalysts, catalyzing a single reaction, or a

set of closely related reactions. There are two proposed models to explain the specificity of the interaction between the substrate molecule and the actIIIe site of an enzyme. (a) The "lock and key" model – in this model the substrate has a shape matching the enzyme's actIIIe site (see figure 2) Figure 2 (b) The "induced fit model" – the actIIIe site has a shape complementary to that of the substrate after the substrate is bound (see figure 3) 4 Figure 3 Enzyme kinetics The mechanism of enzyme catalyzed reactions is often studied by making kinetic measurements on enzyme-substrate reaction systems. These studies include measuring rates of the enzyme-catalyzed reactions at different substrate and enzyme concentrations. Here we will look at a simple model for the catalytic behavior of an enzyme and the kinetic model that arises from this model. For many enzymes, if we were to plot the rate of catalysis, V (also known as the reaction velocity), vs. the substrate concentration, [S] (at a fixed enzyme concentration) we would see a plot as shown in figure 4. Figure 4 Looking at this plot, we see that V varies linearly with [S] for small [S]. As [S] increases, V "plateaus" indicating that V becomes independent of [S] at large values of [S]. The simplest model which accounts for this behavior is:

$$E + S \leftrightarrow k-1\ k1\ ES \rightarrow k2\ E + P \quad (3)$$

where E is the enzyme, S the substrate, ES the enzyme-substrate complex, P the product of the enzyme-catalyzed reaction, k1 the rate constant of the forward reaction of E+S, k-1 the rate of the reverse reaction where the enzyme-substrate complex, ES, falls apart to E+S and k2 the rate constant of the forward reaction of ES forming E+P. In this model, it is assumed that none of 5 the product reacts with the enzyme to form the enzyme-substrate complex, ES (this is true during the initial stages of the reaction when [P] is low, but towards the end of the reaction when [P] is high this may no longer be true). We need to derIIIe an expression that relates the reaction velocity, V, to the concentrations of the substrate and enzyme and the rates of the indIIIidual steps.

From equation (3) the reaction velocity, V can be expressed as:

$$V = k2\ [ES] \quad (4)$$

Since ES is an intermediate and hence its concentration unknown, we have to express [ES] in terms of known values. The rates at which [ES] is formed and falls apart are: Rate of formation

of [ES] = k1 [E] [S] (5) Rate at which [ES] falls apart = (k-1 + k2) [ES] (6) We can use the steady-state approximation to express V in terms of known quantities. Under the steady-state approximation, the concentration of the intermediate [ES] stays a constant, while the concentrations of reactants and product change. The steady state occurs when eqn 5 = eqn 6 i.e. k1 [E] [S] = (k-1 + k2) [ES] (7)

Rearranging, [ES] = [E][S]k1 k −1 + k2 (8)

Define, KM, the Michaelis constant, as KM = k−1 + k2 k1 (9) Substituting (9) into (8)

[ES] = [E][S] KM (10)

Since in most situations the enzyme concentration is very small ([E] << [S]), the concentration of the uncombined S is almost equal to the total concentration of S.

The concentration of 6 uncombined E is equal to the total enzyme concentration [Eo] minus the concentration of the complex

[ES] [E] = [Eo] – [ES] (11) Substituting (11) into (10)

[ES] = ([Eo]− [ES])[S] K M (12)

Solving (12) for [ES], [ES] = [Eo] [S]/ KM 1 + S / KM (13) or, [ES] = [Eo] [S] [S] + KM (14) Substituting (14) into (4) V = k 2[Eo] [S] [S] + KM (15)

The maximum reaction velocity, Vmax, is reached when all enzyme sites are saturated with the substrate. This will happen when [S] >>KM, so that [S]/([S]+KM) approaches 1. In this limit, we can express Vmax (from (15)) as: Vmax = k2 [Eo] (16) Substituting (16) into (15), V = Vmax [S] [S]+ KM (17) If we were to plot V vs S the resulting plot will have a shape as shown in figure 4. Hence, equation (17) describes the kinetic behavior of an enzyme as modeled by the kinetic scheme in equation (3). Looking at equation (17) at very low [S], when [S] << KM, V ~ [S]Vmax/KM, that is, the rate is proportional to [S] (describes the linear region of the plot in figure 4). At high [S], 7 when [S]>>KM, V=Vmax and hence independent of [S] (the “plateau” region of the plot in figure 4). Equation (17) can be re-arranged as: 1 V = 1 Vmax + KM Vmax 1 [S] (18) If we were to plot 1/V vs. 1/[S] we would obtain a straight line with a y-intercept =

1/Vmax and a slope =KM/Vmax (see figure 5). This plot is called a Lineweaver-Burke plot. y = 2.055E+05x + 1.284E+05 1/[S] Figure 5 Significance of KM From equation 18, when [S] = KM, then V=Vmax/2. Hence KM is equal to the substrate concentration at which the reaction rate is half its maximum value. In other words, if an enzyme has a small value of KM, it achieves its maximum catalytic efficiency at low substrate concentrations. Hence, the smaller the value of KM, the more efficient is the catalyst. The value of KM for an enzyme depends on the particular substrate . It also depends on the pH of the solution and the temperature at which the reaction is carried out. For most enzymes KM lies between 10-1 and 10-7 M.

Simple and complex reactions

SIMPLE AND COMPLEX REACTIONS

A simple reaction takes place in a single step. Simple reactions are also known as elementary reactions. One step reactions are elementary reactions. In some reactions many side reactions occur along with the main reaction involving product formation.

Reactions which do not take place in a single step but take place in a sequence of a number of elementary steps are called as complex reactions.

Simple reactions

- Occurs in single step
- Overall order values are small. Total and pseudo order values lie between 0,1,2 and 3.
- No side reactions
- Products are formed directly from the reactants
- Experimental rate constant values agree with the calculated values. Theories of reaction rates apply well on simple reactions.

Complex reactions

- Occurs in multi (or) many steps.
- Overall order values are large and greater than 3.0. Sometimes fractional orders such as 1/2, 1/3, 3/2 etc. are seen.

- Many side reactions are present.
- In some complex reactions
- products are not formed in steps directly involving the reactants.
- Experimental overall rate constant values differ from the calculated values. Theories of reaction rates do not agree well on complex reactions.

	Simple reactions	Complex reactions
1.	Occurs in single step	Occurs in multi (or) many steps.
2.	Overall order values are small. Total and pseudo order values lie between 0,1,2 and 3.	Overall order values are large and greater than 3.0. Sometimes fractional orders such as 1/2, 1/3, 3/2 etc. are seen.
3.	No side reactions	Many side reactions are present.
4.	Products are formed directly from the reactants	In some complex reactions products are not formed in steps directly involving the reactants.
5.	Experimental rate constant values agree with the calculated values. Theories of reaction rates apply well on simple reactions.	Experimental overall rate constant values differ from the calculated values. Theories of reaction rates do not agree well on complex reactions.

Types of Complex reaction

The reactions in which the reactant forms an intermediate and the intermediate forms the product in one or many subsequent reactions are called as consecutive or sequential reactions. In such reactions the product is not formed directly from the reactant. Various steps in the consecutive reaction are shown as below :

A --- ^{k1}--- > B --- ^{k1} --- > C

A = reactant ; B = intermediate ; C = product. Initially only the reactant A will be present. As the reaction starts, A produces an intermediate B through k_1 rate constant. As and when B is formed, it produces the product C through k_2 rate constant. After the completion of reaction only 'C' is present and concentrations of A and B will be zero.

Example of consecutIIIe reactions

Saponification of a diester in presence of an alkali :

$R'OOC-(CH_2)_n-COOR \xrightarrow{k1} R'OOC-(CH_2)_n-COOH \xrightarrow{k2} HOOC-(CH_2)_n-COOH$

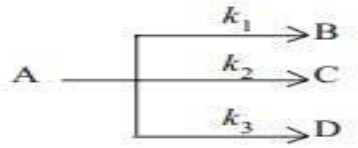
(ii) Parallel reactions

In these group of reactions, one or more reactants react simultaneously in two or more pathways to give two or more products. The parallel reactions are also called as side reactions.

The reactant A reacts to give products B,C,D separately by following three different reaction pathways involving different k_1, k_2, k_3 rate constants respectively. Among the many side reactions, the reaction in which maximum yield of the product obtained is called as the main or major reaction while the other reactions are called as side or parallel reactions.

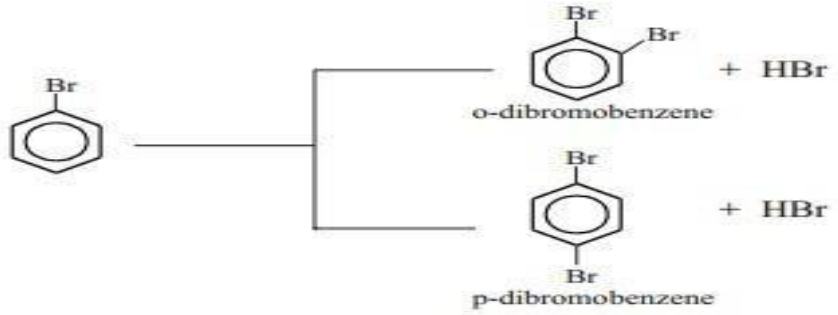
Examples of parallel reaction:

Bromination of bromobenzene :

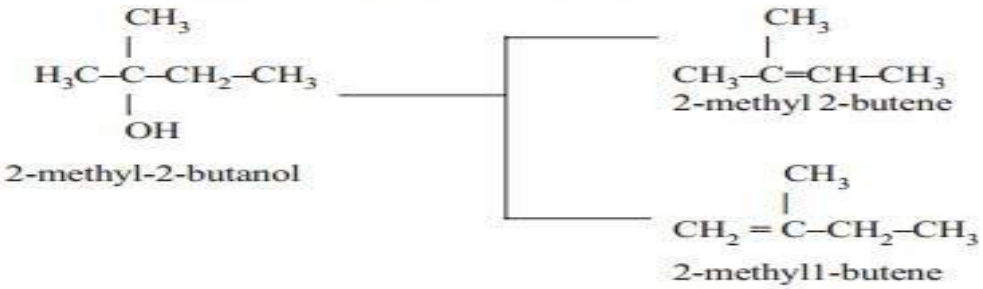


Examples of parallel reaction :

(i) Bromination of bromobenzene :



(ii) Dehydration of 2-methyl-2-butanol



(iii) Opposing reactions

In opposing reactions the products formed react back simultaneously to form the reactants. These reactions are also called as reversible reactions.

A + B -- ^{Kf}-- > < --^{Kr} ---C + D

Examples of opposing reactions

(i) Reaction between CO and NO_2 gases

(ii) Isomerisation of cyclopropane to propene

(iii) Dissociation of hydrogen iodide in gas phase

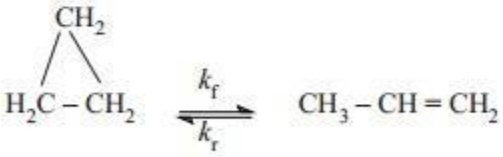
$$A + B \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C + D,$$

Examples of opposing reactions

(i) Reaction between CO and NO_2 gases

$$CO_{(g)} + NO_{2(g)} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} CO_{2(g)} + NO_{(g)}$$

(ii) Isomerisation of cyclopropane to propene



(iii) Dissociation of hydrogen iodide in gas phase

$$2HI_{(g)} \rightleftharpoons H_{2(g)} + I_{2(g)}$$

Inhibition kinetics; effect of pH and temperature on rate of enzyme reactions.

Enzymes-Function and structure

Enzymes are very efficient catalysts for biochemical reactions. They speed up reactions by providing an alternatIIIe reaction pathway of lower actIIIation energy.

Like all catalysts, enzymes take part in the reaction - that is how they provide an alternatIIIe reaction pathway. But they do not undergo permanent changes and so remain unchanged at the end of the reaction. They can only alter the rate of reaction, not the position of the equilibrium.

Most chemical catalysts catalyse a wide range of reactions. They are not usually very selectIIIe. In contrast enzymes are usually highly selectIIIe, catalysing specific reactions only. This specificity is due to the shapes of the enzyme molecules.

Many enzymes consist of a protein and a non-protein (called the cofactor). The proteins in enzymes are usually globular. The intra- and intermolecular bonds that hold proteins in their secondary and tertiary structures are disrupted by changes in temperature and pH. This affects shapes and so the catalytic actIIIity of an enzyme is pH and temperature sensitIIIe.

Cofactors may be:

organic groups that are permanently bound to the enzyme (prosthetic groups)

cations - positIIIely charged metal ions (actIIIators), which temporarily bind to the actIIIe site of the enzyme, gIIIing an intense positIIIe charge to the enzyme's protein

organic molecules, usually vitamins or made from vitamins (coenzymes), which are not permanently bound to the enzyme molecule, but combine with the enzyme-substrate complex temporarily.

For two molecules to react they must collide with one another. They must collide in the right direction (orientation) and with sufficient energy. Sufficient energy means that between them they have enough energy to overcome the energy barrier to reaction. This is called the **activation energy**.

Enzymes have an **active site**. This is part of the molecule that has just the right shape and functional groups to bind to one of the reacting molecules. The reacting molecule that binds to the enzyme is called the **substrate**.

An enzyme-catalysed reaction takes a different 'route'. The enzyme and substrate form a reaction intermediate. Its formation has a lower actIIIation energy than the reaction between reactants without a catalyst.

A simplified picture

Route A reactant 1 + reactant 2 → product

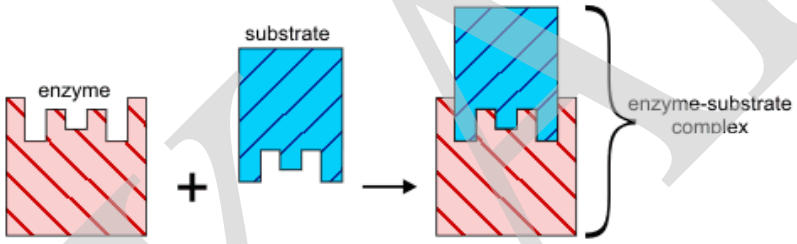
Route B reactant 1 + enzyme → intermediate

intermediate + reactant 2 → product + enzyme

So the enzyme is used to form a reaction intermediate, but when this reacts with another reactant the enzyme reforms.

Lock and key hypothesis

This is the simplest model to represent how an enzyme works. The substrate simply fits into the active site to form a reaction intermediate.

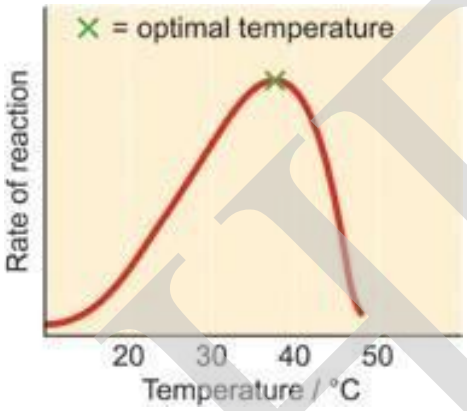


Induced fit hypothesis

In this model the enzyme molecule changes shape as the substrate molecules gets close. The change in shape is 'induced' by the approaching substrate molecule. This more sophisticated model relies on the fact that molecules are flexible because single covalent bonds are free to rotate.

Factors affecting catalytic actIIIity of enzymes

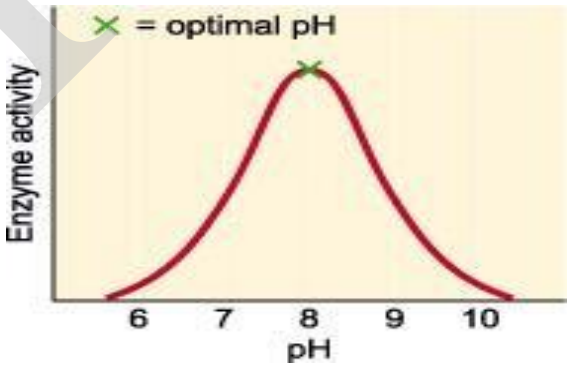
Temperature



As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (37.5 ºC) for the enzymes in human cells.

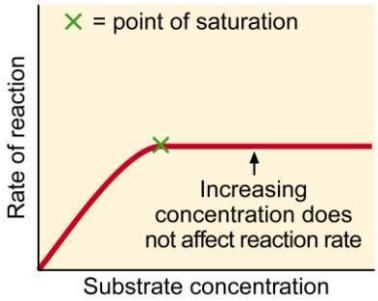
Above this temperature the enzyme structure begins to break down (**denature**) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

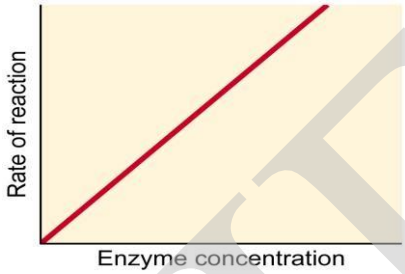
Effect of pH



Each enzyme works within quite a small pH range. There is a pH at which its actIIIity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectIIIeness.

Concentration of enzyme and substrate





The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate. (See graph)

Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration. (See graph)

Inhibition of enzyme activity

Some substances reduce or even stop the catalytic activity of enzymes in biochemical reactions. They block or distort the actIIIe site. These chemicals are called **inhibitors**, because they inhibit reaction.

Inhibitors that occupy the actIIIe site and prevent a substrate molecule from binding to the enzyme are said to be **actIIIe site-directed** (or **competitIIIe**, as they 'compete' with the substrate for the actIIIe site).

Inhibitors that attach to other parts of the enzyme molecule, perhaps distorting its shape, are said to be **non-actIIIe site-directed** (or **non competitIIIe**).

Mathematical derIIIation of growth kinetics

Bacterial growth rates during the phase of exponential growth, under standard nutritional conditions (culture medium, temperature, pH etc.) define the bacterium's generation time. Generation times for bacteria vary from about 12 minutes to 24 hours. The generation time for E. coli in the laboratory is 15-20 min. Symbionts such as Rhizobium tend to have a longer generation time. Some pathogenic bacteria, e.g., Mycobacterium tuberculosis have especially long generation times and this is thought to be an advantage to their virulence. When growing exponentially by binary fission, the increase in a bacterial population is by geometric progression. The generation time is the time interval required for cells (or population) to dIIIide: G =t/n Where G is generation time, n is number of generations and t is time in min/hours The equation for growth by binary fission is: b = B x 2n where b is number of bacteria at end of a time interval, B is number of bacteria at beginning of a time interval, n is the number of generations (number of times the population doubles in the time interval). logb = logB + nlog2 n = logb-logB log2 n = logb-logB .301 n = 3.3logb/B G= t 3.3logb/B 2.5.2

Mathematical derIIIations of batch and continuous culture operations.

GROWTH KINETICS IN BATCH CULTURE

Batch culture occurs in a closed system that contains an initial limited amount of substrate. The inoculated microorganism will pass through a number of growth phases. During the log phase, cell numbers increase exponentially at a constant maximum rate. In mathematical terms, we can write: $dx = \mu x\, dt$ where x is the concentration of microbial biomass, t is the time in hours and μ is the specific growth rate in hours-1 .

If we integrate between time t0 and time t1when the concentrations of the cells are X0 and X1we obtain:

$xt = x0e\ \mu t$

where x0 is the original biomass concentration, xt is the biomass concentration after a time interval t hours and e is the base of the natural logarithm. On taking natural logarithms, the equation becomes:

$\ln x_t = \ln x_0 + \mu t$

Using this equation, a plot of the natural log of biomass concentration versus time should yield a straight line, the slope of which will equal the specific growth rate (μ).

ln x During the exponential phase, nutrients are in excess and the microorganism is growing at maximum specific growth rate μmax for the prevailing conditions. The major problem of the exponential growth equation is that it does not predict an end to growth in a batch environment. According to this model, not only the whole earth, but also the whole solar system could become quickly covered by bacteria. However, growth results in the consumption of nutrients and the excretion of microbial products. After a time, the growth rate of the culture ceases. The cessation of growth may be due to the depletion of some essential nutrient in the medium (substrate limitation), the accumulation of some autotoxic product in the medium or a combination of the two. The nature of the limitation of growth can be explored by growing the microorganisms in a range of substrate concentrations and plotting the biomass concentration in the stationary phase against the initial substrate concentration, the nature of growth limitation may be explored. The effect of initial substrate concentration on the biomass concentration at the onset of stationary phase in batch culture (Stanbury et al. 1995). A proportional increase in biomass is observed to increasing initial substrate concentration in the area between A and B which can be defined as:

$x = Y (SR – S)$

Where x is the concentration of biomass produced,

Y is the yield factor (g biomass produced per g substrate utilized),

SR is the initial substrate concentration and

S is the residual substrate concentration.

In the area between A and B, Sis zero and therefore the equation above could be used to predict the biomass that could be formed from a certain amount of substrate. Between B and C although biomass increases with increasing substrate concentration, there is a diminished effect due to accumulation of toxic products or reduced availability of some other substrate. In the region between C and D, there is no change in biomass with increasing substrate concentration which may be attributed to increasing levels of toxic products or the exhaustion of some other substrate. Biomass concentration at stationary phase Initial substrate concentration A B C D Y, the yield factor is the measure of efficiency of conversion of any one substrate to biomass. Although Y is not a constant and varies according to growth rate, pH, temperature, the limiting substrate and concentration of the substrate in excess, it can be used to predict the substrate concentration required to produce a certain biomass concentration. In the 1930s, Jacques Monod performed a number of initial rate experiments and plotted the specific growth rate against the concentration of growth-limiting substrate. The result was a Langmuir type graph that appeared similar to enzymatic rate-substrate relationships described by MichaelisMenton's model. Monod's model describing the relationship between the specific growth rate and the growth limiting substrate concentration is: Fig.2.5: The effect of residual limiting substrate concentration on specific growth rate of a hypothetical bacterium (Stanbury et al. 1995, adapted). Where μm is the maximum specific growth rate, S is the residual substrate concentration and Ks is the substrate utilization constant, numerically equal to substrate concentration when μ is half μm μm and is a measure of the affinity of the organism for its substrate. Zone A to B represents the exponential phase of growth in batch culture where substrate concentration is in excess and growth is at μm. Zone A to C is the deceleration phase, substrate concentration becomes limiting and cannot support growth at μm. An organism with a high affinity for the limiting substrate (low Ks) will have a short deceleration phase as the growth rate will only be affected when the substrate concentration is very low. Conversely, a microorganism with a low affinity for the substrate will have a very long deceleration phase (growth slows down at high substrate concentrations). The point when growth rate has declined to zero represents the stationary phase. This is a misnomer as many organisms are still metabolically actIIIe and are producing products called secondary metabolites during this phase. Monod's model is widely used to describe the growth of many microorganisms. The equation adequately describes fermentation kinetics and can be used to

describe complex fermentation systems. The equation adequately describes fermentation kinetics and can be used to describe complex fermentation systems, e.g., a commonly used expression to describe product inhibition is: C A B Using the Monod model, a simple model microbial growth can be written as: where Yxs is the biomass yield coefficient. The biomass yield coefficient is the efficiency of conversion of substrate to biomass and is calculated as: Biomass = Dry weight of biomass produced Weight of substrate used The kinetics of product formation may be described as growth-linked products and non-growth linked products. In the first instance – these could relate to primary metabolites synthesized by growing cells and the non-growth-linked products would be secondary metabolites. Formation of growth-linked products can be defined by the following:

$dp/dt = qpx$

where p is the concentration of product, qp is the specific rate of product formation (mg product /g biomass/h). Product formation can also be expressed in relation to biomass as:

$dp/dx = Yp/x$

where Yp/x is the yield of product in terms of biomass (g product/g biomass). Combining these equations:

$qp = Yp/x \, .\mu$

Thus when product formation is linked to growth, the specific rate of product formation increases with specific growth rate and will be highest at μm. In this instance improved output will be obtained by increasing both biomass and μ. Non-growth linked product formation is related to biomass concentration. As these products are produced only under certain physiological conditions (usually limitation of a certain substrate), the biomass needs to be in the correct physiological state before secondary metabolites are produced. Batch fermentations may be used to produce biomass and primary and secondary metabolites. For (i) biomass production: conditions supporting fastest growth rate and maximum cell concentration; For (ii) primary metabolites: conditions to extend exponential phase accompanied by product excretion; For (iii) secondary metabolites: conditions providing a short exponential phase and extended production

phase or conditions gIIIing decreased growth rate in the log phase resulting in earlier secondary metabolite production.

GROWTH KINETICS IN CONTINUOUS CULTURE

Exponential growth in batch culture may be prolonged by the addition of fresh medium, provided that the medium is designed to be substrate-limiting. If the vessel is designed with an overflow mechanism, such that the added medium displaced an equal volume of spent medium, then continuous culture of cells can be achieved. A steady state will be achieved if the medium is fed continuously at a suitable rate, i.e., formation of new biomass by the culture is balanced by the loss of biomass from the vessel. The flow of medium is related to the volume of the vessel by the dilution rate (D) as follows:

$D = F/V$

Where F is the flow rate (l/h) and V is the volume (l). The net change in cell concentration over time may be expressed as:

dx/dt = growth – output or $dx/dt = \mu x - Dx$

Under steady state conditions, the cell concentration remains constant, therefore

$dx/dt = 0$ and $\mu x = dx$ and $\mu = D$ A

continuous culture may be operated at dilution rates below the maximum specific growth rate and so within certain limits, the dilution rate may be used to control the growth rate of the culture. Cell growth in such a continuous culture is controlled by the availability of the growth limiting substrate and the system is referred to as a chemostat. The mechanism underlying the controlling effect of the dilution rate is expressed in the Monod equation:

$\mu = \mu ms/(Ks + s)$ AT steady state, $\mu = D$

Therefore, $D = \mu m /(Ks +)$ where is the steady state concentration of substrate in the chemostat. Rearranging the equation:

$KsD/ (\mu m - D)$

which predicts that the substrate concentration is determined by the dilution rate. This occurs by the growth of the cells depleting the substrate to a concentration that supports the growth rate equal to the dilution rate. If the substrate becomes depleted below the level that supports the growth rate dictated by the dilution rate, the following would occur:

(i) The growth rate of the cells will be less than the dilution rate and they will be washed out of the vessel at a rate greater than they are being produced resulting in a decrease in biomass concentration. (ii) The substrate concentration in the vessel will rise because fewer cells are left in the vessel to consume it.

(iii) The increased substrate concentration in the vessel will result in the cells growing at a rate greater than the dilution rate and biomass concentration will increase. (III) The steady state will be re-established. Therefore a chemostat is a nutrient-limited, self-balancing culture system which may be maintained in a steady state over a wide range of sub-maximum specific rates. The cell concentration in a chemostat is defined by:

= Y(SR - Where is the steady state cell concentration. By combining equation of steady state substrate and biomass concentrations:

= Y[SR – {KsD/(μm – D)}] Therefore biomass concentration at steady state is defined by operational variables SR and D. If SR is increased, increases but remains the same. If D is increased, μ will increase (μ = D), at the new steady state would have increased to support the elevated growth rate and less substrate will be available to be converted to biomass resulting in a lower An alternatIIIe type of continuous culture to a chemostat is a turbidostat. Here the concentration of the cells in the vessel is kept constant by controlling the flow of medium such that the turbidity of the culture is kept within certain narrow limits. To achieve this, biomass is monitored using a photoelectric cell, signals are sent to a pump controlling medium flow into the vessel. If the biomass exceeds a set point, the pump is switched on and if the biomass falls below the set point it is switched off. Other biomass measurement systems include CO2 concentration or pH - biostat. However, the chemostat is the more commonly used system as it has the advantage over the biostat of not requiring complex control systems to maintain steady state.

UNIT III-Possible questions

1. Describe simple and complex reactions in detail.
2. Write in detail about rate equation for enzyme kinetics
3. Make short note on inhibition kinetics fermentation
4. Elaborate derIIIation of growth kinetics in detail
5. Make short note on batch culture operation with mathematical derIIIation
6. Discuss mathematical derIIIations involved in batch operations
7. Make short note on mathematical derIIIations involved in continous operations

UNIT-IV

Production of industrial products: Production of industrial chemicals, biochemicals and chemotherapeutic products. Propionic acid, butyric acid, 2-3 butanediol, gluconic acid, itaconic acid, Biofuels: Biogas, Ethanol, butanol, hydrogen, biodiesel, microbial electricity, starch conversion processes; Microbial polysaccharides; Microbial insecticides; microbial flavours and fragrances, newer antibiotics, anti cancer agents, amino acids.

SYLLABUS

Production of industrial products: Production of industrial chemicals, biochemicals and chemotherapeutic products. Propionic acid, butyric acid, 2-3 butanediol, gluconic acid, itaconic acid, Biofuels: Biogas, Ethanol, butanol, hydrogen, biodiesel, microbial electricity, starch conversion processes; Microbial polysaccharides; Microbial insecticides; microbial flavours and fragrances, newer antibiotics, anti cancer agents, amino acids.

Production of industrial products

Unit IV

Production of industrial products*: production of industrial chemicals, biochemical's and chemotherapeutic products, propionic acid, butyric acid, 2-3 butanediol, gluconic acid, itacnic acid, bio fuels: biogas, ethanol, butane, hydrogen, biodiesel, microbial electricity, starch conversion process; microbial polysaccharide; microbial insecticides; microbial flavours and fragrances, newer antibiotics, anti cancer agents, amino acids.*

Fermentation

The process of anaerobic decomposition of organic substances (primarily carbohydrates); it occu rs under
the influence ofmicroorganisms or the enzymes secreted by them. In the course of fermentation, t

heenergy needed for the vital activity ofthe microorganisms is released as a result of conjugated oxidation
reduction reactions, and chemical compounds used bythe microorganisms for biosynthesis of ami no acid,
proteins, organic acids, fats, and other body components are formed. Atthe same time, there is an accumulation of theend products of fermentation. The different types of fermentation include alcoholic, lactic, butyric-acid, propionic-acid, acetone-butyl alcohol, and acetone
ethyl alcohol fermentation.

The nature, intensity, and direction of fermentation, as well as the quantitative ratios of the end p roducts, depend on thecharacteristics of the fermenting agent and upon the conditions under whic h fermentation takes place (such as the pH,aeration, and the substrate).

Propionic-
acid fermentation. The main products of propionic fermentation, which is caused by several spe cies of bacteriaof the genus *Propionibacterium*, are propionic acid (CH_3CH_2COOH), acetic acid, and CO_2. The chemistry of suchfermentation varies greatly depending on circumstances. This is apparently explained by the ability of propionic bacteria torearrange metabolism—
for example, by aeration. In the presence of oxygen, propionic-
acid bacteria cause oxidation, and inits absence they break down hexoses by fermentation. Propi onic bacteria are capable of fixing CO_2, whereby oxalacetic acidis formed from pyruvic acid and CO_2. The oxalacetic acid changes into succinic acid, from which propionic acid is formed bydeca rboxylation:

$$\underset{\text{(succinic acid)}}{COOHCH_2CH_2COOH} \xrightarrow{-CO_2} \underset{\text{(propionic acid)}}{CH_3CH_2COOH}$$

Types of fermentation that are accompanied by reduction processes also exist. An example of su ch “oxidizing” fermentationis citric-
acid fermentation. Many mold fungi ferment sugar, resulting in the formation of citric acid. The more active strains of*Aspergillus niger* convert up to 90 percent of the sugar consumed into citric acid. A considerable part of the citric acid used inthe food-

processing industry is obtained by microbiological means—
the subsurface and surface culturing of mold fungi.

By tradition, even purely oxidizing processes caused by microorganisms are occasionally called f ermentation. Acetic-acidand gluconic-
acid fermentation may serve as examples of such processes.

Butyric-
acid fermentation. The fermentation of carbohydrates, with the predominant formationof butyric acid, is caused by many anaerobic bacteria belonging to the genus *Clostridium*. Thefirst stages i n the breakdown of carbohydrates by butyric-
acid fermentation are analogous tothe corresponding stages in alcoholic fermentation, up to the p oint of formation of pyruvic acid,from which acetyl coenzyme A (CH_3CO-
CoA) is formed during butyric-acid fermentation.Acetyl-
CoA can serve as a precursor of butyric acid, undergoing the following transformations:

$$2CH_3CO\text{-}CoA \rightarrow \underset{\text{(acetoacetyl-CoA)}}{CH_3COCH_2CO\text{-}CoA}$$
$$CH_3COCH_2CO\text{-}CoA + 2NAD\cdot H \rightarrow CH_3CHOHCH_2CO\text{-}CoA$$
$$\rightarrow \underset{\text{(}\beta\text{-oxybutyryl-CoA)}}{CH_3CH_2CH_2COOH} + CoA$$

$$2CH_3CO\text{-}CoA \rightarrow \underset{\text{(acetoacetyl-CoA)}}{CH_3COCH_2CO\text{-}CoA}$$
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$$\rightarrow \underset{\text{(}\beta\text{-oxybutyryl-CoA)}}{CH_3CH_2CH_2COOH} + CoA$$

Butyric-acid fermentation was formerly used to obtain butyric acid from starch.

2,3-Butanediol is a organic compound with the formula is $(CH_3)_2(CHOH)_2$.[1] 2,3-Butanediol has three stereoisomers, all of which are colorless, viscous liquids. Butanediols have applications as precursors to various plastics and pesticides.

Biotechnological production of 2,3-butanediol (hereafter referred to as 2,3-BD) from wastes and excessive biomass is a promising and attractive alternative for traditional chemical synthesis. In the face of scarcity of fossil fuel supplies the bio-based process is receiving a significant interest,

since 2,3-BD may have multiple practical applications (e.g. production of synthetic rubber, plasticizers, fumigants, as an antifreeze agent, fuel additive, octane booster, and many others). Although the 2,3-BD pathway is well known, microorganisms able to ferment biomass to 2,3-BD have been isolated and described, and attempts of pilot scale production of this compound were made, still much has to be done in order to achieve desired profitability. This review summarizes hitherto gained knowledge and experience in biotechnological production of 2,3-BD, sources of biomass used, employed microorganisms both wild type and genetically improved strains, as well as operating conditions applied.

2,3-**Butanediol fermentation** is anaerobic fermentation of glucose with 2,3-butanediol as one of the end products. The overall stoichiometry of the reaction is

2 pyruvate + NADH --> $2CO_2$ + 2,3-butanediol.

Butanediol fermentation is typical for the facultative anaerobes *Klebsiella* and *Enterobacter*[1] and is tested for using the Voges–Proskauer (VP) test.

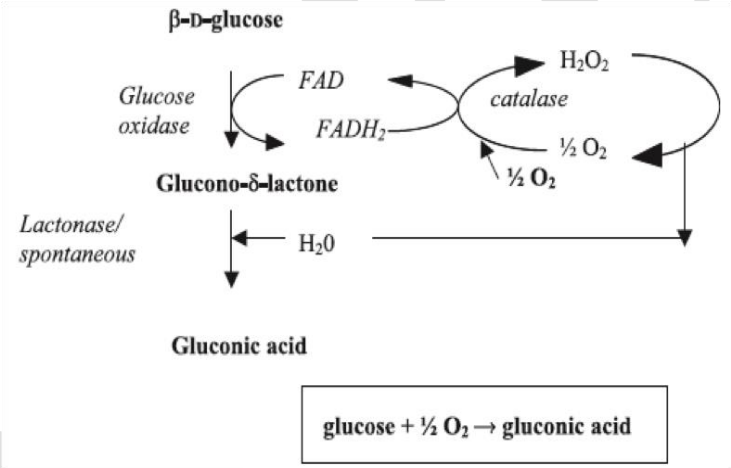
The metabolic function of 2,3-butanediol is not known, although some have speculated that it was an evolutionary advantage for these microorganisms to produce a neutral product that's less inhibitory than other partial oxidation products and doesn't reduce the pH as much as mixed acids.

Gluconic acid production

Introduction: There are different approaches available for the production of gluconic acid, namely, chemical, electrochemical, biochemical and bioelectrochemical.

Gluconic acid, the oxidation product of glucose, is a mild neither caustic nor corrosive, non toxic and readily biodegradable organic acid of great interest for many applications. As a multifunctional carbonic acid belonging to the bulk chemicals and due to its physiological and chemical characteristics, gluconic acid itself, its salts (e.g. alkali metal salts, in especially sodium gluconate) and the gluconolactone form have found extensively versatile uses in the chemical, pharmaceutical, food, construction and other industries. Present review article

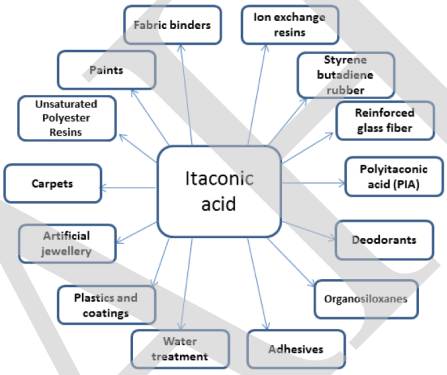
presents the comprehensive information of patent bibliography for the production of gluconic acid and compares the advantages and disadvantages of known processes. Numerous manufacturing processes are described in the international bibliography and patent literature of the last 100 years for the production of gluconic acid from glucose, including chemical and electrochemical catalysis, enzymatic biocatalysis by free or immobilized enzymes in specialized enzyme bioreactors as well as discontinuous and continuous fermentation processes using free growing or immobilized cells of various microorganisms, including bacteria, yeast-like fungi and fungi. Alternatively, new superior fermentation processes have been developed and extensively described for the continuous and discontinuous production of gluconic acid by isolated strains of yeast-like mold Aureobasidium pullulans, offering numerous advantages over the traditional discontinuous fungi processes.

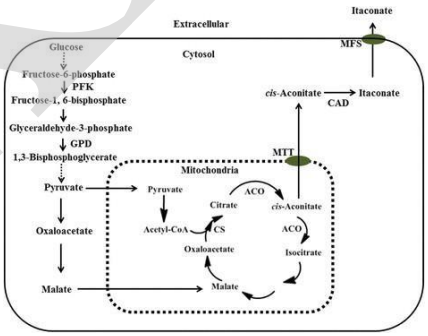


Itaconic acid

Aspergillus terreus is successfully used for industrial production of itaconic acid. The acid is formed from cis-aconitate, an intermediate of the tricarboxylic (TCA) cycle, by catalytic action of cis-aconitate decarboxylase. It could be assumed that strong anaplerotic reactions that replenish the pool of the TCA cycle intermediates would enhance the synthesis and excretion rate of itaconic acid. In the phylogenetic close relative Aspergillus niger, upregulated metabolic flux through glycolysis has been described that acted as a strong anaplerotic reaction.

Deregulated glycolytic flux was caused by posttranslational modification of 6-phosphofructo-1-kinase (PFK1) that resulted in formation of a highly active, citrate inhibition-resistant shorter form of the enzyme. In order to avoid complex posttranslational modification, the native A. niger pfkA gene has been modified to encode for an active shorter PFK1 fragment. By the insertion of the modified A. niger pfkA genes into the A. terreus strain, increased specific productivities of itaconic acid and final yields were documented by transformants in respect to the parental strain. On the other hand, growth rate of all transformants remained suppressed which is due to the low initial pH value of the medium, one of the prerequisites for the accumulation of itaconic acid by A. terreus mycelium.





Biofuels

A **biofuel** is a fuel that is produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than a fuel produced by geological processes such as those involved in the formation of fossil fuels, such as coal and petroleum, from prehistoric biological matter.

Biofuels can be derived directly from plants, or indirectly from agricultural, commercial, domestic, and/or industrial wastes.[1] Renewable biofuels generally involve contemporary carbon fixation, such as those that occur in plants or microalgae through the process of photosynthesis. Other renewable biofuels are made through the use or conversion of biomass (referring to recently living organisms, most often referring to plants or plant-derived materials). This biomass can be converted to convenient energy-containing substances in three different ways: thermal conversion, chemical conversion, and biochemical conversion. This biomass conversion can result in fuel in solid, liquid, or gas form. This new biomass can also be used directly for biofuels.

Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn, sugarcane, or sweet sorghum. Cellulosic biomass, derived from non-food sources, such as trees and grasses, is also being developed as a feedstock for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additiveto increase octane and improve vehicle emissions. Bioethanol is widely used in the United States and in Brazil. Current plant design does not provide for converting the lignin portion of plant raw materials to fuel components by fermentation.

Biodiesel can be used as a fuel for vehicles in its pure form, but it is usually used as a diesel additive to reduce levels of particulates, carbon monoxide, and hydrocarbons from diesel-powered vehicles. Biodiesel is produced from oils or fats using transesterification and is the most common biofuel in Europe.

Biologically produced alcohols, most commonly ethanol, and less commonly propanol and butanol, are produced by the action

of microorganisms and enzymes through the fermentation of sugars or starches (easiest), or cellulose (which is more difficult). Biobutanol (also called biogasoline) is often claimed to provide a direct replacement for gasoline, because it can be used directly in a gasoline engine.

Ethanol fuel is the most common biofuel worldwide, particularly in Brazil. Alcohol fuels are produced by fermentation of sugars derived from wheat, corn, sugar beets, sugar cane, molasses and any sugar or starch from which alcoholic beverages such as whiskey, can be made (such as potato and fruit waste, etc.). The ethanol production methods used are enzyme digestion (to release sugars from stored starches), fermentation of the sugars, distillation and drying. The distillation process requires significant energy input for heat (sometimes unsustainable natural gas fossil fuel, but cellulosic biomass such as bagasse, the waste left after sugar cane is pressed to extract its juice, is the most common fuel in Brazil, while pellets, wood chips and also waste heat are more common in Europe) Waste steam fuels ethanol factory[17] – where waste heat from the factories also is used in the district heating grid.

Ethanol can be used in petrol engines as a replacement for gasoline; it can be mixed with gasoline to any percentage. Most existing car petrol engines can run on blends of up to 15% bioethanol with petroleum/gasoline. Ethanol has a smaller energy density than that of gasoline; this means it takes more fuel (volume and mass) to produce the same amount of work. An advantage of ethanol (CH_3CH_2OH) is that it has a higher octane rating than ethanol-free gasoline available at roadside gas stations, which allows an increase of an engine's compression ratio for increased thermal efficiency. In high-altitude (thin air) locations, some states mandate a mix of gasoline and ethanol as a winter oxidizer to reduce atmospheric pollution emissions.

Ethanol is also used to fuel bioethanol fireplaces. As they do not require a chimney and are "flueless", bioethanol fires[18] are extremely useful for newly built homes and apartments without a flue. The downsides to these fireplaces is that their heat output is slightly less than electric heat or gas fires, and precautions must be taken to avoid carbon monoxide poisoning.

Biogas

Biogas typically refers to a mixture of different gases produced by the breakdown of organic matter in the absence of oxygen. Biogas can be produced from raw materials such as agricultural waste, manure, municipal waste, plant material, sewage, green waste or food waste. Biogas is a renewable energy source.

Biogas can be produced by anaerobic digestion with anaerobic organisms, which digest material inside a closed system, or fermentation of biodegradable materials.[1]

Biogas is primarily methane (CH4) and carbon dioxide (CO_2) and may have small amounts of hydrogen sulfide (H 2S), moisture and siloxanes. The gases methane, hydrogen, and carbon monoxide (CO) can be combusted or oxidized with oxygen. This energy release allows biogas to be used as a fuel; it can be used for any heating purpose, such as cooking. It can also be used in a gas engine to convert the energy in the gas into electricity and heat.

Biogas can be compressed, the same way as natural gas is compressed to CNG, and used to power motor vehicles. In the UK, for example, biogas is estimated to have the potential to replace around 17% of vehicle fuel. It qualifies for renewable energy subsidies in some parts of the world. Biogas can be cleaned and upgraded to natural gas standards, when it becomes bio-methane. Biogas is considered to be a renewable resource because its production-and-use cycle is continuous, and it generates no net carbon dioxide. Organic material grows, is converted and used and then regrows in a continually repeating cycle. From a carbon perspective, as much carbon dioxide is absorbed from the atmosphere in the growth of the primary bio-resource as is released when the material is ultimately converted to energy.

The biogas is a renewable energy that can be used for heating, electricity, and many other operations that use a reciprocating internal combustion engine, such as GE Jenbacher or Caterpillar gas engines.[4] To provide these internal combustion engines with biogas having ample gas pressure to optimize combustion, within the European Union ATEX centrifugal fan units built in accordance with the European directive 2014/34/EU (previously 94/9/EG) are obligatory. These centrifugal fan units, for example Combimac, Meidinger AG or Witt & Sohn AG are suitable for use in Zone 1 and 2 .

Other internal combustion engines such as gas turbines are suitable for the conversion of biogas into both electricity and heat. The digestate is the remaining inorganic matter that was not transformed into biogas. It can be used as an agriculture fertilizers

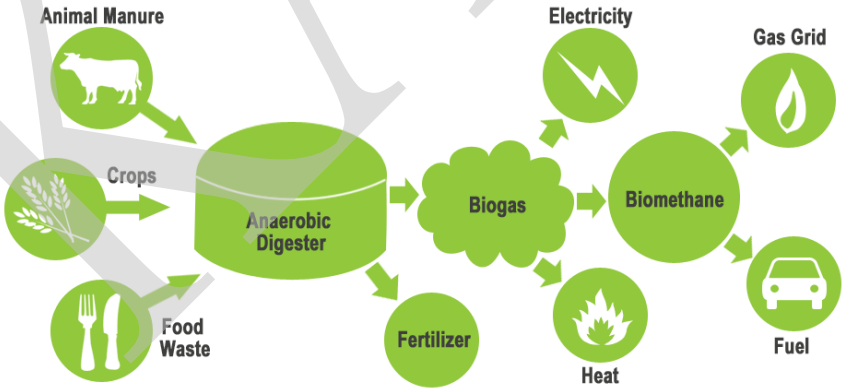
Biogas is produced either as

- as landfill gas (LFG), which is produced by the breakdown of biodegradable waste inside a landfill due to chemical reactions and microbes, or
- as digested gas, produced inside an anaerobic digester.

Projects such NANOCLEAN are nowadays developing new ways to produce biogas more efficiently, using iron oxide nanoparticles in the processes of organic waste treatment. This process can triple the production of biogas[5].

Biogas plants

A *biogas plant* is the name often given to an anaerobic digester that treats farm wastes or energy crops. It can be produced using anaerobic digesters (air-tight tanks with different configurations). These plants can be fed with energy crops such as maize silage or biodegradable wastes including sewage sludge and food waste. During the process, the microorganisms transform biomass waste into biogas (mainly methane and carbon dioxide) and digestate.



Ethanol

Ethanol fuel is ethyl alcohol, the same type of alcohol found in alcoholic beverages, used as fuel. It is most often used as a motor fuel, mainly as a biofuel additive for gasoline. The first

production car running entirely on ethanol was the Fiat 147, introduced in 1978 in Brazil by Fiat. Ethanol is commonly made from biomass such as corn or sugarcane. World ethanol production for transport fuel tripled between 2000 and 2007 from 17×10^9 liters (4.5×10^9 U.S. gal; 3.7×10^9 imp gal) to more than 52×10^9 liters (1.4×10^{10} U.S. gal; 1.1×10^{10} imp gal). From 2007 to 2008, the share of ethanol in global gasoline type fuel use increased from 3.7% to 5.4%.[1] In 2011 worldwide ethanol fuel production reached 8.46×10^{10} liters (2.23×10^{10} U.S. gal; 1.86×10^{10} imp gal) with the United States of America and Brazil being the top producers, accounting for 62.2% and 25% of global production, respectively.[2] US ethanol production reached 57.54×10^9 liters (1.520×10^{10} U.S. gal; 1.266×10^{10} imp gal) in 2017-04.[3]

Ethanol fuel has a "gasoline gallon equivalency" (GGE) value of 1.5, i.e. to replace the energy of 1 volume of gasoline, 1.5 times the volume of ethanol is needed.

Chemistry

During ethanol fermentation, glucose and other sugars in the corn (or sugarcane or other crops) are converted into ethanol and carbon dioxide.

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 + \text{heat}$$

Ethanol fermentation is not 100% selective with other side products such as acetic acid, glycols and many other products produced. They are mostly removed during ethanol purification. Fermentation takes place in an aqueous solution. The resulting solution has an ethanol content of around 15%. Ethanol is subsequently isolated and purified by a combination of adsorption and distillation.

During combustion, ethanol reacts with oxygen to produce carbon dioxide, water, and heat:

$$C_2H_5OH + 3\ O_2 \rightarrow 2\ CO_2 + 3\ H_2O + \text{heat}$$

Starch and cellulose molecules are strings of glucose molecules. It is also possible to generate ethanol out of cellulosic materials. That, however, requires a pretreatment that splits the cellulose into glycose molecules and other sugars that subsequently can be fermented. The resulting product is called cellulosic ethanol, indicating its source.

Ethanol may also be produced industrially from ethylene by hydration of the double bond in the presence of catalysts and high temperature.

$C_2H_4 + H_2O \rightarrow C_2H_5OH$

By far the largest fraction of the global ethanol production, however, is produced by fermentation.

production

Although there are various ways on how ethanol fuel can be produced, we hereby describe the most common way of producing ethanol: via fermentation.

The basic steps for large-scale production of ethanol are: microbial (yeast) fermentation of sugars, distillation, dehydration (requirements vary, see Ethanol fuel mixtures, below), and denaturing (optional). Prior to fermentation, some crops require saccharification or hydrolysis of carbohydrates such as cellulose and starch into sugars. Saccharification of cellulose is called cellulolysis (see cellulosic ethanol). Enzymes are used to convert starch into sugar.[24]

Fermentation

Ethanol is produced by microbial fermentation of the sugar. Microbial fermentation currently only works directly with sugars. Two major components of plants, starch and cellulose, are both made of sugars—and can, in principle, be converted to sugars for fermentation. Currently, only the sugar (e.g., sugar cane) and starch (e.g., corn) portions can be economically converted. There is much activity in the area of cellulosic ethanol, where the cellulose part of a plant is broken down to sugars and subsequently converted to ethanol.

Distillation

For the ethanol to be usable as a fuel, the yeast solids and the majority of the water must be removed. After fermentation, the mash is heated so that the ethanol evaporates.[25] This process, known as distillation, separates the ethanol, but its purity is limited to 95–96% due to the formation of a low-boiling water-ethanol azeotrope with maximum (95.6% m/m (96.5% v/v) ethanol and 4.4% m/m (3.5% v/v) water). This mixture is called hydrous ethanol and can be used

as a fuel alone, but unlike anhydrous ethanol, hydrous ethanol is not miscible in all ratios with gasoline, so the water fraction is typically removed in further treatment to burn in combination with gasoline in gasoline engines.[26]

Dehydration[edit]

There are basically three dehydration processes to remove the water from an azeotropic ethanol/water mixture. The first process, used in many early fuel ethanol plants, is called azeotropic distillation and consists of adding benzene or cyclohexane to the mixture. When these components are added to the mixture, it forms a heterogeneous azeotropic mixture in vapor–liquid-liquid equilibrium, which when distilled produces anhydrous ethanol in the column bottom, and a vapor mixture of water, ethanol, and cyclohexane/benzene.

When condensed, this becomes a two-phase liquid mixture. The heavier phase, poor in the entrainer (benzene or cyclohexane), is stripped of the entrainer and recycled to the feed—while the lighter phase, with condensate from the stripping, is recycled to the second column. Another early method, called extractive distillation, consists of adding a ternary component that increases ethanol's relative volatility. When the ternary mixture is distilled, it produces anhydrous ethanol on the top stream of the column.

With increasing attention being paid to saving energy, many methods have been proposed that avoid distillation altogether for dehydration. Of these methods, a third method has emerged and has been adopted by the majority of modern ethanol plants. This new process uses molecular sieves to remove water from fuel ethanol. In this process, ethanol vapor under pressure passes through a bed of molecular sieve beads. The bead's pores are sized to allow adsorption of water while excluding ethanol. After a period of time, the bed is regenerated under vacuum or in the flow of inert atmosphere (e.g. N_2) to remove the adsorbed water. Two beds are often used so that one is available to adsorb water while the other is being regenerated. This dehydration technology can account for energy saving of 3,000 btus/gallon (840 kJ/L) compared to earlier azeotropic distillation.[27]

Post-production water issues[edit]

Ethanol is hygroscopic, meaning it absorbs water vapor directly from the atmosphere. Because absorbed water dilutes the fuel value of the ethanol and may cause phase separation of ethanol-gasoline blends (which causes engine stall), containers of ethanol fuels must be kept tightly sealed. This high miscibility with water means that ethanol cannot be efficiently shipped through modern pipelines, like liquid hydrocarbons, over long distances.[28]

The fraction of water that an ethanol-gasoline fuel can contain without phase separation increases with the percentage of ethanol.[29] For example, E30 can have up to about 2% water. If there is more than about 71% ethanol, the remainder can be any proportion of water or gasoline and phase separation does not occur. The fuel mileage declines with increased water content. The increased solubility of water with higher ethanol content permits E30 and hydrated ethanol to be put in the same tank since any combination of them always results in a single phase. Somewhat less water is tolerated at lower temperatures. For E10 it is about 0.5% v/v at 70 F and decreases to about 0.23% v/v at −30 F.[30]

Consumer production systems[edit]

While biodiesel production systems have been marketed to home and business users for many years, commercialized ethanol production systems designed for end-consumer use have lagged in the marketplace. In 2008, two different companies announced home-scale ethanol production systems. The AFS125 Advanced Fuel System[31] from Allard Research and Development is capable of producing both ethanol and biodiesel in one machine, while the E-100 MicroFueler[32] from E-Fuel Corporation is dedicated to ethanol only.

Butanol

Butanol (also called **butyl alcohol**) is a four-**carbon** alcohol with a formula of C_4H_9OH, which occurs in five isomeric structures, from a straight-chain primary alcohol to a branched-chain tertiary alcohol;[1] all are a butyl or isobutyl group linked to a hydroxyl group (sometimes represented as **BuOH**, ***n*-BuOH**, and ***i*-BuOH**). These are *n*-butanol, 2 stereoisomers of 2-butanol, *tert*-butanol, and isobutanol. Butanol is primarily used as a solvent, as an

intermediate in chemical synthesis, and as a fuel. It is sometimes also called **biobutanol** when produced biologically.

Isomers[edit]

The unmodified term *butanol* usually refers to the straight chain isomer with the alcohol functional group at the terminal carbon, which is also known as *n*-butanol or 1-butanol. The straight chain isomer with the alcohol at an internal carbon is *sec*-butanol or 2-butanol. The branched isomer with the alcohol at a terminal carbon is isobutanol or 2-methyl-1-propanol, and the branched isomer with the alcohol at the internal carbon is *tert*-butanol or 2-methyl-2-propanol.

The butanol isomers have different melting and boiling points. *n*-butanol and isobutanol have limited solubility, *sec*-butanol has substantially greater solubility, while *tert*-butanol is fully miscible with water above tert-butanol's melting point. The hydroxyl group makes the molecule polar, promoting solubility in water, while the longer hydrocarbon chain mitigates the polarity and reduces solubility. The shorter chain molecules of methanol, ethanol, propanol, and tert-butanol are fully miscible with water, while n-butanol is only moderately soluble because of the diminishing polarity in the longer hydrocarbon group.

Biobutanol

Butanol is considered as a potential biofuel (butanol fuel). Butanol at 85 percent strength can be used in cars designed for gasoline (petrol) without any change to the engine (unlike 85% ethanol), and it contains more energy for a given volume than ethanol and almost as much as gasoline, and a vehicle using butanol would return fuel consumption more comparable to gasoline than ethanol. Butanol can also be added to diesel fuel to reduce soot emissions

Hydrogen

Biodiesel

Biodiesel

Biodiesel is the most common biofuel in Europe. It is produced from oils or fats using transesterification and is a liquid similar in composition to fossil/mineral diesel.

Chemically, it consists mostly of fatty acid methyl (or ethyl) esters (FAMEs). Feedstocks for biodiesel include animal fats, vegetable oils, soy, rapeseed, jatropha, mahua, mustard, flax, sunflower, palm oil, hemp, field pennycress, *Pongamia pinnata* and algae. Pure biodiesel (B100, also known as "neat" biodiesel) currently reduces emissions with up to 60% compared to diesel Second generation B100.[23]

Biodiesel can be used in any diesel engine when mixed with mineral diesel. In some countries, manufacturers cover their diesel engines under warranty for B100 use, although Volkswagen of Germany, for example, asks drivers to check by telephone with the VW environmental services department before switching to B100. B100 may become more viscousat lower temperatures, depending on the feedstock used. In most cases, biodiesel is compatible with diesel engines from 1994 onwards, which use 'Viton' (by DuPont) synthetic rubber in their mechanical fuel injection systems. Note however, that no vehicles are certified for using pure biodiesel before 2014, as there was no emission control protocol available for biodiesel before this date.

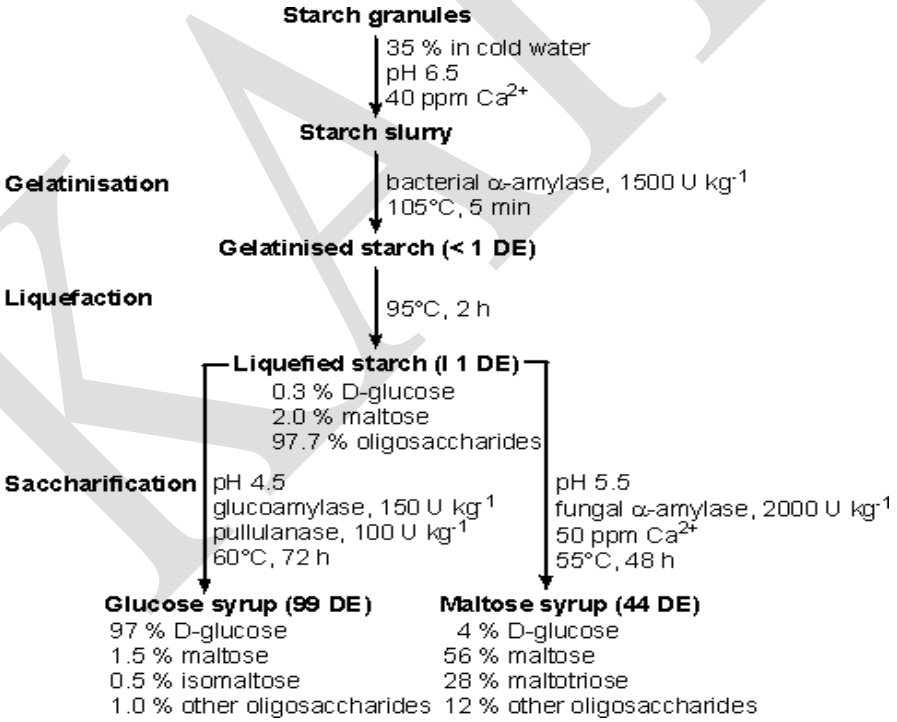
Electronically controlled 'common rail' and 'unit injector' type systems from the late 1990s onwards may only use biodiesel blended with conventional diesel fuel. These engines have finely metered and atomized multiple-stage injection systems that are very sensitive to the viscosity of the fuel. Many current-generation diesel engines are made so that they can run on B100 without altering the engine itself, although this depends on the fuel rail design. Since biodiesel is an effective solvent and cleans residues deposited by mineral diesel, engine filters may need to be replaced more often, as the biofuel dissolves old deposits in the fuel tank and pipes. It also effectively cleans the engine combustion chamber of carbon deposits, helping to maintain efficiency. In many European countries, a 5% biodiesel blend is widely used and is available at thousands of gas stations.[24][25] Biodiesel is also an oxygenated fuel, meaning it contains a reduced amount of carbon and higher hydrogen and oxygen content than fossil diesel. This improves the combustion of biodiesel and reduces the particulate emissions from unburnt carbon. However, using pure biodiesel may increase NO_x-emissions[26]

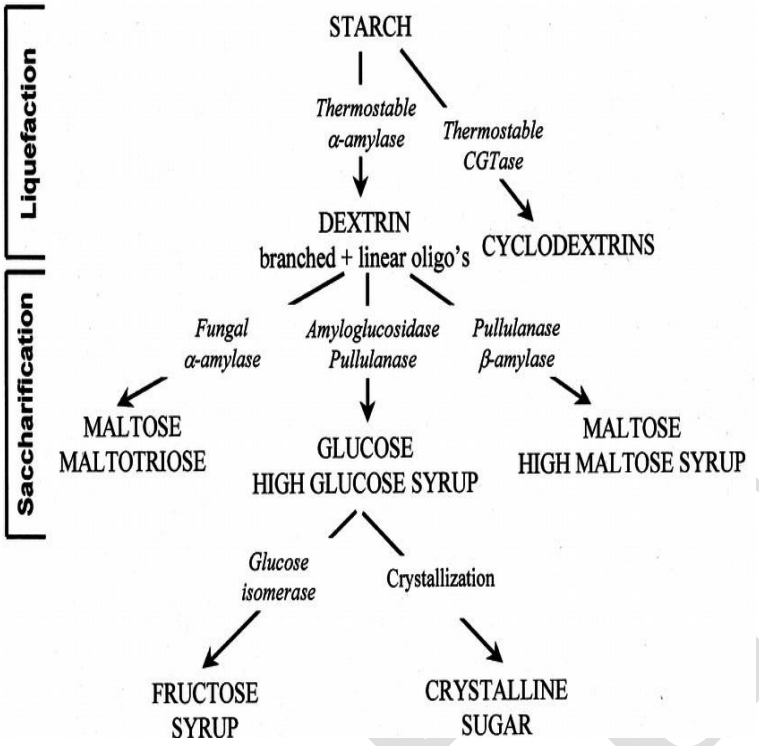
Biodiesel is also safe to handle and transport because it is non-toxic and biodegradable, and has a high flash point of about 300 °F (148 °C) compared to petroleum diesel fuel, which has a flash point of 125 °F (52 °C).[27]

In the USA, more than 80% of commercial trucks and city buses run on diesel. The emerging US biodiesel market is estimated to have grown 200% from 2004 to 2005. "By the end of 2006 biodiesel production was estimated to increase fourfold [from 2004] to more than" 1 billion US gallons (3,800,000 m^3).[28]

In France, biodiesel is incorporated at a rate of 8% in the fuel used by all French diesel vehicles.[29] Avril Group produces under the brand Diester, a fifth of 11 million tons of biodiesel consumed annually by the European Union.[30] It is the leading European producer of biodiesel.

Starch conversion process





Microbial polysaccharide

Polysaccharides:

The microorganisms can produce large amounts of polysaccharides in the presence of surplus carbon source. Some of these polysaccharides (e.g. glycogen) serve as storage compounds. The polysaccharides excreted by the cells, referred to as exopolysaccharides, are of commercial importance. The exopolysaccharides may be found in association with the cells or may remain in the medium.

The microbial polysaccharides may be neutral (e.g. dextran, scleroglucan) or acidic (xanthan, gellan) in nature. Acidic polysaccharides possessing ionized groups such as carboxyl, which can function as polyelectrolytes, are commercially more important.

Applications of Microbial Polysaccharides:

Microbial polysaccharides have immense commercial importance. They are employed in the stabilization of foods, and production of several industrial and pharmaceutical compounds. The commercial value of a polysaccharide is based on its ability to modify the flow characteristics of solutions (technically known as rheology). Polysaccharides can increase the viscosity and, are therefore useful as thickening and gelling agents.

Microbial polysaccharides are of great importance in oil industry. By conventional methods, only 50% of the oil can be extracted. And the rest is either trapped in the rock or too viscous to be pumped out. It is now possible to recover such oils also by a technique called microbial enhanced oil recovery (MEOR). This can be done by injecting surfactants and viscosity decreasing biological agents (i.e. the microbial polysaccharides e.g. xanthan and emulsan).

Production of Microbial Polysaccharides:

The synthesis of polysaccharides favourably occurs in the excess supply of carbon substrate in the growth medium while limiting nitrogen supply. A carbon/nitrogen ratio of around 10: 1 is considered to be favourable for optimal polysaccharide synthesis. The production process is mostly carried out by batch culture fermentation.

By manipulating the nutrient supply, differential synthesis of polysaccharides can be achieved. By limiting nitrogen supply in the medium, mostly neutral polysaccharides are produced. When metal ions are limited, acidic polysaccharides are mainly synthesized. Molecular oxygen supply of around 90% saturation is ideal for good growth and polysaccharide synthesis.

Biosynthesis of polysaccharides:

Microorganisms are capable of producing a large number of polysaccharides. The pathways for their biosynthesis are comparable to the processes that occur for the formation bacterial cell wall. It is estimated that there are well over 100 enzymatic reactions, directly or indirectly involved in the synthesis of polysaccharides. Starting with glucose, appropriate sugars (by transforming glucose to others) are incorporated in the formation of polysaccharides.

Recovery of polysaccharides:

As the polysaccharide production increases, there occurs a marked increase in viscosity of the culture broth. The polysaccharides can be precipitated by salts, acids or organic solvents, and recovered by employing appropriate techniques.

Microbial polysaccharides versus plant polysaccharides:

There is a lot of competition between microbial and plant polysaccharides for industrial applications. Production of plant polysaccharides is relatively cheap, although it is uncontrolled and occurs for a short period in a year. In contrast, production of microbial polysaccharides is well controlled and can be continued throughout the year. However, fermentation processes for manufacture of cheap (from plant sources) polysaccharides is not advisable.

General Features of Microbial Polysaccharides:

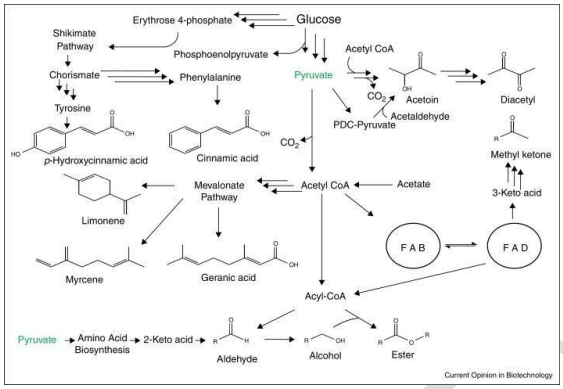
Of the several microbial polysaccharides, around 20 are of industrial importance. As already stated, the commercial value of a polysaccharide is mostly dependent on its rheological properties i.e. its ability to modify the flow characteristics of solutions. A selected list of commercially important polysaccharides, the microorganisms used for their production, and their applications are given in the Table 30.1. Some of the important features of individual microbial polysaccharides are briefly described hereunder.

Table 30.1 Commercially important microbial polysaccharides and their applications

Polysaccharide	*Producing organism(s)*	*Application(s)*
Xanthan	*Xanthomonas campestris*	As a food additive for stabilization, gelling and viscosity control, i.e. for the preparation of soft foods e.g. ice cream, cheese. In oil industry for enhanced oil recovery. In the preparation of toothpastes, and water based paints.
Dextran	*Leuconostoc mesenteroides,* *Acetobacter sp,* *Streptococcus mutans*	Blood plasma expander Used in the preventions of thrombosis, and in wound dressing (as adsorbent). In the laboratory for chromatographic and other techniques involved in purification. As a foodstuff.
Alginate	*Pseudomonas aeruginosa* *Azobacter vinelandi*	In food industry as thickening and gelling agent. Alginate beads are employed in immobilization of cells and enzymes. Used as ion-exchange agent.
Scleroglucan	*Sclerotium glucanicum* *S. rolfsii, S. delphinii*	Used for stabilizing latex paints, printing inks, and drilling muds.
Gellan	*Pseudomonas elodea*	In food industry as a thickner and solidifying agent.
Polluan	*Aureobasidium pollulans*	Being a biodegradable polysaccharide, it is used in food coating and packaging.
Curdlan	*Alcaligenes faecalis*	As a gelling agent in cooked foods (forms a strong gel above 55°C) Useful for immobilization of enzymes.
Emulsan	*Acinetobacter calcoaceticus* *Arthrobacter sp*	In oil industry for enhanced recovery. For cleaning of oil spills.

Microbial flavour fragrance

production



Newer antibiotic

Newer Antibiotics

- FDA Approved Antibiotics which are marketed internationally
  - Linezolid
  - Daptomycin
  - Tigecycline
  - Dalfopristin-quinupristin
  - Newer Fluroquinolones
  - Newer Beta-Lactam Antibiotics
  - Tetracycline
  - Macrolides

Daptomycin

• Active against Gram-positive bacteria

• Binds to bacterial membrane with rapid depolorization of membrane potential

• Proven activity in vitro against enterococci (including VRE) and Staphylococcus aureus (including MRSA)

• Binds avidly to pulmonary surfactant and thus, it cannot be used in pneumonia

Daptomycin-FDA indications

• Complicated skin and skin structure infections (cSSSI)

• Staphylococcus aureus bloodstream infections (bacteremia) including infections (bacteremia), including those with right-sided infection endocarditis .

Linezolid

Works on the initiation of protein synthesis; binds to 50S ribosome

• This disruption occurs earlier in the process than other protein synthesis inhibitors (chloramphenicol, clindamycin, aminoglycosides, and macrolides)

• Effective against gram positives: enterococcus (VRE), staphylococcus (MRSA)

• Some anaerobic activity

• No gram negative activity

• Excellent lung penetration Antimicrobial Agents Chemotherapy Excellent bioavailability

• Predictable thrombocytopenia typically >14 days

• Neuropathy when given longer time p (yp y eriods (typically >6-12 weeks) – Optic: usually reversible – Peripheral: may persist; painful sensory

• Mitochondrial toxicity: lactic acidosis

Tigecycline

• Active against many gram positives (including MRSA), gram negative bacilli, and anaerobes; no activity against Pseudomonas or Proteus

• Licensed against skin and soft tissue infections, intra-abdominal infections, and community-acqui db t i l i ired bacterial pneumonia caused by Streptococcus pneumoniae (penicillin-susceptible isolates), including cases with concurrent bacteremia, Haemophilus influenzae (beta-lactamase negative isolates), and Legionella pneumophila

Tigecycline-Adverse Effects

• Abdominal pain, diarrhea, nausea, vomiting

• Headache

• Serious: septic shock, pancreatitis, elevated liver ALT anaphylaxis

Ceftraoline

• Broad-spectrum oxyiminocephalosporin

• Activity against Gram-positive org gg anisms including MRSA and drugresistant S pneumoniae and a variety of Gram-negative organisms

• Antimicrobial activity correlates with T>MIC

Ceftaroline-Adverse Effects

• Diarrhea, nausea, uriticaria, rash

• Increased transaminases, hypokalemia, phlebitis, fever

• Anemia, neutropenia, thrombocytopenia

• Anaphylaxis, positive Direct Coomb's test

• Dizziness, seizures

• bradyarrythimias

Colisitin

• Mixture of cyclic polypeptides (polymixin A and B); polycationic with both hydrophilic and lipophilic moieties

• Disrupts cell membrane

• Active against gram negative bacteria esp Pseudomonas and Acinetobacter

• Previous concerns for neurotoxicity and nephrotoxicity

• Resistance currently is rare

Colistin resistance

• 265 isolates of Acinetobacter from 2 Korean hospitals

• Categorized into 3 subgroups: – Subgroup I (142 isolates [53.6%]) – Subg ([ ]) roup II (54 [20.4%]) – Subgroup III (18 [6.8%])

• Forty-eight isolates (18.1%) and 74 isolates (27.9%) were resistant to polymyxin B and colistin, respectively

Fidaxomicin

• Inhibits bacterial RNS polymerase resulting in the death of C. difficile

• FDA indications: treatment of C. difficile infections

Fidaxomicin-Adverse Effects

• Abdominal pain, nausea, vomiting

• Anemia, neutropenia

• Bowel obstruction

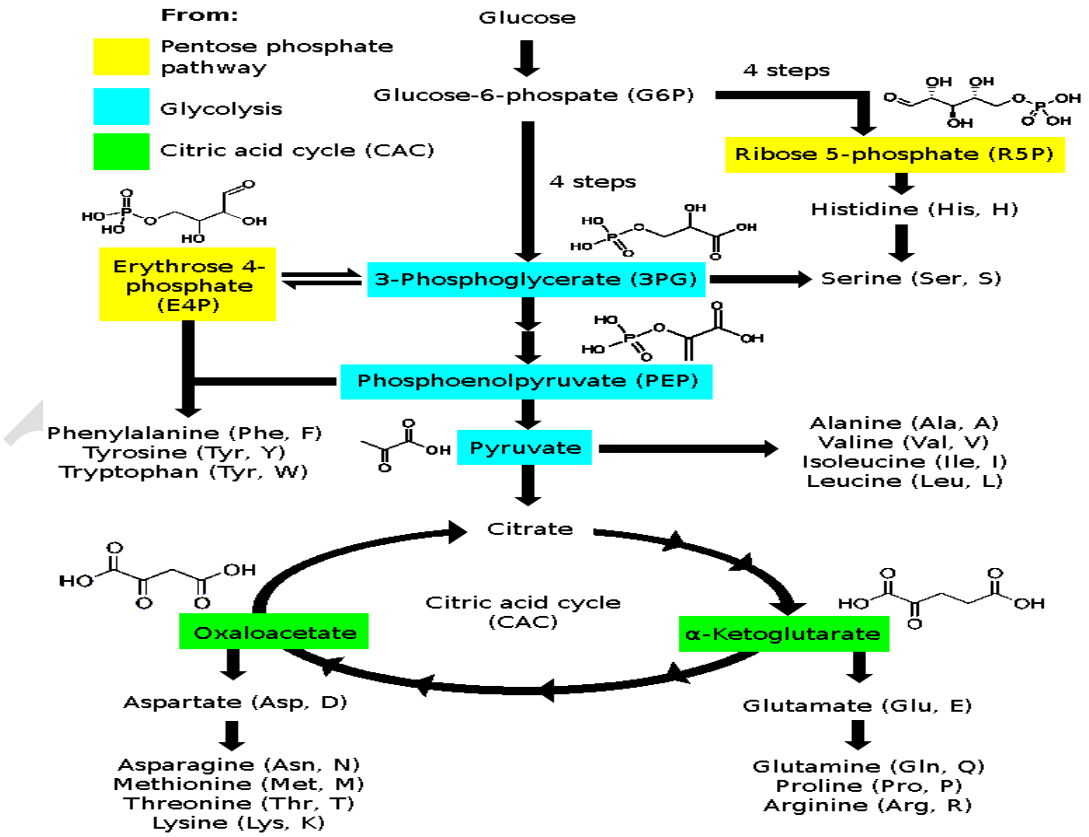
Anticancer agents

Classification of anticancer agents

Cytotoxic drugs

1. Alkylating agents
2. Platinum coordination: Cisplatin, Carboplatin, Oxaliplatin
3. Antimetabolites
4. Microtubule damaging agents: Vincristine, Vinblastine, Vinorelbine, Paclitaxel, Docetaxel
5. Topoisomerase-2 inhibitor: Etoposide
6. Topoisomerase-1 inhibitor: Topotecan, Irinotecan
7. Antibiotics: Actinomycin D, Doxorubicin, Daunorubicin, Epirubicin, Bleomycins, Mitomycin C.
8. Miscellaneous: Hydroxyurea, L-Asparaginase, Tretinoin, Arsenic trioxide

Amino acid synthesis pathway



Production of industrial products: Production of industrial chemicals, biochemicals and chemotherapeutic products. Propionic acid, butyric acid, 2-3 butanediol, gluconic acid, itaconic acid, Biofuels: Biogas, Ethanol, butanol, hydrogen, biodiesel, microbial electricity, starch

conversion processes; Microbial polysaccharides; Microbial insecticides; microbial flavours and fragrances, newer antibiotics, anti cancer agents, amino acids.

UNIT I-Possible questions

1. Describe chemotherapeutic products.
2. Write in detail about Production of industrial chemicals
3. Make short note on Propionic acid, butyric acid
4. Elaborate Over production of microbial metabolite
5. Make short note on 2-3 butanediol, gluconic acid, itaconic acid
6. Write in detail about Biofuels and Biogas
7. Make short note on Microbial polysaccharides
8. Describe in detail about newer antibiotics, anti cancer agents
9. Discuss Enzyme immobilization techniques in industrial processing

UNIT-V

Mass transfer determination and Metabolic engineering: Single stage CSTR; mass transfer in aerobic fermentation; resistances encountered; overall mass transfer co-efficient (Ka) determination, factors depending on scale up principle and different methods of scaling up. Metabolic engineering of antibiotic biosynthetic pathways.

SYLLABUS

Mass transfer determination and Metabolic engineering: Single stage CSTR; mass transfer in aerobic fermentation; resistances encountered; overall mass transfer co-efficient (Ka) determination, factors depending on scale up principle and different methods of scaling up. Metabolic engineering of antibiotic biosynthetic pathways.

CSTR

Continuous reactors (alternatively referred to as **flow reactors**) carry material as a flowing stream. Reactants are continuously fed into the reactor and emerge as continuous stream of product. Continuous reactors are used for a wide variety of chemical and biological processes within the food, chemical and pharmaceutical industries. A survey of the continuous reactor market will throw up a daunting variety of shapes and types of machine. Beneath this variation however lies a relatively small number of key design features which determine the capabilities of the reactor. When classifying continuous reactors, it can be more helpful to look at these design features rather than the whole system.

Batch versus continous

Reactors can be divided into two broad categories, batch reactors and continuous reactors. Batch reactors are stirred tanks sufficiently large to handle the full inventory of a complete batch cycle. In some cases, batch reactors may be operated in semi batch mode where one chemical is charged to the vessel and a second chemical is added slowly. Continuous reactors are generally smaller than batch reactors and handle the product as a flowing stream. Continuous reactors may

be designed as pipes with or without baffles or a series of interconnected stages. The advantages of the two options are considered below.

Benefits of batch reactors

Batch reactors are very versatile and are used for a variety for different unit operations (batch distillation, storage, crystallisation, liquid-liquid extraction etc.) in addition to chemical reactions.

- There is a large installed base of batch reactors within industry and their method of use is well established.
- Batch reactors are excellent at handling difficult materials like slurries or products with a tendency to foul.
- Batch reactors represent an effective and economic solution for many types of slow reactions.

Benefits of continuous reactors

- The rate of many chemical reactions is dependent on reactant concentration. Continuous reactors are generally able to cope with much higher reactant concentrations due to their superior heat transfer capacities. Plug flow reactors have the additional advantage of greater separation between reactants and products giving a better concentration profile.
- The small size of continuous reactors makes higher mixing rates possible.
- The output from a continuous reactor can be altered by varying the run time. This increases operating flexibility for manufacturers.

Heat transfer capacity

The rate of heat transfer within a reactor can be determined from the following relationship:

where:

q_x: the heat liberated or absorbed by the process (W)

U: the heat transfer coefficient of the heat exchanger ($W/(m^2K)$)

A: the heat transfer area (m^2)

T_p: process temperature (K)

T_j: jacket temperature (K)

From a reactor design perspective, heat transfer capacity is heavily influenced by channel size since this determines the heat transfer area per unit volume. Channel size can be categorised in various ways however in broadest terms, the categories are as follows:

Industrial batch reactors : 1 – 10 m^2/m^3 (depending on reactor capacity)

Laboratory batch reactors : 10 – 100 m^2/m^3 (depending on reactor capacity)

Continuous reactors (non micro) : 100 - 5,000 m^2/m^3 (depending on channel size)

Micro reactors : 5,000 - 50,000 m^2/m^3 (depending on channel size)

Small diameter channels have the advantage of high heat transfer capacity. Against this however they have lower flow capacity, higher pressure drop and an increased tendency to block. In many cases, the physical structure and fabrication techniques for micro reactors make cleaning and unblocking very difficult to achieve.

Temperature control

Temperature control is one of key functions of a chemical reactor. Poor temperature control can severely affect both yield and product quality. It can also lead to boiling or freezing within the reactor which may stop the reactor from working altogether. In extreme cases, poor temperature control can lead to severe over pressure which can be destructive on the equipment and potentially dangerous.

Single stage systems with high heating or cooling flux

In a batch reactor, good temperature control is achieved when the heat added or removed by the heat exchange surface (qx) equals the heat generated or absorbed by the process material (qp). For flowing reactors made up of tubes or plates, satisfying the relationship qx = qp does not deliver good temperature control since the rate of process heat liberation/absorption varies at different points within the reactor. Controlling the outlet temperature does not prevent hot/cold spots within the reactor. Hot or cold spots caused by exothermic or endothermic activity can be

eliminated by relocating the temperature sensor (T) to the point where the hot/cold spots exists. This however leads to overheating or overcooling downstream of the temperature sensor.

Hot/cold spots are created when the reactor is treated as a single stage for temperature control

Hot/cold spots can be eliminated by moving the temperature sensor. This however causes overcooling or overheating downstream of the temperature sensor.

Many different types of plate or tube reactors use simple feed back control of the product temperature. From a user's perspective, this approach is only suitable for processes where the effects of hot/cold spots do not compromise safety, quality or yield.

Single stage systems with low heating or cooling flux

Micro reactors can be tube or plates and have the key feature of small diameter flow channels (typically less than <1 mm). The significance of micro reactors is that the heat transfer area (A) per unit volume (of product) is very large. A large heat transfer area means that high values of qx can be achieved with low values of Tp – Tj. The low value of Tp – Tj limits the extent of over cooling that can occur. Thus the product temperature can be controlled by regulating the temperature of the heat transfer fluid (or the product).

Overheating/overcooling is prevented by the limited temperature difference between the product and heat transfer fluid.

The feedback signal for controlling the process temperature can be the product temperature or the heat transfer fluid temperature. It is often more practical to control the temperature of the heat transfer fluid.

Although micro reactors are efficient heat transfer devices, the narrow channels can result in high pressure drops, limited flow capacity and a tendency to block. They are also often fabricated in a manner which makes cleaning and dismantling difficult or impossible.

Multistage systems with high heating or cooling flux

Conditions within a continuous reactor change as the product passes along the flow channel. In an ideal reactor the design of the flow channel is optimised to cope with this change. In practice, this is achieved by breaking the reactor into a series of stages. Within each stage the ideal heat

transfer conditions can be achieved by varying the surface to volume ratio or the cooling/heating flux. Thus stages where process heat output is very high either use extreme heat transfer fluid temperatures or have high surface to volume ratios (or both). By tackling the problem as a series of stages, extreme cooling/heating conditions to be employed at the hot/cold spots without suffering overheating or overcooling elsewhere. The significance of this is that larger flow channels can be used. Larger flow channels are generally desirable as they permit higher rate, lower pressure drop and a reduced tendency to block.

Mass transfer in aerobic fermentation

Mass transfer is the net movement of mass from one location, usually meaning stream, phase, fraction or component, to another. Mass transfer occurs in many processes, such as absorption, evaporation, drying, precipitation, membrane filtration, and distillation. Mass transfer is used by different scientific disciplines for different processes and mechanisms. The phrase is commonly used in engineering for physical processes that involve diffusive and convective transport of chemical species within physical systems.

Some common examples of mass transfer processes are the evaporation of water from a pond to the atmosphere, the purification of blood in the kidneys and liver, and the distillation of alcohol. In industrial processes, mass transfer operations include separation of chemical components in distillation columns, absorbers such as scrubbers or stripping, adsorbers such as activated carbon beds, and liquid-liquid extraction. Mass transfer is often coupled to additional transport processes, for instance in industrial cooling towers. These towers couple heat transfer to mass transfer by allowing hot water to flow in contact with air. The water is cooled by expelling some of its content in the form of water vapor.

Mass Transfer

Mass Transfer

- Correlations for convection mass transfer coefficients associated with evaporation or sublimation from liquid or solid surfaces in external flow may be inferred from the corresponding heat transfer correlations for an isothermal surface by invoking the heat-and-mass transfer analogy:

$$Nu \rightarrow Sh$$
$$\Pr \rightarrow Sc$$

- Example: Flat Plate in Parallel Flow
  - Laminar Flow:

$$Sh_x \equiv \frac{h_m x}{D_{AB}} = 0.332\ \mathrm{Re}_x^{1/2} Sc^{1/3}$$
$$\overline{Sh}_L \equiv \frac{\overline{h}_m L}{D_{AB}} = 0.664\ \mathrm{Re}_L^{1/2} Sc^{1/3}$$

  - Laminar-to-Turbulent Flow:

$$\overline{Sh}_L = (0.037\ \mathrm{Re}_L^{4/5} - A) Sc^{1/3}$$
$$A = 0.037 \mathrm{Re}_{x,c}^{4/5} - 0.664\ \mathrm{Re}_{x,c}^{1/2}$$

  - Turbulent Flow:

$$\overline{Sh}_L = 0.037\ \mathrm{Re}_L^{4/5} Sc^{1/3}$$

The general form for the mass transfer is:

$$N_A = K_C' (C_{A_1} - C_{A_2}) \qquad \ldots 121$$

All these individual mass transfer coefficients are related to each other.
For gases:

$$N_A = K_C' (C_{A_1} - C_{A_2}) = K_G' (P_{A_1} - P_{A_2}) = K_y' (y_{A_1} - y_{A_2}) \qquad \ldots 122$$

So

$$K_C' = \frac{K_G'}{RT} = \frac{K_y'}{C_T}$$

Home work: find the relations for the liquid phase.
2- For uni - molecular mass transfer
A) For gases:

$$N_A = \frac{-(D_{AB} + E_D)}{R * T} * \frac{dP_A}{dZ} + \frac{P_A}{P_T} * (N_A + N_B) \qquad \ldots 114$$

For uni – molar transfer: $(N_B = 0)$
Then equation 114 will be:

$$N_A = \frac{-(D_{AB} + E_D)}{R * T} * \frac{dP_A}{dZ} + \frac{P_A}{P_T} * (N_A) \qquad \ldots 123$$

By integrating equation 123 from P_{A_1} at Z_1 to P_{A_2} and Z_2 then

$$N_A = \frac{(D_{AB} + E_D) * P_T}{R * T * P_{BLM}} * \frac{(P_{A_1} - P_{A_2})}{(Z_2 - Z_1)} \qquad \ldots 124$$

Similarly as done in section 1:

$$N_A = K_G (P_{A_1} - P_{A_2}) = K_y (y_{A_1} - y_{A_2}) \qquad \ldots 125$$

B) For Liquids:
Similar to what done for gases, the rate of mass transfer is:

$$N_A = K_L (C_{A_1} - C_{A_2}) = K_x (X_{A_1} - X_{A_2}) \qquad \ldots 126$$

The general form for the mass transfer is:

$$N_A = K_C (C_{A_1} - C_{A_2}) \qquad \ldots 127$$

Home work: find the relations for the gas phase and liquid phase. Also write the units of each coefficient.

Mass transfer coefficient (ka) determination

Oxygen acts a limiting nutrient for an aerobically growing culture in the bioreactor. Even in a reactor having non limiting availability of normal Carbon, Nitrogen and other sources, the limiting or no availability to aerobically growing culture may lead to death of the microbial population in no time. Therefore the air (oxygen) has to be adequately supplied either by sparger or in head space to enhance the dissolved oxygen so that microorganisms are able to breathe through the pores. It is important to note that the capacity of water to retain oxygen is rather limited. Under the normal condition of 25º C and 1 Atmosphere pressure, the dissolved oxygen will be 8 mg/L. Air has to be continuously purged and agitated so as to enhance the depleting oxygen in the bioreactor. For adequately aerated cultivation, the supply of oxygen has to be greater than the demand of the culture to maintain the healthy growth & metabolite production by the microorganisms at any point of time. Assume that the bioreactor is in well mixed condition and dissolved oxygen concentration is constant through-out the reactor, the following equation will hold true.

Rate of dissolution of dissolved oxygen (dC_L/dt) = {Supply of oxygen (K_La (C^*– C_L)} – {Demand by microorganism (rX)}

Where, '**K_La'** is termed as volumetric mass transfer coefficient or 'aeration efficiency', C^* saturation concentration of dissolved oxygen, C_L is the dissolved oxygen concentration in the bulk of the fermentation broth, 'r' is respiratory rate constant and 'X' is available biomass (g/L).

This measurement technique for aeration efficiency is dependent on actively growing culture in bioreactor in which aeration is stopped and decrease in dissolved oxygen due to respiration of the cells is measured by a fast response Dissolved Oxygen probe to estimate the rate of oxygen uptake by the total available microbial population. Dissolved oxygen concentration is recorded before a critical level is reached. Thus when aeration is stopped

Upon resumption of aeration

Rate of dissolution of dissolved oxygen (dC_L/dt) = {Supply of oxygen (K_La (C^*– C_L)} – {Demand by microorganism (rX)}

Where, '**K_La'** is termed as volumetric mass transfer coefficient or 'aeration efficiency', C^* saturation concentration of dissolved oxygen, C_L is the dissolved oxygen concentration in the bulk of the fermentation broth, 'r' is respiratory rate constant and 'X' is available biomass (g/L).

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Upon resumption of aeration

Which can be rearranged to give C_L as follows:
Therefore a plot of C_L vs (dC_L / dt + rX) on arithmetic coordinates will give linear revlationship and from this the slope is (-1/K_La) & C^* is the intercept on the y axis. Therefore K_La and C^* can be determined.

Factors depending on scale up principle

- Definition
    - Scale up studies refers to the act of using results obtained from laboratory studies for designing a prototype and a pilot plant process; construction a pilot plant and using pilot plant data for designing and constructing a full scale plant or modifying an existing plant.

Why conduct scale up studies

- A pilot plant allows investigation of a product and process on an intermediate scale before large amounts of money are committed to full-scale production.
- It is usually not possible to predict the effects of a many-fold increase in scale.
- Scale up studies are studies carried out at the laboratory or even pilot plant scale fermentors to yield data that could be used to to extrapolate and build the large scale

industrial fermentors with sufficient confidence it will function properly with all its behaviours anticipated.

Importance of scale up studies

- More important during scale up exercises is that we are trying to build industrial size fermentor capable or close of producing the fermentation products as efficient as those produced in small scale fermentors.
- It must be appreciated as the size of fermentation increases during scale up various parameters measured might not show a predictable linear co-relationships. Certain parameters changes. Some remained constant. Some parameters need to be modified and adjusted during scale up studies. The objective is to try to get the same fermentation efficiency as obtained in small scale fermentors at the most economical values.

There are few crucial studies which will only be answered by carrying it out on the pilot plant such as:

- Determining the various operational parameters for optimized oxygen supply to the fermentation process.
- Selection of optimum operative modes of the fermentor
- Determining the changes in rheological properties and its effect on the fermentation process.
- Modeling and formulation of process controls
- Sensors and controls

Steps in scale up

- Define product economics based on projected market size and competitive selling and provide guidance for allowable manufacturing costs
- Conduct laboratory studies and scale-up planning at the same time

- Define key rate-controlling steps in the proposed process
- Conduct preliminary larger-than-laboratory studies with equipment to be used in rate-controlling step to aid in plant design
- Design and construct a pilot plant including provisions for process and environmental controls, cleaning and sanitizing systems, packaging and waste handling systems, and meeting regulatory agency requirements
- Evaluate pilot plant results (product and process) including process economics to make any corrections and a decision on whether or not to proceed with a full scale plant development

Scale up studies are studies carried out at the laboratory or even pilot plant scal fermentors to yield data that could be used to to extrapolate and bui;ld the large scale industrial fermentors with sufficient confidence it will function properly with all its behaviours anticipated. More important during scale up exercises you are trying to build industrial size fermentor capable or close of producing the fermentation products as efficient as those produced in small scale fermentors

INITIAL SCALE UP STUDIES

Most scale up studies are usually carried at different phases involving different scales of fermentors.Preliminary work are carried out at the level of petri dishes and small scale laboratory fermentors to establish whether the process is:

1 Technically viable, meaning it is possible to produce such fermentation process and the products on the small scale. Additional parameters not provided by petri dishes studies and for more confidence are obtained by carrying further studies using submerged liquid fermentation using various sizes laboratory scale fermentors and even a pilot plant fermentor.

There are a few rules of the thumb followed when doing scale up studies such as:

1 Similarity in the geometry and configuration of fermentors used in scaling up

2 A minimum of three or four stages of increment in the scaling up of the volume of fermentation studies. Each jump in scale should be by a magnitude or power increase and not an increase of a few litres capacity. Slight increase in the working volume would not yield significant data for scale up operation

It must be appreciated as the size of fermentation increases during scale up various parameters measured might not show a predictable linear co relationships. Certain parameters changes. Some remained constant. Some parameters need to be modified and adjusted during scale up studies. The objective is to try to get the same fermentation efficiency as obtained in small scale fermentors at the most economical values

Challenges in scale up in fermentation

Microbes are prolific sources of novel secondary metabolites with a range of biological activities that may ultimately find application as anti-infective, anti-cancer agents or other pharmaceutically useful compounds. Hence many drug discoverycompanies are engaged in isolation of novel bioactive metabolites from these microbial sources. Till date, numerous metabolitesfrom these sources have been identified and few of them are undergoing developmental process through pre-clinical or clinicalstages or have been launched in the market.

Many of such new secondary metabolites are produced using microbial fermentation process rather than chemical synthesis because the fermentations are economically competitive and produce biologically active isomers. To carry out further drug development work including pre-clinical and clinical trials, sufficient quantities of these compounds are needed. For this purpose, the processes are usually scaled up from flask to pilot scale fermenter level. The scale up process is very challenging as the conditions followed during shake flask or lab fermentation may not suit for high volume cultivation. The recovery of the product is also a difficult job and needs lots of efforts to arrive at the right process conditions. Also, at the initial stages of discovery, the microbial strains (wild type) generally have very low production yields of the desired novel metabolites when cultivated in flasks or laboratory fermenter. In order to make the scale up

process cost effective, the strains need to be improved to achieve several fold improvement in titers.

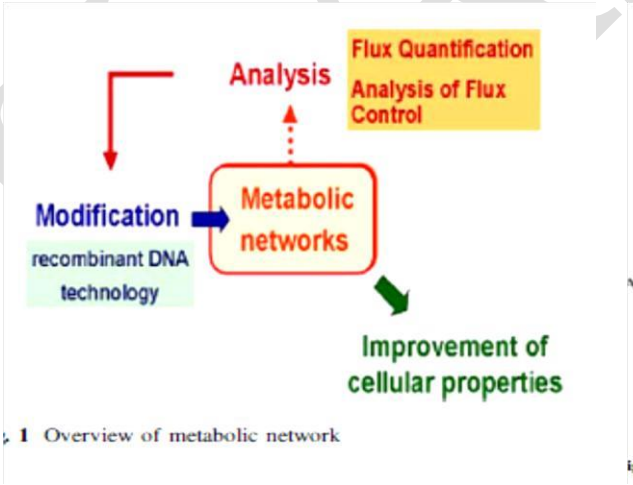
Metabolic engineering of antibiotic biosynthesis pathways

The term ‘‘metabolic engineering’’ is itself tells about the concept. It is made up of two different words metabolism and engineering. Metabolism is the entire set of enzyme catalyzed transformation of organic molecule that occurs in cell of an organism and engineering is to manipulating things to make it more fruitful. Metabolic engineering is generally referred to as the targeted and purposeful alteration of metabolic pathways found in an organism in order to better understand and utilize cellular pathways for chemical transformation, energy transduction, and supramolecular assembly. Number of microorganisms produces antibiotics that can inhibit or kill the other microbes. The production of some antibiotics is not sufficient in native host rather difficult to synthesize chemically and to extract in large amounts for commercialization. Metabolic engineering plays an increasingly significant role in the production of antibiotics and its precursors. Thus, we engineer biosynthetic pathways in desire host for the production of sufficient quantity of antibiotics. In this chapter, we illustrated bioengineering of different microbes using synthetic biology and metabolic engineering approaches for production and regulation of antibiotics.

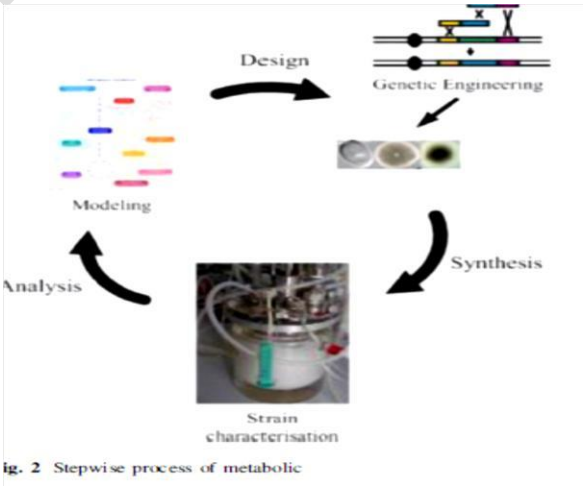
Need of Metabolic Engineering Microbes are used for the production of many valuable product in various industries such as pharmaceutical, agriculture, food, etc. due to wide spread use of microbes in industries. There is a need of metabolic engineering to achieve many goal such as (1) to increase product formation, (2) to speed up the process, (3) to save energy, (4) to stop production of byproduct, and (5) to develop strain with resistance to environmental stress. One way of grouping the different targets for metabolic engineering is as follows: extension of substrate range; improvements of productivity and yield; elimination of by-products; improvement of process performance; improvements of cellular properties; and extension of product range including heterologous protein production

Steps of Metabolic Engineering The process of metabolic engineering can be divided into three main steps. The first and important step in metabolic engineering is to study metabolic pathway

which we want to engineer. For that we need to develop the analysis of dynamic changes occurring in a cell. A detailed biochemical study of particular pathway is required. Radiotracer technique is generally used if a pathway is unknown for biochemical studies [13]. The second step is a use of computational approach. On the basis of available information about particular metabolic pathway in silico models has been designed to engineered particular metabolic pathway. For example a computational approach known as ‘‘Knock’’ was developed for identifying the gene deletions needed to maximize the production of a desired chemical. Likewise it is developed for the up and down regulation of gene expression needed to engineered metabolic pathway. The third steps are to applied computational suggested design at experimental level using different genetic engineering approaches (Fig. 2). Traditionally, mutagenesis programs have been used for strain and production improvements. In the last few years, the development of technology such as recombinant DNA technology and gene shuffling has provided new tools for approaching yields improvement by means of genetic manipulation of biosynthetic pathways.



1 Overview of metabolic network



ig. 2 Stepwise process of metabolic

Approaches for Metabolic Engineering

Several approaches have been used for the improvement of secondary metabolites produced by certain bacteria in metabolic engineering. Some of these approaches are (1)heterologous expression of entire gene clusters, (2) engineeringregulatory networks, (3) gene insertion and deletion,(4) redirecting metabolic pathway, (5) stimulation byprecursors, (6) genetic knockout of loci, and (7) quorumsensing.Heterologous Expression of Entire Gene ClusterAdvances in developing DNA manipulation tools and theimprovement in genome sequencing technologies haveproved fruitful for the isolation of many gene clustersinvolved in natural products biosynthesis. However, insome cases production titers of the encoded compound arelow, and there are no genetic tools optimized for metabolicengineering in the particular producer strain. The solutionof these problems is to transfer the whole metabolite pathway to new hosts where genetic tools are available orthey have been previously engineered for the heterologousproduction of bioactive compounds. Cloning and heterologousexpression of the complete gene cluster have been reported for the biosynthesis of secondary metabolites.Tetrangulol and tetrangomycin have been synthesized in S. rimosus NRRL 3016.Another important example is the genes encoding the polyhydroxybutyrate (PHB) biosynthetic pathway of Ralstonia eutropha is transferred into industrially convenientorganism Saccharomyces cerevisiae. Yeast strains were equipped in their cytoplasm with the phaABCRe operon containing genes phbA, phbB and phbC of the PHA biosynthetic pathway of R. eutropha, which encode b-ketothiolase, NADPH linked acetoacetyl-CoA reductase and PHA synthase, respectively. Resultant recombinant yeast was able to synthesize PHA and PHB in sufficient amount. This PHB is used to produce bioplastics which is biodegradable.

Engineering Regulatory Network

Genes for the biosynthesis of metabolic pathways are commonly grouped together in clusters on the chromosome including their pathway specific regulatory genes. Pathway specific regulators can have either positive (activators) or negative (repressors) effects on the expression of gene cluster elements. In microbes generally all the genes are not expressed at a time. Although microorganisms are

extremely good in presenting us with an amazing array of valuable products, they usually produce them only in amounts that they need for their own benefit; thus, they tend not to

overproduce their metabolites. But in industries we want continuous synthesis of products. For that we engineered microbes by up and down regulation approach.

Unit –V Possible Questions

1. Describe Mass transfer in aerobic fermentation detail.
2. Write in detail about Metabolic engineering
3. Make short note on Single stage CSTR
4. Elaborate overall mass transfer co-efficient (Ka) determination
5. Make short note on Metabolic engineering of antibiotic biosynthetic pathways.
6. Discuss scale up process and challenges in scale up
7. Make short note on techniques in biosynthesis of antibiotic

S.NO	**Questions**	**Option 1**	**Option 2**	**Option 3**	**Option 4**	**Answer**
Unit-4						
1	Basic chemicals are divided into_____	1	2	3	4	3
2	Which is an example of petrochemicals?	ammonia	ethanol	methanol	acetone	methanol
3	Which is used for making Nitric acid?	nitrogen	nitrate	Nitrite	ammonia	ammonia
4	What are the major components to make the fertilizer?	Nitrate and ammonia	Ammonia and nitric acid	Ammonia and nitrite	Sulphate and ammonia	Ammonia and nitric acid
5	Ammonia is also converted into_____	cyanide	nitrate	Nitrite	hydrogen cyanide	hydrogen cyanide
6	Which is used in the process to make methyl 2-methylpropenoate?	hydrogen cyanide	Methane	ethanol	acetic acid	Hydrogen cyanide
7	The hydrocarbons are first separated by this unit_____	Filtration	Evaporation	Distillation	Magnetism	Distillation
8	The main use for petrochemicals is in the manufacture of_____	polymers	monomer	Dimer	None of the above	polymers.
9	Which industry is a very important contributor to the wealth of a country?	Mining industry	chemical industry	oil industry	agricultural industry	chemical industry
10	The source of many C2 chemicals was_____	Fermentation	Dehydration	Oxidation	None of the above	Fermentation
11	Asparagine is a nutritional requirement for both_____	Ubnormal	normal and tumor cell	normal	tumor	normal and tumor cell.
12	_____is widely distributed among the microorganism, animals, and plant	L- Aspartic acid	Ammonia	L-asparaginase	aspargin	L-asparaginase
13	_____are also a good source of L-asparaginase.	Streptomyces	Actinomycetes	Pseudomonas fluoresces	None of the above	Actinomycetes
14	which is break down the starches to smaller carbohydrates for commercial use?	Amylase	Pepsin	Renin	HCL	Amylase
15	Propionic acid (PA) is widely used as an _____agent in food.	Antiviral	antifungal	anticancer	None of the above	antifungal

16	The conversion of natural raw materials into biochemicals by the process of_____	Dehydration	enzymatic reaction	degradation	None of the above	enzymatic reaction
17	Which is regulates adipokine production in human adipose tissue?	Acetic acid	citric acid	glutaric acid	propionic acid	propionic acid
18	Propionic acid is also useful as an intermediate in the production	Aspirin	polymers	vinegar	detergent	polymers
19	Which is a saturated short-chain fatty acid with a 4-carbon backbone?	Propionic acid	itaconic acid	butyric acid	gluconic acid	Butyric Acid
20	Butyric, acetic and propionic acids have___of the short-chain fatty acids (SCFAs) in the human colon	83%	68%	74%	55%	83%
21	The concentration of these acids in the intestinal lumen ranges from_____	60 mmol/kg to 150 mmol/kg	50 mmol/kg to 140 mmol/kg	40 mmol/kg to 170 mmol/kg	60 mmol/kg to 150 mmol/kg	60 mmol/kg to 150 mmol/kg
22	_____acid is prepared by fungal fermentation process	itaconic acid	butyric acid	gluconic acid	Propionic acid	gluconic acid
23	Gluconic acid solution contains _____structure	glucono delta lactone	lactone	glucono	glucagon	glucono delta lactone
24	Gluconic acid occurs naturally_____	Honey	Milk	Juice	All of the above	Honey
25	Gluconic acid helps cleaning up_____deposit	metal	Mineral	silicate	phosphate	Mineral
26	Gluconic acid is a strong chelating agent, especially in_____	acidic solution	Alkaline solution	Neutral solution	None of the above	Alkaline solution
27	Methylenesuccinic acid was otherwise known as_____	Itaconic acid	Gluconic acid	propionic acid	Butyric acid	Itaconic acid
28	Itaconic acid was obtained by the distillation of _____	Citric acid	acetic acid	glutaric acid	All of the above	Citric acid
29	Which is novel substitute monomer to replace petroleum-based chemicals?	butyric acid	propionic acid	butyric acid	Itaconic acid	Itaconic acid

30	_____is a process that takes inorganic carbon and converts it into organic compounds.	Nitrogen fixation	carbon fixation	oxygen fixation	All of the above	carbon fixation
31	If this process occurs in a living organism, it was called us _____	biological carbon fixation	chemical carbon fixation	physical carbon fixation	None of the above	biological carbon fixation
32	_____is naturally produced from the decomposition of organic waste	hydrogen	Ethanol	butanol	Biogas	Biogas
33	_____examples of organic matter that can produce biogas by anaerobic digestion.	Animal manure	Plastic	Soil	Water	Animal manure
34	_____gas has approximately 20 to 30 times the heat-trapping capabilities of carbon dioxide.	Hydrogen	Methane	Nitrogen	Oxygen	Methane
35	Ethanol is a renewable fuel made from various plant materials collectively known as _____	biomass	biogas	biofilm	All of the above	biomass
36	_____fuel can be used instead of gasoline in cars and other engines	ammonia	nitrogen	ethanol	methanol	ethanol
37	Butanol (also called butyl alcohol) is a four-carbon alcohol with a formula _____	C4H9OH	C3H8OH	C2H7OH	CHOH	C_4H_9OH
38	The unmodified term butanol usually refers to the_____isomer with the alcohol functional group	Branched chain	straight chain	Homo cyclic	Hetero cyclic	straight chain
39	Butanol is an alcohol with a _____ carbon structure and the molecular formula of C4H10O	4	3	2	1	4
40	Which is a colorless, odorless, tasteless, non-toxic, nonmetallic, highly combustible diatomic gas?	oxygen	hydrogen	nitrogen	carbon	hydrogen
41	Which gas has two different isotopes?	nitrogen	oxygen	carbon	oxygen	hydrogen

42	Deuterium has____neutron and tritium has ____	one,two	one, three	one,four	two, one	one,two
43	It acts like a_____when it is solid.	Silver	metal	Gold	iron	metal
44	Which is process used to convert these oils to Biodiesel	transesterificatio n	emulsification	purification	None of the above	transesterificatio n
45	_____is a technique to quantitatively assess the environmental impact and the energy requirements of a product	LCA	ALC	CLA	CAL	All of the above
46	100% biodiesel is referred to as_____	B20	B5	B100	B2	B100
47	Microbial fuel cell is otherwise known as	biofilm	biological fuel cell	Biogas	None of the above	biological fuel cell
48	bio-electrochemical system that drives an electric current by using_____	fungi	virus	Bacteria	All of the above	Bacteria
49	_____is the commonest storage carbohydrate in plants	maltose	starch	sucrose	lactose	starch
50	which is an example of microbial polysaccharides may be neutral?	dextran	xanthan	Gellan	None of the above	dextran
51	A carbon/nitrogen ratio of around _____ is considered to be favourable for optimal polysaccharide synthesis.	01:10	10:01	02:10	10:02	10:01
52	Microbial polysaccharides are _____ soluble biopolymers produced by many bacteria.	base	Acid	Water	All of the above	
53	Bacterial cellulose (BC) is produced by_____	Rhizobium	Acetobacter xylinum	Azotobacter pullulans	All of the above	Acetobacter xylinum
54	microbial polysaccharides are resistant to_____used as substitutes for starch in low-calorie foods	digestive enzymes	excretion enzymes	both	None of the above	digestive enzymes
55	Microbial insecticides are essentially	toxic	nontoxic	both	None of the above	non-toxic
56	_____and_____sources are an	animal	Plant	both	None of the	both

	important source of bioflavours				above	
57	_____are the drugs that prevent or inhibit the maturation and proliferation of neoplasms	antibacterial	antifungal	Anticancer drugs	antimalarial	Anticancer drugs
58	Each molecule contains a central _____ atom, called the α-carbon	Hydrogen	carbon (C)	oxygen	All of the above	carbon (C)
59	____are essential for the structure, function, and regulation of the body's tissues and organs.	Amino acids	starch	fatty acids	All of the above	Amino acids
60	Kidney was affected by_____tumour	Gynecological Cancer	Wilms tumour	Cervical Cancer	Bladder	Wilms tumour
Unit-1						
62	Biocatalytic potential of microorganisms have been employed for_____	wine	fruit juice	jam	All of the above	wine
63	The microbial enzymes act as_____to perform reactions in bio-processes	autotrophs	bio-catalysts	heterotrophs	None of the above	bio-catalysts
64	Traditional industrial microbiology was merged with_____to yield improved recombinant processes	molecular biology	microbiology	bioinformatics	immunology	molecular biology
65	_____involves transfer of DNA via cell-to-cell contact.	transformation	elongation	Conjugation	termination	Conjugation
66	_____involves uptake and expression of naked DNA by competent cells	elongation	Conjugation	transformation	termination	transformation
67	Primary metabolites are microbial products made during the_____of growth	exponential phase	stationary phase	lag phase	log phase	exponential phase
68	Metabolic engineering has been used in C. glutamicum to improve____ production	histidine	isoleusine	valine	L-lysine	L-lysine
69	Steroid hormones and their derivatives	cosmetic	therapeutic	industrial	All of the	therapeutic

	have been used for a wide range of _____purposes				above	
70	____are the most active hydroxylating microorganisms	Fungi	virus	bacteria	Actinomycetes	Fungi
71	actinomycetes are sources of antibiotics such as _____	amoxillin	cephalexin	streptomycin	Penicillin	streptomycin
72	steroids are_____alcohols	liquid	solid	both	None of the above	solid
73	_____are essential for lipid digestion and absorption and	Acetic acid	Hcl	bile acids	Citric acid	bile acids
74	cardiac aglycones are used for_____failure treatment	heart	lungs	liver	kidney	heart
75	bacterial metabolites mediate communication between the commensal microbiota and_____	Physiological system	immune system	Nerve system	All of the above	immune system
76	To determine whether butyrate is capable of promoting extrathymic Treg-cell generation _____	in vivo	in vitro	both	None of the above	in vivo
77	Which is primary metabolites?	quinine	penicillin	morphine	vitamin	vitamin
78	Which is essential in the normal growth, development, or reproduction of an organism?	Primary metabolites	secondary metabolites	tertiary metabolites	All of the above	Primary metabolites
79	Which is essential in important functions such as protection, competition, and species interactions?	Primary metabolites	secondary metabolites	tertiary metabolites	None of the above	secondary metabolites
80	secondary metabolic can_____different species in varying ways	benefit	affect	partially affect	partially benefit	affect
81	Monarch butterflies have evolved to be able to eat	milkweed	rotting fruit	pollen	tree sap	milkweed
82	Many secondary metabolites aid the plant in gaining essential nutrients_____	nitrogen	oxygen	hydrogen	carbon	nitrogen

83	Many plants utilize_____are high in nutrients and advantageous for human consumption	Primary metabolites	secondary metabolites	both	None of the above	secondary metabolites
84	Plant secondary metabolites can be divided into_____major groups	One	Two	three	Four	three
85	___are also found in some organisms such as algae, fungi, some bacteria, and certain species of aphids	Carotenoids	pheopigment	haemoglobin	hemocyanin	Carotenoids
86	Which is First Immobilized enzyme?	Calcium alginate	Aminoacylase	sodium alginate	calcium chloride	Aminoacylase
87	Xanthophylls are carotenoids with molecules contains_____	nitrogen	oxygen	hydrogen	carbon	oxygen
88	In plants_____can occur in roots, stems, leaves, flowers, and fruits	Carotenoids	Pheopigment	hemoerythrin	hemocyanin	Carotenoids
89	Enzymes are_____protein	structural	catalytic	transport	regulatory	catalytic
90	The enzyme is bound_____to an insoluble support (such as silica gel)	hydrogen bond	chemical	covalentely	All of the above	covalentely
91	which enzymes used in the cancer treatment?proteolytic enzymes	proteolytic enzymes	hydrolytic enzymes	lygase enzyme	oxidoreductases	proteolytic enzymes
92	_____are synthesized from DOXP precursors in plants and some bacteria.	5,6-epoxyxanthophylls	violaxanthin	Tetraterpenes	epoxylutein	Tetraterpenes
93	which is secrete the hydrolase enzyme?	lactbacillus jensenii	lactbacillus acidopihilus	lactbacillus bulgaricus	lactbacillus crispatus	lactbacillus acidopihilus
94	Glucose isomerase molecular weight_____	153000 daltons	163000 daltons	173000 daltins	183000 daltons	173000 daltins
95	when this Glucose isomerase enzyme denatured?	above pH 5.0	below pH 5.0	below pH 6.0	below pH 8.0	below pH 5.0
96	Metabolic engineering and pathway optimization with the aim to reduce_____	cost	productivity	both	None of the above	cost

97	How much of modern drugs are derived from an edible product	15%	25%	35%	45%	25%
98	which is disadvantages of immobilizd enzyme?	Reuse of enzyme	Continuous of use enzyme	minimum reaction time	Unstable after immobilizatio n	Unstable after immobilization
99	which food enzyme was accelerate the cheese ripening?	Lacto peroxidase	lipases	lysozyme	beta galactosidase	Lipases
100	Which enzyme is used in syrup production?	Pectinases	Urease	Glucose oxidase	Penicillinase	Pectinases
101	Which oil was used to produce bio diesel?	vegetable oil	fish oil	Liver oil	All of the above	vegetable oil
102	Which fungus was produce the pectinase enzyme?	Aspergillus fumigatus	Aspergillus niger	Aspergillus flavus	Aspergillus oryzae	Aspergillus niger
103	In detergent industry which enzyme was used to remove dirt from clothes?	Lipase enzyme	protease	carbohydrase	isomerasse	Lipase enzyme
104	Which polymer are inert and water holding capacity?	collagen	alginate	chitosan	gelatin	alginate
105	Nitrous oxide generated from _____	arginine	aspartic acid	asparagine	lysine	arginine
106	Which is the simple and oldest method of enzyme immobilization?	covalent bonding	absorption	entrapment	encapsulation	absorption
107	_____is produced from a selected strain of Streptomyces murinus.	proteolytic enzymes	hydrolytic enzymes	glucose isomerase	lygase enzyme	glucose isomerase
108	Glucose isomerase is used in the_____to produce high-fructose corn syrup	Food industry	chemical industry	agricultural industry	None of the above	food industry
109	____are now replacing other improving additives in breadmaking, indirectly affecting bread flavor.	bacteria	fungi	Enzyme	actinomycete s	Enzyme
110	What is the meaning of no growth of organisms after maximum growth?	lag phase	log phase	stationary phase	mobile phase	stationary phase
111	Each fermenter should be filled with	65-80%	75-80%	85-90%	80-95%	75-80%

	range between_____					
112	What is the diameter of column fermenter?	>3	<3	<1.5	>1.5	>3
113	How many number of baffle will there in a fermenter ?	5	7	10	4	4
114	Which enzymes is joints two molecules in catalytic reaction?	Ligase	lyases	hydrolases	isomerases	ligases
115	Tower fermentors are used for	continuous penicillin production	continuous beer production	batch production of beer	production of enzymes	continuous beer production
116	In an airlift bioreactor, the air sparging region is called	downcomer	disengagement zone	air riser	none of these	none of these
117	enzymes are present in____of all cells	mitochondria	cytoplasm	nucleus	ribosome	cytoplasm
118	Enzyme has_____strucutres	complex three dimensional	two dimensional	both	none of the above	complex three dimensional
119	enzymes catalyst requires specific reaction condition	temperature	pH	sovent	All of the above	All of the above
120	The function of a mechanical seal is to	prevent contaminants entering the reactor	prevent cells from leaving the reactor	both (a) and (b)	prevent air to enter	both (a) and (b)
121	The term organic means	Mineral	artificial product	carbon basis	nitrogen basis	carbon basis
Unit-2						
123	The first attempts at isolating substances from plants having similar properties to_____	Egg yolk	Egg albumin	both (a) and (b)	none of the above	Egg albumin
124	_____was the first crystalline protein obtained	ovalbumin	Egg yolk	both (a) and (b)	none of the above	ovalbumin
125	Purification techniques focus mainly on _____	density	Shape	size & charge	All of the above	size & charge
126	Building blocks of protein	amino acid	sucrose	fructose	lactose	amino acid

127	In protein purification which step is essential/	Filtration	Precipitation	Evaporation	magnetic	Precipitation
128	chromatography has become an essential tool for_____purification	carbohydrate	lipid	protein	All of the above	protein
129	Tannin acyl hydrolase also referred as_____	Tannin	tannase	tannease	None of the above	tannase
130	DSP means	digital signal processor	digital system Processor	downstream processing	digital systematic programme	downstream processing
131	Fed-batch reactors can also be used in the_____digestion of sewage sludge	Aerobic	Anaerobic	both	none of the above	Anaerobic
132	Example for renewable resources	Petroleum	Natural gas	coal	Sun rise	Sun rise
133	Example for Non renewable resources	Wind	water	nuclear fission	Sun rise	Coal
134	_____replaced by a renewable raw material	Petroleum	wind	biological fussion	water	Petroleum
135	BTAC means	Biological technique advisory committee	Biomass Technical Advisory Committee	Biotact	None of the above	Biomass Technical Advisory Committee
136	Which is not commodity products	organic fuels	chemicals	Effluents	materials	Effluents
137	_____gradually replace gasoline as well as diesel due to its high energy content	Butanol	ethanol	methanol	acetone	Butanol
138	butanol is a valuable_____compound for chemical synthesis	C4	C3	C2	All of the above	C4
139	An alternative source for n-butanol is anaerobic_____bacterial fermentation in conjunction	E.coli	Clostridium	cyanobacteria		Clostridium
140	In_____fermentation bacteria conjunction with acetone and a small	AB	BC	BA	AC	AB

	amount of ethanol					
141	AB fermentation with modernized technology for fermentation and_____ processing	upstream	downstream	both	none of the above	downstream
142	In fermentation_____can be regarded as the oldest biotechnology	carbohydrate to starch	starch to ethanol	starch to ethanol	All of the above	starch to ethanol
143	Butanol was an unnecessary by-product during the _____	Cyclone	War	Land slide	Earth quake	War
144	Basic chemicals and fuels were also produced cheaper from _____	petroleum oil	Natural products	fossil fuels	coal	petroleum oil
145	___used extensively for microalgae fermentation,having yielded high biomass growth and lipid productivity	Protein	starch	carbohydrate	lipid	glucose
146	Which is also produce other metabolites,such as astaxanthin, lutein?	Macroalgae	bacteria	fungi	microalgae	microalgae
147	Recently_____has been used as an alternative carbon source instead of glucose	Glycerol	glutaric acid	glutamine	All of the above	Glycerol
148	PUFAs means	Pacific Ultralight Flying Association	People United For Animals	Polyunsaturated fatty acids	Purdue University Financial Aid	Polyunsaturated fatty acids
149	____have many benefits,including the potential use of their lipid rich biomass in biodiesel production	Aplanochytrids	Schizochytrids	Thraustochytrids	Aurantiochytrids	Thraustochytrids
150	_____is a particularly important omega-3 fatty acid due to its health benefits in humans	ADH	HAD	DHA	DAH	DHA
151	_____have potent antioxidant activities and are therefore potentially beneficial to human health	Carotenoids	pheopigment	hemocyanin	hemoerythrin	Carotenoids
152	____are endogenous in humans and are an important component of eye retina	zeaxanthin	Carotenoids	Flavoin	Xanthophyll	zeaxanthin

153	_____is secrete enzymes, polysaccharides and carotenoids, squalene and co-enzymes	Thraustochytrids	Aplanochytrids	Schizochytrids	DAH	Thraustochytrids
154	A raw organic feed is converted into product by the action of microbes____	putrification	Fermentation	emulsification	none of the above	Fermentation
155	which is depends on concentration and morphology of organisms and carriers	Rheology	biology	entomology	embryology	Rheology
156	In fermentation techniques_____ requires gas sparging	Anaerobic	Aerobic	both	none of the above	Aerobic
157	How many types of fermenters used in industrial?	5	4	3	2	5
158	Diameter of the vessel in aerobic fermenter	5.7m	5.8m	5.9m	5.10m	5.8m
159	Most biotechnical products are produced by	Fermentation	putrification	emulsification	All of the above	Fermentation
160	_____biological catalysts and are produced as secondary metabolites of enzyme fermentation.	Hormones	pigments	Enzymes	nutrients	Enzymes
161	During the batch reacor process there is no	Input	output	both	antifoamig agent	both
162	In_____process, one substrate component is added in order to control the reaction rate by its concentration	batch	fed-batch	continuous processes	All of the above	fed-batch
163	most of the bacteria, require an organic compound as the_____source.	calcium	carbon	magnesium	silicate	carbon
164	organisms that use an organic carbon source and a chemical source of energy_____	Autotrophs	heterotrophs	chemoheterotrophs	All of the above	chemoheterotrophs
165	In aerobic processes____is a vital component.	oxygen	hydrogen	carbon	ammonia	oxygen
166	Required amounts of_____can be derived from water and organic	carbon	nitrite	hydrogen	oxygen	hydrogen

	substrates.					
167	Organisms that cannot use oxygen are called	aerobes	anaerobes	thermophilic	hemophilic	anaerobes
168	is an important in the bioreactors and by its efficient control can be increased.	Heat removal	Blend time	Aeration	Food concentration	Aeration
169	The rate of change of dissolved oxygen is about_____times faster than the cell mass	5	10	15	20	10
170	In the past, only the fermentation of extracellular enzymes, such as	Amylase	pectinase	hydrolase	urease	Amylase
171	The main advantages of the fed-batch operation are the possibilities to control ___by substrate feeding rate.	reaction rate and metabolic reaction reaction rate		metabolic reaction	none of the above	reaction rate and metabolic reactions
172	In fed-batch operation the limitations caused by oxygen transfer and cooling	oxygen transfer	cooling	oxygen transfer and cooling	none of the above	oxygen transfer and cooling
173	In fed-batch the growth rate can be controlled by the substrate concentration to avoid	Anabolite repression	catabolite repression	metabolite repression	none of the above	catabolite repression
174	____is a repression of the respiration on the enzyme synthesis level	catabolite repression	metabolite repression	Anabolite repression	none of the above	catabolite repression
175	catabolite repression occurs during the long-term exposure of the cell to the high	glycerol concentration	Ammonia concentration	glucose concentration	nitrate concentration	glucose concentration
176	If no reaction rate control is used, and the cells are grow in exponentially, limited by	heat	oxygen	both (a) and (b)	cool	both (a) and (b)
177	The biggest problem in the bioprocess, there is no suitable sensors for ____ measurements	software sensor	on-line	both (a) and (b)	reliable sensor	on-line
178	The important of the bioprocess to develop____	high-yield processes	reduced the work and energy	process with minimum number of steps	All of the above	All of the above

179	Which is product recovery from unit operations?	Biomass	crude extract	broth from bioprocess	All of the above	All of the above
180	The separation of solid particles, from a solution depends on	size	density	properties of liquid media	All of the above	All of the above
181	Separation and isolation procedures for lipids include	crystallization	urea fractionation	distillation,	All of the above	All of the above
182	The downstream process has direct contact with_____through the finished product	Owner	customers	retailer	customers	customers
Unit-3						
184	In biochemistry, Michaelis–Menten kinetics is one of the best-known models of _____	enzyme kinetics	enzymology	enzymes dynamics	All of the above	enzyme kinetics
185	_____an enzyme-catalyzed reaction	E+S−→k1[ES]−→k2E+P	E+P−→k1[ES]−→k2E+S	P+E−→k1[ES]−→k2E+S	E+P−→k2[ES]−→k1E+S	E+S−→k1[ES]−→k2E+P
186	The enzyme interacts with the substrate by binding to its_____	allosteric site	active site	negative allosteric site	inhibition site	active site
187	Enzymes are_____molecular weight proteins that act on a substrate	low	medium	high	None of the above	high
188	Which is not correct in the Michaelis-Menten model	enzyme-substrate interaction	antigen-antibody binding,	DNA-DNA hybridization	carbohydrate-protein interaction	carbohydrate-protein interaction
189	empirical equation used in the _____	enzymatic activity	microbial growth	enzyme kinetics	enzyme dynamics	microbial growth
190	In Michaelis-Menten Enzyme Kinetics Km means _____	enzyme's original concentration	rate constant	Michaelis constant	All of the above	Michaelis constant
191	In Michaelis-Menten Enzyme Kinetics Kcat means _____	turnover number	enzyme concentration	maximum rate	substrate concentration	turnover number
192	_____one of the fastest enzymes	glucoamylase	sucrase	Acetylcholinesterase	peptidase	Acetylcholinesterase

193	which is the chemistry of intermediate compounds?	Chemical kinetics	Chemical dynamics	Physical dynamics	Physical kinetics	Chemical kinetics
194	An example of enzymes that bind a single substrate and release multiple products are_____	protienase k	proteases	pepsin A	Prolyl endopeptidase	proteases
195	An enzyme E combines with substrate S to form an ES complex, with a rate_____	constant k-1	constant k-2	constant k1	constant k2	constant k1
196	The ES complex has two possible fates, can dissociate to E and S and other one _____	form product P	form product S	form product E	All of the above	form product P
197	Rate formation of ES	K1[S][E]	K1[E][S]	K1[E][P]	None of the above	K1[E][S]
198	Structure-based enzyme mechanism studies useful for understanding the mechanism used by an _____	stimulator	inhibitor	both	none of the above	inhibitor
199	To determine the_____is to perform Km determinations at several different inhibitor concentrations.	Ki	Km	Vmax	All of the above	Ki
200	The measured values of Km in the presence of the inhibitor are altered, and are called the _____	apparent Km	non-competitive inhibition	competitive inhibition	none of the above	apparent Km
201	Demonstration plots rate data for Michaelis-Menten enzyme kinetics for _____of inhibition.	five types	four types	three types	two types	four types
202	_____an inhibitor forms an inactive complex with the enzyme.	competitive inhibtion	non-competitive inhibition	mixed inhibition	self-inhibited	competitive inhibtion
203	_____an inhibitor forms an inactive complex with the enzyme-substrate complex	self-inhibition	competitive inhibtion	uncompetitive inhibition	All of the above	uncompetitive inhibition
204	In_____the inhibitor forms both types of inactive complexes.	competitive inhibtion	non-competitive inhibition	mixed inhibition	none of the above	mixed inhibition

205	In_____the substrate itself forms an inactive complex with the enzyme-substrate complex.	self-inhibited	competitive inhibtion	uncompetitive inhibition	none of the above	self-inhibited
206	_____is selected, the substrate concentration is the inhibitor concentration, so the slider is disabled.	uncompetitive inhibition	self-inhibition	competitive inhibtion	none of the above	self-inhibition
207	Enzymes are basically the proteins which speed up the rate of reactions in _____	living cells	non- living cells	plant cells	animal cells	living cells
208	catalysts are made by the cells in very small amounts which are not consumed during a_____	physical reaction	chemical reaction	biological reaction	All of the above	chemical reaction
209	Enzymes digest food substances in cells to release the energy required for _____	Respiration	Digestion	Circulation	All of the above	Respiration
210	Every enzyme shows maximum activity at an optimum	Temperature	pH	cooling	All of the above	pH
211	Activity is_____above or below the optimum pH	High	Medium	slow	none of the above	slow
212	A pH of about_____is the optimum	6	7	8	9	7
213	Every enzyme shows highest activity at a specific temperature which is called	Medium temperature	Low temperature	optimum temperature	All of the above	optimum temperature
214	These active sites are damaged or in other words their_____is changed by changing the pH	size	shape	density	none of the above	shape
215	_____Celsius rise in the temperature, the activity of enzyme doubles until the optimum temperature is reached	10 degree	9 degree	8 degree	7degree	10 degree
216	At temperatures higher than_____ Celsius enzymes are basically completely denatured	70 degrees	60 degrees	80 degrees	90 degrees	60 degrees
217	The activity of enzymes is affected by_____and_____	temperature	pH	both (a) and (b)	none of the above	both (a) and (b)

218	Intestinal enzymes have an optimum pH of about	6.5	7.5.	8.5	9.5	7.5.
219	____is also a factor in the stability of enzymes	pH	Temperature	cooling	All of the above	pH
220	animal enzymes rapidly become denatured at temperatures above	40°C	50°C	60°C	70°C	40°C
221	Storage of enzymes____at generally the most suitable	4°C	5°C	6°C	7°C	5°C
222	Initial studies of microbial surface colonization_____is used to determine specific growth rate	lag phase	exponential growth	stationary phase	decline phase	exponential growth
223	Bacterial growth is a_____process involving numerous anabolic and catabolic reactions	simple	complex	both	none of the above	complex
224	In a batch culture the growth of a single organism or a group of organisms, called a	consortium	individual	group of organisms	All of the above	consortium
225	The materials are often produced in large batches_____also called large-scale fermentations.	500,000 liters	up to 500,000 liters	upto to 6,00,000 liters	All of the above	up to 500,000 liters
226	The lag phase may also be due to low initial densities of organisms that result in dilution of	exoenzymes	co-enzymes	endoenzymes	All of the above	exoenzymes
227	—the most rapid growth possible under the conditions present in the batch system	lag phase	exponential growth	stationary phase	death phase	exponential growth
228	In stationary phase, growth is simply balanced by an equal number of cells	living	dying	slow growth	fast growth	dying
229	Growth on dead cells is called _____ metabolism	endogenous	exogenous	both (a) and (b)	None of the above	endogenous
230	_____phase, there may be individual cells that are metabolizing and dividing,	stationary phase	lag phase	exponential growth	death phase	death phase

	but more viable cells are loss.					
231	The vessel that is used as a growth container in continuous culture is called	Petridish	bioreactor	Conical flask	None of the above	bioreactor
232	The mass balance equation can be used to estimate the amount of _____	oxygen or nitrogen	nitrogen or hydrogen	oxygen or methane	All of the above	oxygen or nitrogen
233	This is useful for wastewater treatment for production of high value microbial products _____	proteins	antibiotics	nutrients	All of the above	antibiotics
234	In Anaerobic fermentation the absence of oxygen,organic substrates can be mineralized to____by fermentation	oxygen	hydrogen	carbon dioxide	nitrogen	carbon dioxide
235	Under anaerobic conditions, organic compounds are degraded by _____	consortium of microorganisms	fungi	endosymbiotic	ectosymbiotic	consortium of microorganisms
236	The final step of anaerobic degradation is	Neogenesis	methanogenesis	Parthanogenesis	none of the above	methanogenesis
237	Methanogenesis results in the production of	Ammonia	Nitrate	Nitrite	Methane	Methane
238	Which is the most important type of metabolism in anoxic freshwater?	Pond sediments	lake sediments	rock sediments	All of the above	lake sediments
239	Carbohydrates are converted to approximately equal amounts of____and CO	CH 4	CO2	H2SO4	none of the above	CH 4
240	Kinetics is a branch of	Life science	Natural science	Nanoscience	Information Technology	Natural science
241	Stoichiometry is the quantitative relationship between____ and products in a chemical reaction	substrate	enzyme	reactants	All of the above	reactants
242	____are those, which involve a single elementary reaction?	simple	complex	Unimolecular	bimolecular	simple
243	In____reaction, a reactant A transforms itself to two different products B and C.	complex	simple	series	parallel	parallel

Unit V						
245	Uniform suspension of microbial cells in homogenous medium is known as	Aeration	Agitation	Transportation	Mixing	Agitation
246	Rheology refers to_____of fluids	Uplift	Unload	Mixing	Flow	Flow
247	Stresses versus rate of strain curve in newtonian fluid is	bell shaped	linear	j shaped	non-linear	linear
248	Viscosity depends upon	Oxygen	Temperature & Pressure	CO2	Salinity	Temperature & Pressure
249	Air bubbling is performed during	Agitation	Aeration	Transportation	Mixing	Aeration
250	A single constant value of viscosity is described by	newtonian fluid	Non-Newtonian fluid	heat transfer	Agitation	Non-Newtonian fluid
251	Oxygen mass transfer capability is characterized by	q0	q0X	kLa	C-C	kLa
252	Air bubbles are formed at	Sparger	Open pipe	Porous diffusers	Complex injectors	Sparger
253	The air flow dominates the flow pattern known as	Flooding	Impelling	Scaleup	Stirring	Flooding
254	If broth viscosity increases, kLa will	Decrease	Increase	Equal	No reaction	Decrease
255	High degree of aeration and agitation will lead to	Dilution	Gaseous reaction	Evaporation	Foam formation	Foam formation
256	Oxygen transfer may be reduced by	Evaporation	Foaming	Dilution	Flooding	Foaming
257	Fermentation which is carried by yeast is called	Pyruvic	Acrylic	Lactic acid	Alcoholic	Lactic acid
258	Applications of fermentation includes	Cereals	Dairy	Beverages	All of the above	All of the above
259	Common example of fermented beverage product is	Pickles	Beer	Bread and buns	Cheese and yoghurt	Beer
260	Microorganism Bacillus is used to form	Ethanol	Formimc acid	Acrylic acid	Glycerol	Acrylic acid
261	How many ethanol molecules are produced from one glucose molecule	One	Two	Three	Four	Two
262	How many pyruvate molecules are broken down from one glucose molecule	One	Two	Three	Four	Two
263	How many lactic acid molecules are	One	Two	Three	Four	Two

	converted from one glucose molecule					
264	The process of one glucose molecule is broken down into two pyruvate molecules is	Glycolysis	Oxidative phophorylation	Gluconeogenesis	TCA cylce	Glycolysis
265	Penicillin is produced by _____ condition	Aerobic	Anaerobic	Facultative	None of the above	Aerobic
266	Penicillin is produced by _____ type of fermentation	Suspensive	Submerged	Flowt	None of the above	Submerged
267	Penicillin is_____a metabolite	Primary	Secondary	Tertiary	None of the above	Secondary
268	Penicillin is produced in the _____ phase	Lag	Log	Stationary	Death	Stationary
269	Penicillin requires _____ fermenter	Continious	Batch	Cooling	None of the above	Batch
270	The optimum tempertature for Pencillin production is between	10-15°C	15-20°C	25-27°C	35-45°C	25-27°C
271	What is the carbon source for penicillin?	Glucose	Lactose	Mannose	Maltose	Lactose
272	What is the energy source for penicillin?	Glucose	Lactose	Mannose	Maltose	Glucose
273	Streptomycin is an _____	Antibiotic	Antifungals	Alkaloids	All of the above	Antibiotic
274	Streptomycin is a_____synthesis inhibitor	Nucleic acid	Protein	Vitamin	None of the above	Protein
275	Streptomycin is produced by _____ type of fermentation	Suspensive	Submerged	Flowt	None of the above	Submerged
276	The optimum tempertature for Streptomycin production is between	10-15°C	15-20°C	28-30°C	35-45°C	28-30°C
277	The optimum pH for Streptomycin production is between	5-6 pH	7-7.2 pH	7.6-8 pH	8-8.2 pH	7.6-8 pH
278	An antibody is a	Nucleic acid	Protein	Mineral	None of the above	Protein
279	Identical antibodies that are produced from one type of cell is known as	Immunoglobulin	Monoclonal antibody	Polyclonal antibody	None of the above	Monoclonal antibody
280	The antibody which can cross placenta is	IgG	IgD	IgM	IgA	IgG
281	The antibody which involve in secretion is	IgG	IgD	IgM	IgA	IgA

282	The antibody which involve in immediate hypersensitivity is	IgG	IgD	IgE	IgA	IgE
283	The first Ig molecule produced in a primary response to an antigen is	IgG	IgD	IgM	IgA	IgM
284	An antibody class is determined by its _____	Light chain	Heavy chain	Epitopes	None of the above	Heavy chain
285	A single antigenic determinant in the variable domains of an antibody known as	Idiotype	Idiotope	Allotype	Isotype	Idiotope
286	The antibodies that are derived from different cell line is known as	Immunoglobulin	Monoclonal antibody	Polyclonal antibody	None of the above	Polyclonal antibody
287	How many identical light chain does an antibody have?	1	2	3	4	2
288	What % of mouse dervied antibodies to develop human antimouse antibody (HAMA)?	10	33	100	None of the above	100
289	What % of mouse dervied antibodies to develop Chimeric antibody?	23	33	43	None of the above	33
290	What % of mouse dervied antibodies to develop humanized antibody (HAMA)?	10	100	1000	None of the above	10
291	Ethanol fermentation is classifed under	Aerobic	Anaerobic	Facultative	None of the above	Anaerobic
292	The optimum pH for Ethanol production is between	4.8-5.0 pH	7-7.2 pH	7.6-8 pH	8-8.2 pH	4.8-5.0 pH
293	During ethanol production, alcohol is converted from	Protein	Lipids	Carbohydrate	Nucleic acid	Carbohydrate
294	The antibiotic bacitracin is produced from	*Bacillus subtilis*	*Streptomyces*	*Saccharomyces*	*Penicillium*	*Bacillus subtilis*
295	The mode of action of antibiotic bacitracin on	Wall synthesis	Protein synthesis	Cell membrane	All of the above	Wall synthesis
296	The antibiotic Tetracyclin is produced from	*Bacillus subtilis*	*Streptomyces*	*Saccharomyces*	*Penicillium*	*Streptomyces*
297	The mode of action of antibiotic Tetracyclin on	Wall synthesis	Protein synthesis	Cell membrane	All of the above	Protein synthesis
298	Griseofulvin is an_____antibiotic	Antifungal	Antibacterial	Antiviral	All of the above	Antifungal
299	Which one of the following is not a Viral	Malaria	Hepatitis B	Influenza	Anthrax	Anthrax

	disease					
300	Which one of the following is not a Bacterial disease	*Brucellosis*	*Typhoid*	*Measles*	*Shigellosis*	*Measles*
301	The carbon substrate for *spirulina* species is	CO2	Methane	Ethanol	Mollases	CO2
302	Which of the following microbe have more composition of protein content	Fungi	Algae	Yeast	Bacteria	Bacteria
303	Spirulina belongs to the group _____	Fungi	Algae	Yeast	Bacteria	Algae
304	Candida belongs to the group	Fungi	Algae	Yeast	Bacteria	Yeast